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The work presented in this thesis is concerned with the effect of several antibiotics and quaternary ammonium sanitizers upon growth of lactic acid bacteria. Section I reports the purification of beta-lactamase from Lactococcus cremoris PR-108, by ion exchange chromatography, using the chromogenic substrate pyridine-2-azo-p-dimethylaniline (PADAC) as the enzymatic indicator. Section II reports a study of the influence of antibiotics on lactococcal growth, where the effects of incubation time, culture dilution and the use of seeded and spread agar plate techniques are investigated. These studies were extended, in section III, to include investigations of the effect of quaternary ammonium base sanitizer (Ster-bac) on lactic starters. In addition, this section describes an reverse phase high performance liquid chromatography assay for the detection of quaternary ammonium compounds in milk.

GROWTH OF LACTOCOCCI RELATIVE TO ANTIBIOTIC AND QUATERNARY AMMONIUM COMPOUNDS

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Typed by the author, Marilin Valladao

To my sons

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GROWTH OF LACTOCOCCI RELATIVE TO ANTIBIOTIC AND QUATERNARY AMMONIUM COMPOUNDS

CHAPTER 1

STUDIES ON BETA-LACTAMASE OF LACTOCOCCUS LACTIS SUBSP. CREMORIS: PURIFICATION OF ENZYME FROM STRAIN PR-108 USING SEPHADEX CHROMATOGRAPHY

ABSTRACT

The exoenzyme beta-lactamase of Lactococcus cremoris PR-108 confers resistance to benzyl-penicillin and other lactam antibiotics. Attempts were made to purify the enzyme by precipitation with ammonium sulfate, adsorption on DEAE-Sephadex A-50 and CM-Sephadex C-50, and gel filtration on Sephadex G-50, with small yields of up to 570 mg/L culture. The penicillinase had a pH optimum of 6 to 7 for hydrolysis of the chromogenic cephalosporin substrate pyridine-2-azo-p-dimethylaniline cephalosporin (PADAC). Enzyme activity was inhibited by dicloxacillin.

INTRODUCTION

Antibiotics occur from time to time in raw milk as a result of treating udder infections in dairy herds. These infections, called mastitis, are very costly to dairy producers, causing 3 billion dollars per year loss for the 180,000 U.S. producers. For example, Gonzales et al. (11) recently reported a clinical mastitis incidence of 49% from a 3-year study involving two large commercial California dairy herds. Clinical mastitis is defined as an inflammatory abnormality of the udder, frequently accompanied by a watery discharge and milk clots.

Penicillin and other antibiotics frequently are used by farmers to treat mastitis and other infections. When this occurs, milk from such treated cows should be witheld from the bulk supply for at least 72 hours or until it tests negative for antibiotic (20). If this is not done and the milk is supplied to commercial cheese factories, antibiotic residues may interfere with the acid producing activity of cheese vat starter cultures (14), especially when the more sensitive Lc. cremoris strains are being used.

In general, the milk used for starter propagation is heated at 87.8°C (190°F) for about 45 minutes, while cheese milk is high temperature, short time (HTST) pasteurized at 65.6°C - 71°C (150°F - 160°F) for 16 seconds (2). Even though milk may be pasteurized before cheesemaking, antibiotic may be expected to remain intact. For example, Watts and McLeod (34) working with solutions of penicillin in milk heated to 100°C (212°F), detected no destruction within 15 minutes, but after 60 minutes 75% appeared to be destroyed. In addition, after milk pasteurization at 60°C (140°F) for 30 minutes, it was found that very little loss in penicillin potency occurred (9).

The main economic significance of the presence of antibiotic residues in milk is inhibition of acid production by lactic starters. Several laboratory tests are available for detection of drugs in milk. For example, the Charm screening assay for beta-lactam residues is a fast, accurate and sensitive method. This test detects the binding of radioactive carbon-14 at certain sites on the cell wall of microorganisms. Since beta-lactam antibiotics interfere with such binding, they can be detected at very low levels (i.e., $\geq$.01 IU/ml). In addition, using H^3 labeled reagents the Charm Test also can detect other antibiotics, including tetracycline, streptomycin, erythromycin, sulfa drugs and

chloramphenicol (24). The modified Whiteside Mastitis Test (WMT - 12) is based on the reaction between nucleated somatic cells and NaOH in the presence of the pH indicator bromocresol purple while the California Mastitis TEST (CMT - 27, 28) relies on a reaction between a detergent and DNA liberated from somatic cells. Milk having concentrations of somatic cells in excess of 500,000 per milliliter are abnormal (2) and will give positive reactions in the WMT and CMT tests. Other mastitis detection procedures include the Electroconductivity (19), DNA-Somata Count (4), MAST (26), Wisconsin Mastitis Test (3) and the Fossomatic Count (17) tests.

Enzymatic and physiological reactions of lactic acid bacteria inhibited by the beta-lactam antibiotics have been shown to vary widely, depending on the structure of the drug, the type of bacterium, and the growth conditions (10, 13). In this regard, penicillin appears to act on the bacterial cell by binding to either a transpeptidase or carboxypeptidase, each of which is involved in terminal reactions necessary to complete the peptidoglycan structure. Consequently, the cytoplasm continues to grow, leading to aberrant morphology and subsequent rupture of the cell wall (3, 8, 34). Affinity chromatography has revealed several penicillin binding proteins (PBP) in bacteria; gel electrophoresis was used to separate and enumerate bacterial proteins that can bind radioactive penicillin (26). One such

protein, beta-lactamase, was discovered in 1940 by Abraham and Chain (1) in an extract of Escherichia coli. It has since been found in many other species of bacteria such as Staphylococcus aureus, Bacillus cereus and Bacillus licheniformis (6, 25). Beta-lactamase hydrolyzes the amide bond in the beta-lactam ring of penicillin or cephalosporins, resulting in the liberation of penicilloic and cephalosporic acids, respectively.

Penicilloic acid is a stable molecule and can be assayed by various methods. These include a traditional iodometric method (5), which uses the reactions between iodine and starch as an indicator of whether or not penicilloic acid is formed from penicillin, following the mixing with the test organism. Under the low pH conditions created by beta-lactamase activity, starch is hydrolyzed to other sugars and cannot maintain binding with iodine, dissipating the dark blue color in the medium. In the broth or disk acidometric method (7), phenol-red or other pH indicators denotes the presence or absence of penicilloic acid. A third, more sensitive method depends on changes of chromogenicity resulting from the disruption of the beta-lactam ring due to beta-lactamase action against certain cephalosporins. Cephalosporin substrates that possess this chromogenic characteristic are nitrocefin (yellow to red reaction), CENTA (2-nitro-5-mercaptobenzoic acid; faint to intense yellow) and PADAC

(pyridine-2-azo-p-dimethylaniline cephalosporin; purple to yellow) (18).

PADAC hydrolysis was the assay of choice in the present study, since it is highly reproducible and sensitive. Its chromophore moiety resides in the third substituted position of the cephalosporin nucleus. The intact compound has a distinct purple to violet color (570 nm), which changes to a bright yellow when the beta-lactam ring is opened, and the chromophore site released (29). Since beta-lactamase produced by the mutant Lc. cremoris PR-108, created by N-methyl-N-nitro-N-nitrosoguanidine mutagenesis (15), is extracellular, isolation and purification was based on extraction of enzyme from M-17 medium. The separation method employed in this study involved ammonium sulphate fractionation, ion exchange chromatography and gel filtration. Ion exchange chromatography was first developed by Peterson and Sober (21). It is based on the principle that protein mixtures and chromatographic matrixes of opposite charge are bound by reversible electrostatic interactions, bringing about separation. The enzyme solution was further purified by gel filtration, first developed by Porath and Flodin (23). In this procedure, separation results from differences in molecular size, that is, smaller molecules will enter and leave the pores of the gel matrix more often than larger molecules and in doing so, will elute at a slower rate from the chromatographic column.

The objective of the work reported in this chapter was to follow up on the study of Khosravi and Sandine (15) in characterizing beta-lactamase of Lactococcus cremoris. At the start of the work resistance to penicillin by Lc. cremoris PR-108 was confirmed but the level of resistance was reduced by 20% over that originally reported. Nonetheless, attempts were made to purify the enzyme but its low level of activity made this difficult.

MATERIALS AND METHODS

Medium

M-17 broth (32) (Difco Laboratories) was reconstituted (37.25 g/L) in distilled water, autoclaved for 15 minutes at 15 pounds pressure (121°C), cooled to 45°C in a water bath, and supplemented with .5% sterilized lactose (Sigma Chemical Company). Final pH was 6.8. M-17 powder constituents were prepared as above, supplemented with 1% Bacto agar (Difco Laboratories) (32) and 15 ml quantities were added to sterile petri plates. The plates were stored inverted in a sealed plastic bag at 4°C.

Bacterial Growth

Lyophilized cultures of Lactococcus cremoris 108 and Lactococcus cremoris PR-108 were initially grown in M-17 broth at 22°C for 16-18 hr. An inoculum (1 ml and 150 ml of each respective culture) was then subcultured into 100 ml or 15 L of fresh M-17 broth (1% inoculum) and incubated at 22°C for 18 hr (15). Bacillus cereus (B. cereus spore suspension from Difco Laboratories) was grown initially in 10 ml M-17 broth

(1% inoculum) at 22°C for 16-18 hr. The culture was then subcultured (1.5 ml) into 150 ml of M-17 broth (1% inoculum) at 22°C for 18 hr. For the experiment concerning beta-lactamase production by whole resting cells of B. cereus, 108 and PR-108 were grown in tubes of M-17 broth (10 ml) supplement with .5% sterile lactose, in the presence and absence of 1 μg/ml Penicillin G (United States Biochemical Corp.).

Preparation of Crude Bacterial Extracts

Each culture was tested after growth for 18 hr by immersing 1 ml samples in duplicate in an ice bucket containing a mixture of ice and water. After 20 minutes, bacterial densities were measured by reading optical densities (O.D.) at 600 nm in a Beckman Scanning Spectrophotometer, model DU-40 (Beckman Instruments Inc.), at a temperature of 26°C. M-17 broth was used as blank. Cells of 108 (90 ml) and PR-108 (14.48 L), both in logarithmic growth phase, were then harvested, using a Beckman, model J2-2 centrifuge, at 10,000 X g for 15 minutes. Enzyme activity in the supernatants were measured; 2 ml 108 and 10 ml PR-108 fluid supernatant were stored at -70°C in a cabinate freezer (Kelvinator Commercial products Inc.). To the PR-108 supernatant, 0.016% polyethylene glycol (PEG) antifoam (grade III, molecular weight (MW) 3,000, density 1.20 g/ml-Sigma Chemical Company) was added.

All consecutive steps for beta-lactamase purification were performed with either .05 M trizma-hydrochloric acid or .05 M sodium phosphate buffers and carried out at 4°C.

Preparation of Buffers

Sodium phosphate buffers were prepared as follows: 6.702 g $Na_2HPO_4 \cdot 7H_2O$ (dibasic), MW = 268.07 (Aldrich Chemical Company Inc.), was added to 500 ml distilled water and 12 g NaH_2PO_4 (monobasic), MW = 120 (Sigma Chemical Company) was added to 2 L distilled water. To make a .05 M buffer solution, 1754 ml monobasic was added to 246 ml dibasic to make a 4 L final solution using distilled water. To make a .5 M buffer solution, 139 g monobasic was added to 2 L distilled water, and 268.25 g dibasic was added to 2 L distilled water. Then 1370 ml monobasic was added to 630 ml dibasic to make a 4 L final solution as before. Buffers were divided into 1/2 volume and autoclaved for 30 minutes at 15 pounds pressure (121°C), cooled to room temperature or to 4°C, depending on time of use; the pH was then adjusted using NaOH or HCl to the desired value.

Trizma-hydrochloric acid (tris-HCl) buffers were prepared as follow: To make a .05 M solution, 28.08 g trizma-HCl (Sigma Chemical Company) was added to 2.68 g trizma-base (Sigma Chemical Company) and mixed in distilled water to a

final volume of 4 L. Four liters of .5 M tris-HCl buffer solution were also prepared.

Preparation of washed cells

Beta-lactamase production by whole resting cells of Lc. cremoris PR108 and the controls 108 and B. cereus were prepared as follows: Each respective bacterial culture was grown in M-17 tubes (10 ml) at 22°C for 18 hr, then centrifuged at 10,000 X g for 15 minutes. Pellets were resuspended in 5 ml .05 M tris-HCl buffer, pH 7.0 and centrifuged as before. The fluid supernatant (1 ml) was analyzed spectrophotometrically for beta-lactamase activity, in the presence of 70 μl PADAC (125 μg/ml) at 570 nm. The second pellet was wash treated as before and the second fluid supernatant analyzed accordingly.

Preparation of Beta-Lactamase

A commercial preparation of beta-lactamase from B. cereus was used to make a standard curve. The sample contained 30 to 50 units/mg of 75% protein (type II), and was assayed using benzylpenicillin or cephaloridine (Sigma Chemical Company). Then enzyme at .5 units/mg protein was dissolved in either .05 M tris-Hcl buffer, pH 7.0 or .05 M sodium phosphate buffer, pH 7.0. In both cases, final

concentrations ranged from .048 to 1 μg/ml.

Enzyme Inhibitor

Dicloxacillin, MW = 492.3, 1% water and with a potency of 917 mg was purchased from Sigma Chemical Company. Dicloxacillin was dissolved in .05 M sodium phosphate buffer, pH 7.0 at various concentrations.

Preparation of PADAC

According to Calbiochem-Behring technical services, PADAC solutions should be first dissolved as follow: 2.5 mg PADAC / 5 ml methanol + 5 μl acetic acid, glacial. In this experiment, the PADAC solution was prepared by dissolving .125 mg PADAC in .25 ml methanol (T.J. Baker Chemical Company), containing .1% glacial acetic acid (T.J. Baker Chemical Company); to this solution, .05 M sodium phosphate buffer, pH 7.0 at room temperature was added to make a 2 ml total volume. Alternatively, .05 M tris-HCl buffer, pH 7.0 at room temperature, was added also to make a 2 ml final solution. In both cases, the final PADAC concentration was 125 μg/ml. PADAC solutions were kept at -20°C and remained stable for several days.

Beta-Lactamase Assay

Enzyme activities in the culture supernatant and in the various fractions collected during purification were assayed spectrophotometrically at 26°C with PADAC. The change in O.D. at 570 nm/minute was measured in a 1 cm pathlength microcuvette with a 1 ml volume (American Scientific Products).

Preparation of Antibiotic

Penicillin G (United States Biochemical Corp.), 1650 units/mg (1 μg = 1.650 units) (15) was dissolved in .05 M sodium phophate buffer, pH 6.10 at room temperature to a final concentration of 100 μg/ml. The solution was sterilized by filtration through a .2 μm acrodisc filter (Gelman Sciences Inc.) and stored at 4°C; it was stable for at least five days. Sterile susceptibility paper disks of .45 μm pore size (Schleicher & Schnell) were soaked in various penicillin concentrations ranging form .5 to 2.5 μg/ml, gently squeezed on the side of the test tube and placed on the M-17 agar surface.

Selection of Buffer pH

According to Pharmacia (22), a range of pH 5 - 9 should be used for anion-exchange buffers and pH 4 - 8 for cation

exchangers with .5 pH-unit intervals between tubes; .1 g DEAE-Sephadex ion exchanger was added to 9 test tubes and .1 g CM - Sephadex ion exchanger was added to the remaining 9 test tubes. The powdered gel in each tube was equilibrated to a different pH by washing 10 times with buffers as follow: .5 M sodium phosphate for CM-Sephadex and Sephadex G-50, and .5 M tris-HCl for DEAE-Sephadex. Equilibration of gel in each tube was followed, using a lower ionic strength buffer (.05 M), with washing 5 times with 10 ml of respective .05 M buffers; 2 ml of crude enzyme preparation was added to each tube, mixed for 5 minutes, and allowed to settle for 10 minutes. The supernatant of each tube was then assayed spectrophotometrically at 570 nm, using 50 μl PADAC (128 μg/ml) in 1 ml solution in a 1 cm cuvette pathlength. Experiments were run in triplicate.

Swelling of DEAE-Sephadex A-50

Diethylaminoethyl (DEAE) Sephadex A-50 (Pharmacia LKB Biotechnology Inc., 5.5 g) was soaked in 150 ml of .2 M NaCl (EM Science, a Division of EM Industries, Inc.) for 5 minutes. To complete the swelling process, the DEAE-NaCl solution was mixed into 225 ml .05 M tris-HCl buffer, pH 8.0 (at 100°C tris-HCl has a pH 7.1) and boiled at 100°C for 2 hr in a water bath to deaerate the gel (22). After cooling to room temperature, the gel was washed two times with 400 ml of

.05 M tris-HCl buffer, pH 7.0 at 4°C. The gel was allowed to settle, excess buffer discarded and the final slurry was poured into a glass column (1.6 by 70 cm, Pharmacia Fine Chemicals); the ion exchange bed had a final height of 44 cm and was allowed to equilibrate and stabilize by running 3 column-volumes (265 ml) of the same starting buffer.

Swelling of CM-Sephadex C-50

Carboxymethyl (CM) Sephadex C-50 (Pharmacia LKB Biotechnology Inc., 7.87 g) was soaked in 250 ml .2 M NaCl for 5 minutes. To complete swelling, 300 ml .05 M sodium phosphate buffer, pH 6.5 was added and the solution was allowed to boil at 100°C for 2 hr in water bath. After cooling to room temperature, the gel was washed two times with 600 ml .05 M sodium phosphate buffer, pH 6.5 at 4°C. The gel was allowed to settle, and excess buffer discarded. The final slurry was poured into a glass column (2.6 by 40 cm, Pharmacia LKB Biotechnology Inc.). The ion exchange bed had a final height of 25 cm, and was equilibrated and stabilized by running 3 column-volumes (400 ml) of the same buffer.

Swelling of Sephadex G-50

Sephadex G-50 (Pharmacia LKB Biotechnology Inc., 14.1 g) was mixed into 500 ml .05 M sodium phosphate buffer, pH 6.5

and boiled at 90°C for 3 hr in water bath. After cooling to room temperature, the gel was washed two times with 500 ml .05 M sodium phosphate buffer, pH 6.5 at 4°C. The gel was allowed to settle and excess buffer discarded. The final slurry was poured into a glass column (2.6 by 40 cm, Pharmacia Fine Chemicals). The ion exchange bed had a final height of 33.4 cm and was equilibrated and stabilized by running 3 column-volumes (530 ml), using the same buffer conditions.

Purification of Beta-Lactamase

Step 1. Enzyme concentration: Using a laboratory-constructed design (Figure 1.7), the PR-108 supernatant was filtered simultaneously through two Amikon stirred ultrafiltration cell systems, models 8050 and 8400, over a YM-30 membrane of 43 and 76 mm diameters with a 30,000 MW cut-off (Amikon Corp.). The ultrafiltration units were connected to a pressure vessel, which was connected to a nitrogen gas tank at 65 pounds pressure per square inch (psi) (Liquid Air Corporation).

The YM-30 concentrates (2.4 L) were checked for enzyme activity and discarded. Enzyme activity in each respective filtrate was assayed. The 11.9 L filtrate was passed through YM-10 membranes of 43 and 76 mm diameters and 10,000 MW cut-off; all other parameters remained unchanged.

Step 2. Ammonium Sulfate Fractionation

The enzyme activity in the YM-10 concentrate culture supernatant (569 ml) was measured, then brought to 20% (354 g/L) (30) saturation with solid ammonium sulfate [$(NH_4)_2SO_4$] grade III, which had reduced heavy metal content (Sigma Chemical Company) and held for 3 hr with gentle stirring. The pH was maintained at 7.0 by the addition of .05 M trizma-base (Sigma Chemical Company). The solution was then centrifuged at 18,000 X g for 30 minutes. Fluid supernatant (595 ml) was then brought to 90% (511 g/L) (30) saturation with $(NH_4)_2SO_4$ and held for 18 hr with gentle stirring as before. The precipitate was collected by centrifugation at 18,000 X g for 30 minutes. The pellet was dissolved in a minimum volume of .05 M tris-HCl buffer (pH 7.0). The solution was dialyzed (Spectrum Medical Industries, Inc.) for 30 hrs against 4 L of the same buffer, with 3 buffer changes. The 287 ml from the dialysis bag was further concentrated using YM-10 membranes to 8 ml. Enzyme activity was associated with the precipitate occurring between 20 and 90% ammonium sulfate saturation.

Step 3. DEAE-Sephadex A-50 Anion Exchange Chromatography: The concentrated dialyzed material was adjusted to pH 7.0 with .05 M trizma-base and applied to DEAE-Sephadex A-50 column (1.6 X 70 cm). Tris-HCl (.5 M) pH 7.0 was used to elute the

beta-lactamase from the column. A flow rate of 10 ml/hr was maintained, and fractions of 5 ml collected. Enzyme activities were measured, and fractions # 40 through # 54 were pooled and concentrated to 12 ml in an Amikon YM-10 membrane. An aliquot (1 ml) was stored at -70°C; The remaining 11 ml was used in the PADAC assay.

Step 4. CM-Sephadex C-50 cation exchange chromatography: The concentrated active fraction from step 3 was adjusted to pH 7.0 as before and loaded on a CM-Sephadex C-50 column (2.6 X 40 cm) at flow rate of 18 ml/hr. Sodium phosphate buffer (.5 M), pH 6.5 was used to elute beta-lactamase. Enzyme fractions # 28 through # 44 were pooled and concentrated to 10 ml, using Amikon YM-10 membrane. An aliquot (1 ml) was stored at -70°C; The remaining 11 used in the PADAC assay.

Step 5. Sephadex G-50, super fine grade, gel filtration chromatography: The concentrated active fraction from step 4 (8 ml) was applied to a Sephadex G-50 column (2.6 X 40 cm). The beta-lactamase was eluted from the column with .5 M of the same buffer at a flow rate of .5 ml/min. Enzyme activity was measured, and fractions # 28 through # 48 pooled and concentrated to 8 ml, using the Amikon YM-10 membrane as before.

RESULTS AND DISCUSSION

This study began by checking the actual resistance levels of strains 108 and PR-108 against Penicillin G. At 22°C inhibition zones on M-17 plates were measured from the edge of a paper disk or a well (Figures 1.1 and 1.2) to the termination of growth for each lactococcal strain analyzed. Inhibition zones ranged from .49 to 8.35 mm (Table 1.1). Strain 108 was very sensitive to the different concentrations of Penicillin G while PR-108 was able to hydrolyze the beta-lactam ring of this drug up to a concentration of 2.0 μg/ml. In addition, the agar diffusion assay showed higher inhibition zones when compared to the disk assay; this was true for all the Penicillin G concentrations employed. The difference was probably due to the fact that some penicillin remained on the paper disk and did not totally diffuse through the agar, resulting in lower readings, while each one of the wells was completely dried and no visible residue of antibiotic solution was seen. It is interesting to point out that the tolerance level seen for PR-108 against Penicillin G differed from previous data of Khosravi and Sandine (15) which indicated 2.5 μg/ml (4.13 units) tolerance level for this bacterial strain. This small change (20% tolerance loss) could have

been caused by storage. According to Mayhall and Appolo (16), cultures of Staphylococcus aureus showed a 25% decrease in level of antibiotic resistance after being stored for one year at -70°C.

Next, penicillinase activity in whole resting cells of PR-108 and the controls 108 and Bacillus cereus was checked to ensure that an exoenzyme type molecule was being produced. Table 1.2 shows the excellent levels of growth of these lactic acid strains in M-17 broth, used in these studies. Figure 1.3 indicates very small enzymatic activity in the first buffer washing of PR-108 cells when compared to controls Bacillus cereus and Lc. cremoris 108 and PR-108 grown in the presence and absence of penicillin. There was probably some enzyme left from the M-17 supernatant as these mutant cells appear to have acquired excess lipid/carbohydrate which did not allow good cell aggregation after centrifugation. The second tris-HCl buffer washing confirmed these findings as the enzymatic activity from PR-108 cells was not detected to any degree (Figure 1.4). As expected in each case, the controls Bacillus cereus and 108 demonstrated the indicative curves for the presence and absence of penicillinase respectively. Figure 1.5 demonstrates the presence of the exoenzyme, beta-lactamase, from PR-108 culture supernatant as determined by PADAC hydrolysis measured at 570 nm. Table 1.3 summarizes Figures 1.2 through 1.4 by listing the various enzyme

activities as the change in optical density over the 15 minute assay period (Δ O.D.). From these data it may be seen that in comparison to the control there was negligeable to no activity in the whole cell washings but slight activity in the cell supernatant. These data are typical of that found in repeated experiments. While the Δ O.D. values are small for the supernatant enzyme they were repeatly demonstrable and thus enzyme purification was attempted, using PADAC as the chromogenic substrate.

PADAC is a colored cephalosporin and has been found to be very useful for the detection of beta-lactamase inactivating compounds like dicloxacillin (16). Table 1.4 shows the resistance pattern of various beta-lactamases against different concentrations of dicloxacillin. Beta-lactamase from PR-108 was readily inhibited in the presence of .013 μg/ml inhibitor while a commercial preparation of 75% pure beta-lactamase from Bacillus cereus (.048 μg/ml) required at least 3.1×10^{-3} μg/ml Dicloxacillin to inhibit PADAC hydrolysis, indicating that beta-lactamase from PR-108 was present in higher concentration. Table 1.4 shows a O.D. value of only .010 per minute per ml for the PR-108 supernatant enzyme. From this, it became clear that in order to obtain measurable enzyme activity during purification where activity would be lost, a large sample volume would be required. Therefore, 15 L was used.

Once the preliminary work demonstrated the presence of beta-lactamase in the culture supernatant of PR-108 cells, it was necessary to determine a suitable operational pH to maintain enzyme integrity throughout the various steps in the purification of this enzyme. The choice of the pH in the starting buffer should allow the enzyme to adsorb to the ion exchanger, to be close to the pH of elution and not to denature any sensitive area on the enzyme structure.

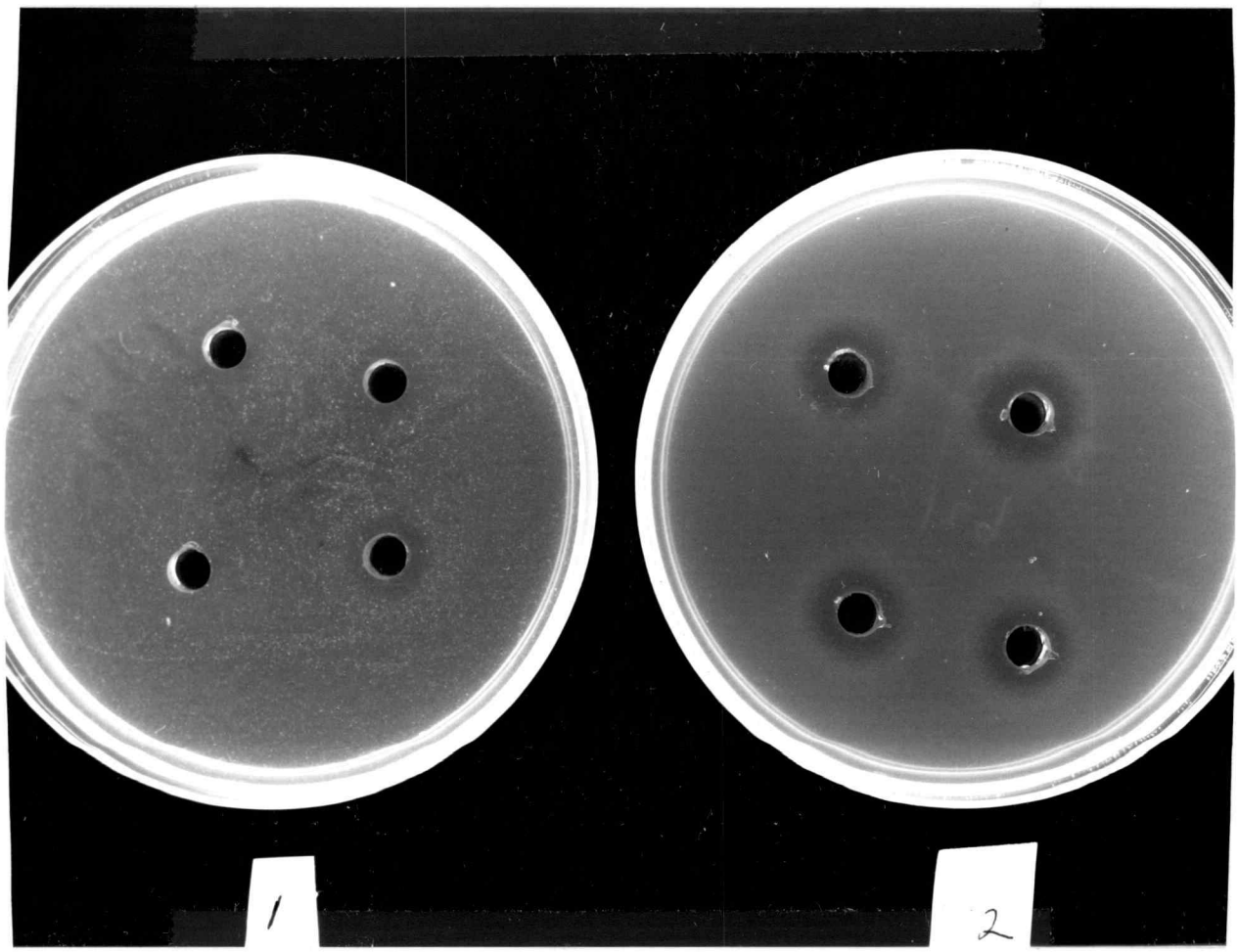
Figure 1.6 illustrates a simple test tube method developed by Pharmacia (21), used here to determine the starting pH for the purification of crude preparation of beta-lactamase from PR-108. Tables 1.5 and 1.6 show the decrease in optical density readings from PR-108 related to PADAC hydrolysis, indicating that PR-108 produces an amphoteric type of enzyme. As a result a pH between 6.44 and 7.2 was appropriate for use in DEAE-Sephadex while pH 6.5 was suitable for CM-Sephadex. Using the same approach, the ionic strength of each solution was determined, so that the cationic buffer tris-HCl (.05 M/ .5 M), pH 7.0 was chosen for the anion exchanger, DEAE-Sephadex, while the anionic buffer, sodium phosphate (.05 M/ .5 M) at pH 6.5 for the cationic exchanger, CM-Sephadex. Figure 1.7 shows the laboratory-constructed design used in the purification of beta-lactamase from PR-108. On DEAE-Sephadex A-50 anion exchange chromatography, the elution of enzyme from the bulk of other proteins was slow

(Figure 1.8), indicating the presence of many negatively charged molecules on the enzyme's surface; however, most other molecules that lacked penicillinase activity remained bound to the matrix and a considerable amount of beta-lactamase activity via PADAC hydrolysis, was detected after this step (Figure 1.11). The column matrix virtually turned to a brown color after passage of the sample. Figure 1.11 also illustrates the further purification of beta-lactamase by CM-Sephadex C-50 cation exchange chromatography and Figure 1.9 shows late enzyme elution as one major peak as indicative of the amphoteric nature of this enzyme. Some enzyme loss occurred during this step due to fragile equipment connections. Gel filtration eliminated the smaller molecules, but the beta-lactamase activity was practically lost after this step (Figure 1.11) due to the amount of time accumulated to run the entire procedure. However, when gel-filtration followed DEAE-Sephadex, enzyme elution occurred in two consecutive peaks (Figure 1.10) but still resulted in small degree of purification. Figure 1.12 illustrates the effect of time at 4°C on the stability of the beta-lactamase from PR-108. After the time required to carry out this entire experiment, 15 days, enzyme activity started to decrease after passage through either CM-Sephadex. Due to the fact that absorbance of a solution is directly related to its concentration, a direct relationship was drawn, between known concentrations of commercially available beta-lactamase (75%

pure) and change in optical density per minute, at 570 nm. Standard curves were plotted for enzyme solutions prepared in M-17 broth (Figure 1.13), .05 M tris HCl buffer, pH 7.0 (Figure 1.15), and .05 M sodium phosphate buffer, pH 6.5 (Figure 1.17). Each respective standard curve was determined from the degradation of 125 μg/ml PADAC in .05 M tris-HCl buffer, pH 7.0, in the presence of known concentrations of beta-lactamase from Bacillus cereus (Figures 1.14, 1.16 and 1.18). The degree of beta-lactamase purification from the various steps was determined from each respective standard curve, and expressed as mg enzyme per L solution and as units of enzyme activity per L solution. PADAC has a molar coefficient of 5.7×10^4, and it was used to calculate enzymatic hydrolysis as micromoles of PADAC hydrolyzed per minute per milliliter of enzyme solution. Enzyme purified through DEAE-Sephadex had the highest hydrolysis rate corresponding to an average yield of 24%, which was based upon values obtained from culture fluid supernatant (Table 1.7). The great amount of M-17 components present in the medium concentrate seems to have a negative effect on the enzymatic assay used in this study, so that numerical values could not be set for this step.

In summary, using PADAC as the chromogenic cephalosporin substrate, the concentration of beta-lactamase produced by PR-108 was calculated in each enzymatic purification step,

based on respective standard curves plotted for 75% pure beta-lactamase from Bacillus cereus. Gel filtration gave the least satisfactory step in the purification procedure since by the time it was performed, enzyme degradation started to occur. This final enzyme material can, however, be used for future experiments on the properties of the beta-lactamase.



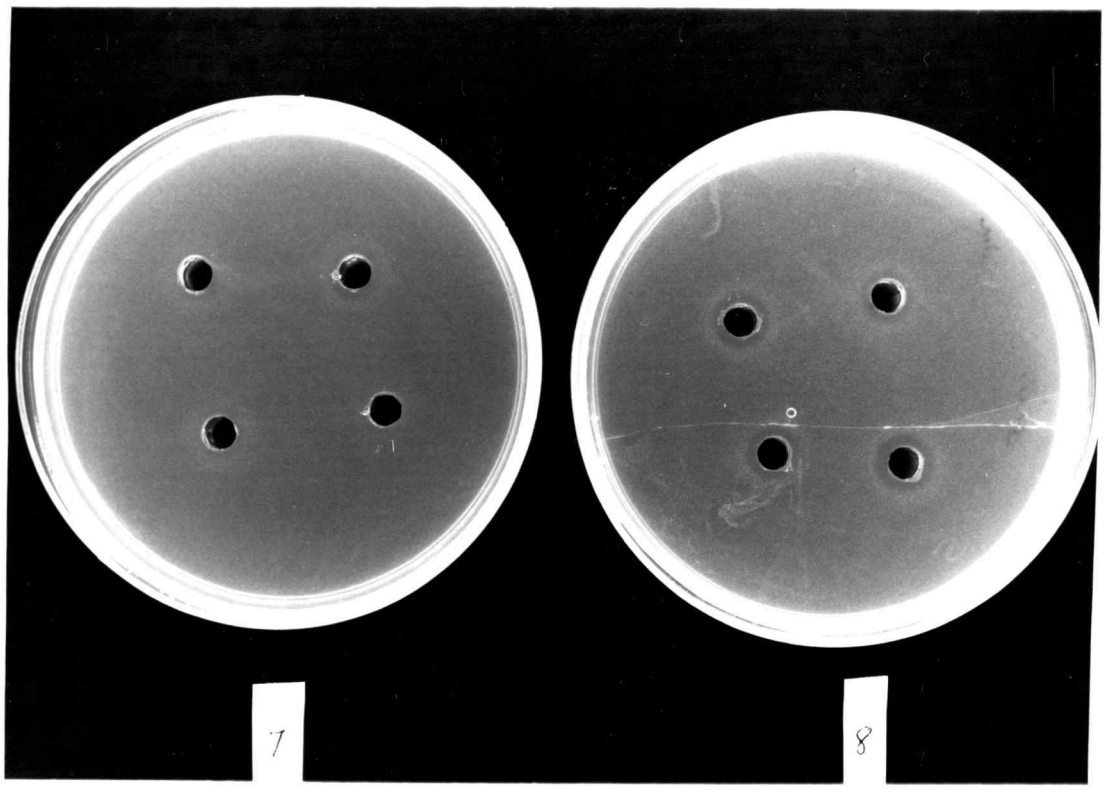
Lactococcus cremoris 108

(1) Wells in M-17 agar plates contain 0.5 μg/ml of Penicillin G (1.650 U/μg) in .05 M sodium phosphate buffer, pH 6.10.

Lactococcus cremoris 108

(2) Wells on M-17 agar plates contain 1.0 μg/ml of Penicillin G (1.650 U/μg) in .05 M sodium phosphate buffer, pH 6.10.

Figure 1.1 Effect of Penicillin G on cells of Lc. cremoris 108 measured by the plate diffusion assay method.



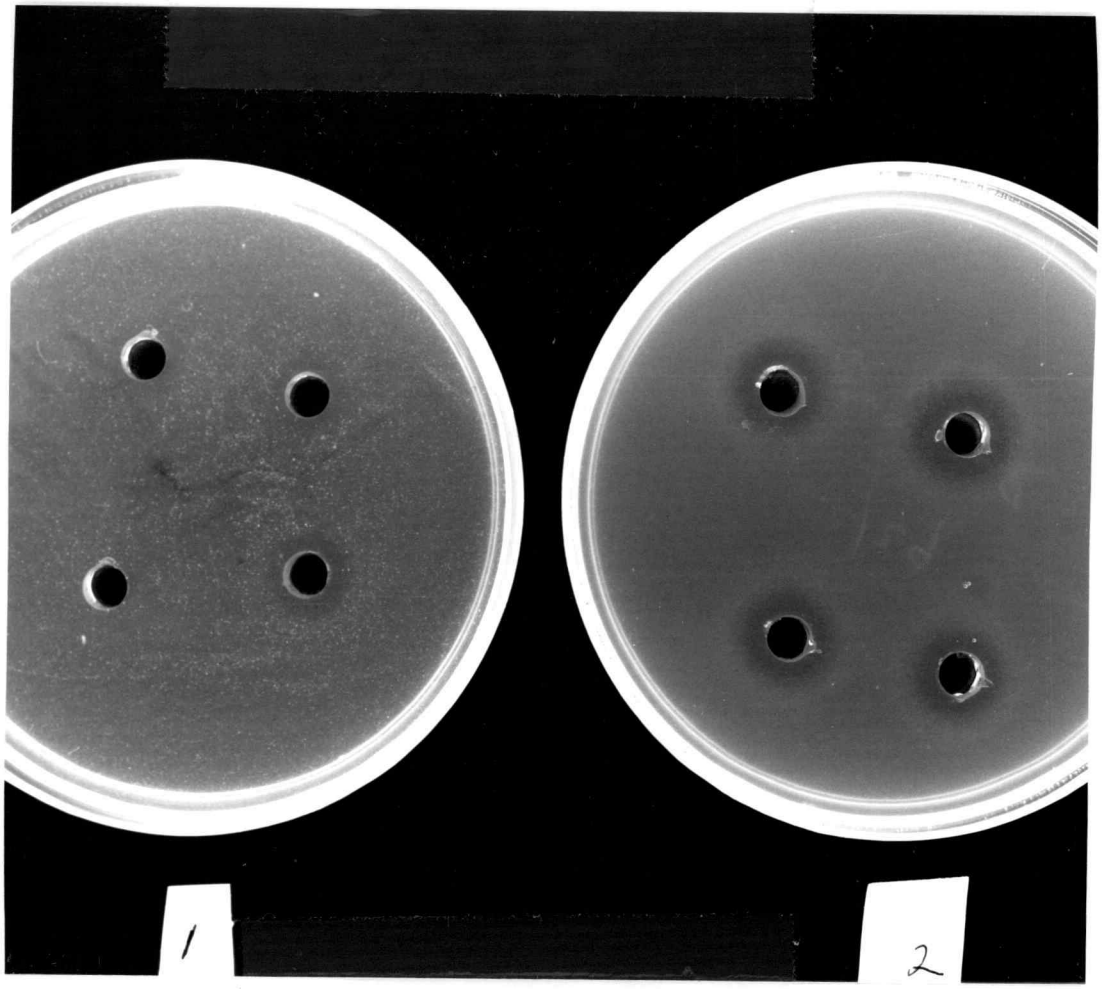
Lactococcus cremoris PR-108

(7) Wells in M-17 agar plates contain 2.0 μg/ml of Penicillin G (1.650 U/μg) in .05 M sodium phosphate buffer, pH 6.10.

Lactococcus cremoris PR-108

(8) Wells on M-17 agar plates contain 2.5 μg/ml of Penicillin G (1.650 U/μg) in .05 M sodium phosphate buffer, pH 6.10.

Figure 1.2 Effect of Penicillin G on cells of Lc. cremoris 108 measured by the plate diffusion assay method.



Lactococcus cremoris PR-108

(7) Wells in M-17 agar plates contain 2.0 μg/ml of Penicillin G (1.650 U/μg) in .05 M sodium phosphate buffer, pH 6.10.

Lactococcus cremoris PR-108

(8) Wells on M-17 agar plates contain 2.5 μg/ml of Penicillin G (1.650 U/μg) in .05 M sodium phosphate buffer, pH 6.10.

Figure 1.2 Effect of Penicillin G on cells of Lc. cremoris 108 measured by the plate diffusion assay method.

Table 1.1 Comparison of inhibition zones, measured in mm, by the agar diffusion method vs the disk assay technique, as a result of the action of different concentrations of Penicillin G in .05 M sodium phosphate buffer, pH 6.1. Each culture (.1 ml) was inoculated on M-17 plates and incubated at 30°C for 16 hr.

	Inhibition zone (mm)			
	Lc. cremoris 108		Lc. cremoris PR-108	
Penicillin G (µg/ml)[a]	agar diffusion	paper disk	agar diffusion	paper disk
0.0	0.0	0.0	0.0	0.0
0.5	3.98	3.95	0.0	0.0
1.0	4.85	4.75	0.0	0.0
1.5	7.00	5.05	0.0	0.0
2.0	7.70	5.70	0.0	0.0
2.5	8.35	6.25	0.60	0.49

[a] 1650 units/mg (1.650 units = 1 µg)

Table 1.2 Optical density readings as measured at 600 nm for Lc. cremoris 108 and PR-108 after growth in M-17 broth for 18 hr at 22°C. M-17 was used as blank.

	Optical density at 600 nm	
Bacterial strain	Sample 1	Sample 2
Lc. cremoris 108	0.747	0.706
Lc. cremoris PR-108	0.739	0.704

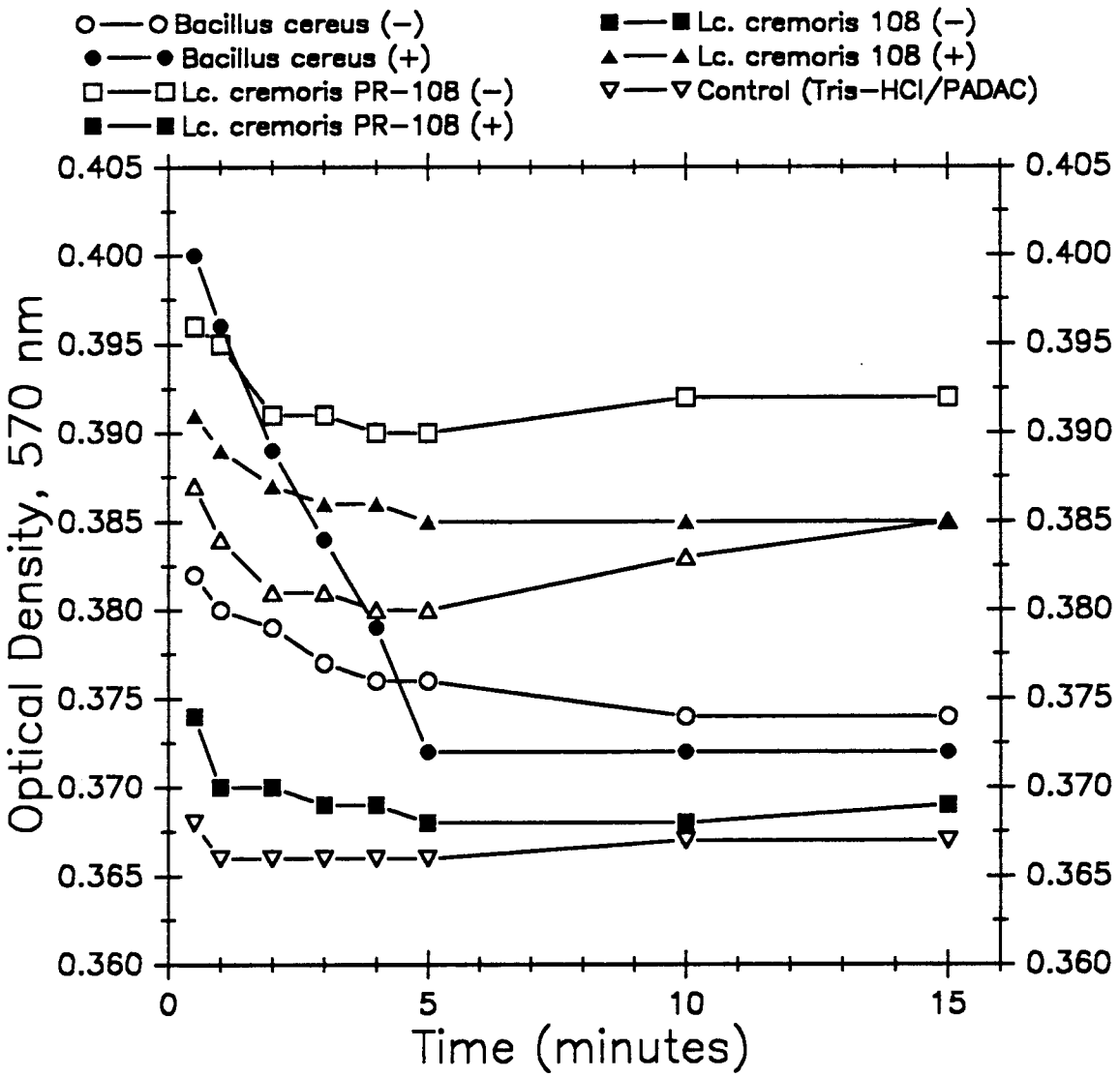


Figure 1.3 Optical density readings as measured at 570 nm for beta-lactamase in whole resting cells of Lc. cremoris PR-108, and the controls Lc. cremoris 108 and Bacillus cereus. The cultures were grown in tubes of M-17 broth (10 ml) supplemented with .5% sterile lactose solution in the presence (+) and absence (-) of 1 μg/ml Penicillin G. The cells were washed once in .05 M tris-HCl buffer, pH 7.0; 70 μl PADAC (125 μg/ml) added to 1 ml buffer wash.

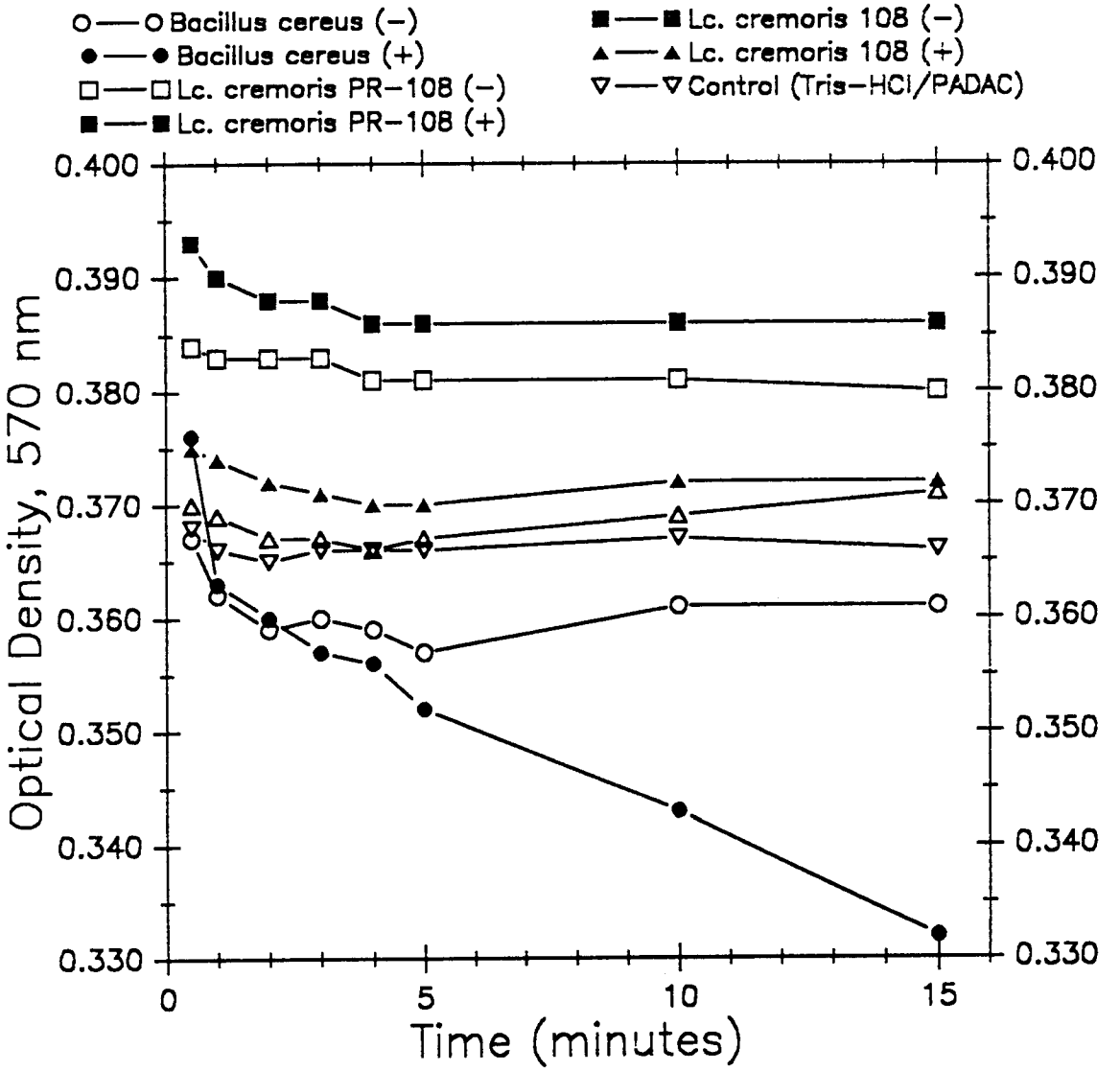


Figure 1.4 Optical density readings as measured at 570 nm for beta-lactamase in whole resting cells of Lc. cremoris PR-108, and the controls Lc. cremoris 108 and Bacillus cereus. The cultures were grown in tubes of M-17 broth (10 ml) supplemented with .5% sterile lactose solution in the presence (+) and absence (-) of 1 μg/ml Penicillin G. The cells were washed twice in .05 M tris-HCl buffer, pH 7.0; 70 μl PADAC (125 μg/ml) added to 1 ml second buffer wash.

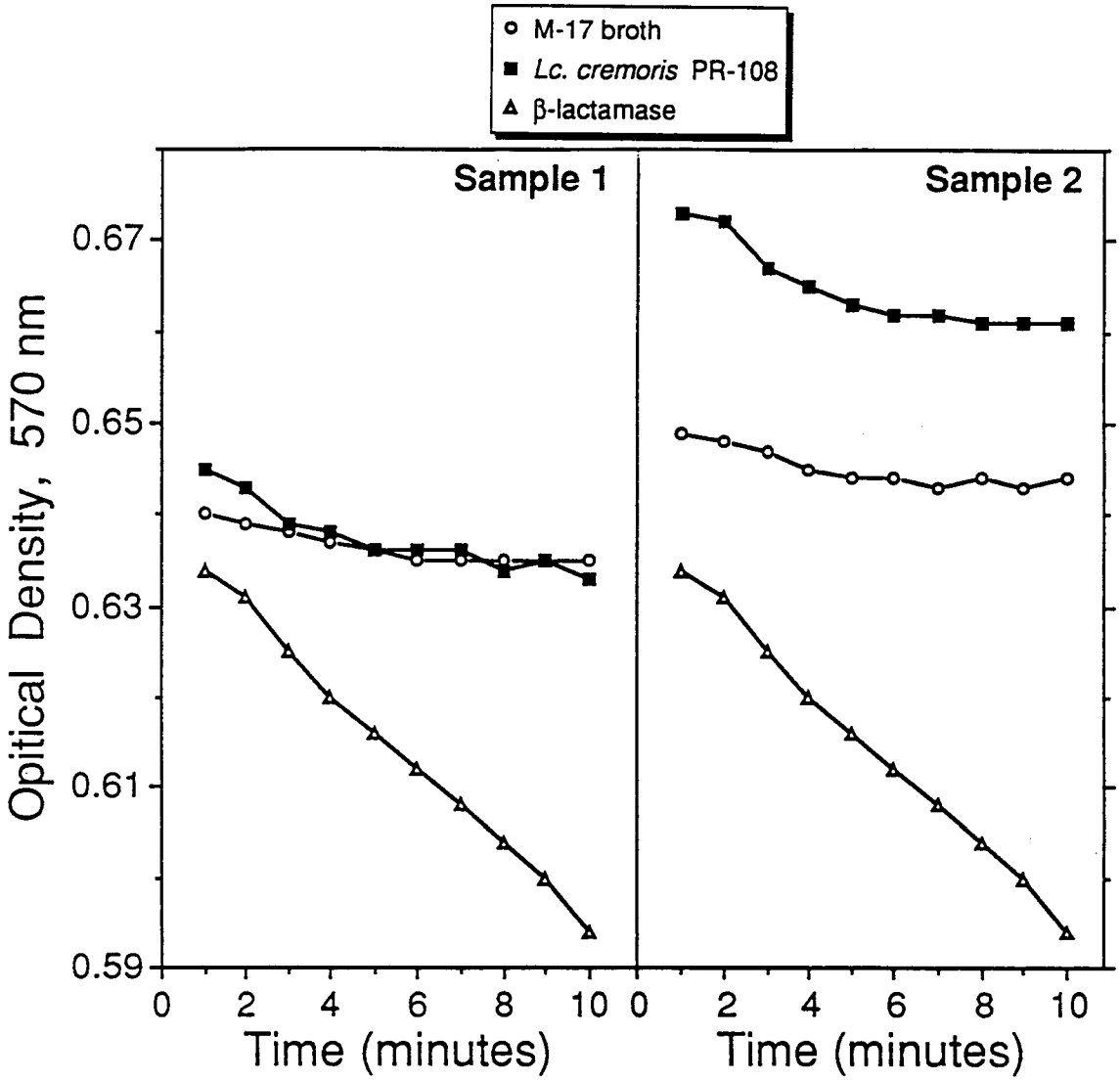


Figure 1.5 Beta-lactamase hydrolysis of PADAC as determined by optical density readings as measured at 570 nm. Reaction mixtures were composed of 1 ml fluid supernatant from Lc. cremoris PR-108 or 75% pure beta-lactamase type II from Bacillus cereus (Sigma Chemical Company, .1 μg/ml), M-17 broth and 70 μl PADAC (125 μg/ml) in .05 M phosphate buffer, pH 7.0. Cultures grown in M-17 broth (10 ml) for 18 hr at 22°C and centrifuged at 10,000 x g for 15 minutes.

Table 1.3 Summary of beta-lactamase activity found in different sample preparations. The change in optical density readings was measured at 570 nm over 15 minutes period.

SAMPLE	PENICILLIN G (1 ug/ml)	PENICILLIN G (0 ug/ml)
Supernatant		
Bacillus cereus	.307	.045
Lc. cremoris PR-108	.012	.014
Lc. cremoris 108	.007	.005
Control M-17/PADAC	-	.003
First Cell Washing		
Bacillus cereus	.028	.008
Lc. cremoris PR-108	.006	.004
Lc. cremoris 108	.006	.002
Control tris-HCl/PADAC	-	.001
Second Cell Washing		
Bacillus cereus	.044	.006
Lc. cremoris PR-108	.003	.003
Lc. cremoris 108	.003	.001
Control tris-HCl/PADAC	-	.001

[a] Penicillin G solution prepared in .05 M sodium phosphate buffer, pH 6.10

Table 1.4 Optical density readings as measured at 570 nm for beta-lactamases from Lc. cremoris PR-108, Bacillus cereus, and 75% pure enzyme preparation (Sigma Chemical Company) in M-17 broth (.048 μg/ml). Culture supernatants obtained after 18 hr growth at 22°C in M-17 broth, centrifuged at 10,000 x g for 15 minutes. The various concentrations of dicloxacillin were prepared in .05 M sodium phosphate buffer, pH 7.0; 70 μl PADAC (125 μg/ml) was added to 1 ml sample; M-17 broth used as blanck.

Time (minutes)	Dicloxacillin concentration (μg/ml)															
	0			4.92×10^{-5}			3.1×10^{-3}			0.013			0.026		0.049	
	Lc. cremoris PR-108	β-lac[a]	*Bacillus cereus*	*Lc. cremoris* PR-108	β-lac	*Bacillus cereus*	*Lc. cremoris* PR-108	β-lac	*Bacillus cereus*	*Lc. cremoris* PR-108	β-lac	*Bacillus cereus*	*Lc. cremoris* PR-108	*Bacillus cereus*	*Lc. cremoris* PR-108	*Bacillus cereus*
0.5	.309	.502	.360	.311	.483	.360	.293	.311	.317	.216	.275	.238	.205	.276	.340	.330
1	.308	.499	.283	.305	.484	.283	.292	.307	.306	.215	.271	.235	.205	.275	.339	.327
2	.305	.496	.273	.305	.481	.273	.286	.307	.607	.213	.267	.233	.204	.273	.336	.323
3	.303	.495	.256	.302	.480	.256	.285	.304	.298	.212	.266	.232	.200	.271	.334	.321
4	.303	.492	.231	.303	.478	.231	.285	.304	.295	.210	.266	.232	.200	.272	.334	.319
5	.301	.490	.215	.302	.478	.215	.286	.306	.291	.212	.267	.229	.198	.271	.333	.317
10	.299	.485	.218	.303	.478	.318	.286	.306	.289	.212	.269	.227	.200	.267	.336	.317
Δ O.D.	.01	.017	.142	.008	.005	.042	.007	.005	.028	.004	.006	.011	.005	.009	.004	.013

[a] β-lac = 75% pure β-lactemase (0.95 μg/ml)

pH measurements

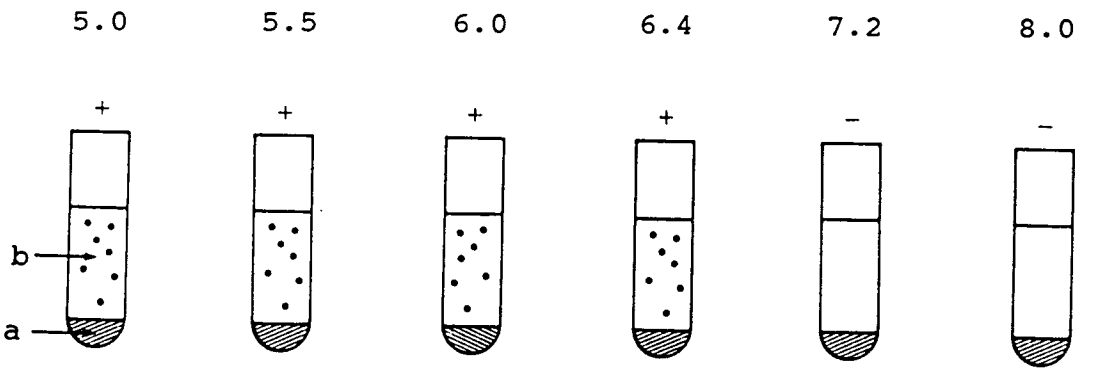


Figure 1.6 pH selection for DEAE-Sephadex (a). Beta-lactamase from Lc. cremoris PR-108 (b) bound at pH 7.2 as determined by optical density readings at 570 nm in the presence of PADAC (125 μg/ml) in .05 M tris-Hcl, pH 7.0.

Table 1.5 Optical density readings as measured at 570 nm for beta-lactamase from Lc. cremoris PR-108. Culture supernatant obtained after 12 hr at 30°C in M-17 broth, centrifuged at 10,000 x g for 15 minutes; 50 μl PADAC (128 μg/ml) in .05 M tris-HCl buffer, pH 7.0 added to 1 ml mixture (DEAE-Sephadex in .05 M tris-HCl buffer at different pH's); control used PADAC in specified buffer pH.

Time	pH Values															
	5.0		5.48		6.0		6.44		7.2		7.4		8.0		8.5	
(minutes)	Sample	Control	Sample	Control	Sample	Control	Sample	Control	Sample	Control	Sample	Control	Sample	Control	Sample	Control
0.5	.354	.372	.392	.337	.344	.327	.379	.362	.384	.347	.359	.342	.364	.340	.370	.345
1	.350	.366	.380	.338	.339	.327	.379	.360	.382	.343	.357	.342	.362	.338	.368	.338
2	.349	.366	.379	.341	.336	.326	.370	.361	.382	.342	.355	.339	.362	.339	.366	.337
3	.348	.365	.380	.336	.335	.326	.368	.362	.382	.341	.354	.338	.362	.340	.364	.338
4	.348	.366	.379	.335	.334	.326	.368	.362	.381	.342	.354	.337	.361	.338	.363	.337
5	.348	.366	.380	.336	.333	.326	.368	.362	.379	.342	.353	.337	.360	.339	.363	.338
Δ O.D.	.006	.006	.012	.001	.011	.001	.011	0	.005	.005	.006	.005	.004	.001	.007	.007

Table 1.6 Optical density readings as measured at 570 nm for beta-lactamase from Lc. cremoris PR-108. Culture supernatant obtained after 12 hr at 30°C in M-17 broth, centrifuged at 10,000 x g for 15 minutes; 50 μl PADAC (128 μg/ml) in .05 M sodium phosphate buffer, pH 7.0 added to 1 ml mixture (CM-Sephadex in .05 M sodium phosphate buffer at different pH's); control used PADAC in specified buffer pH.

Time (minutes)	pH Values													
	5.0		5.5		6.0		6.5		7.0		7.5		8.0	
	Sample	Control	Sample	Control	Sample	Control	Sample	Control	Sample	Control	Sample	Control	Sample	Control
0.5	.379	.402	.349	.336	.583	.336	.385	.340	.372	.308	.356	.358	.353	.403
1	.372	.400	.345	.334	.576	.334	.386	.333	.372	.307	.355	.356	.352	.403
2	.370	.397	.345	.334	.576	.332	.383	.337	.371	.306	.354	.358	.351	.403
3	.370	.397	.345	.334	.572	.331	.383	.337	.370	.302	.353	.356	.351	.402
4	.368	.396	.344	.334	.572	.331	.383	.337	.369	.302	.353	.356	.350	.402
5	.368	.396	.340	.333	.571	.331	.380	.337	.369	.302	.352	.357	.352	.402
Δ O.D.	.011	.006	.009	.003	.012	.005	.005	.003	.003	.006	.004	.001	.001	.001

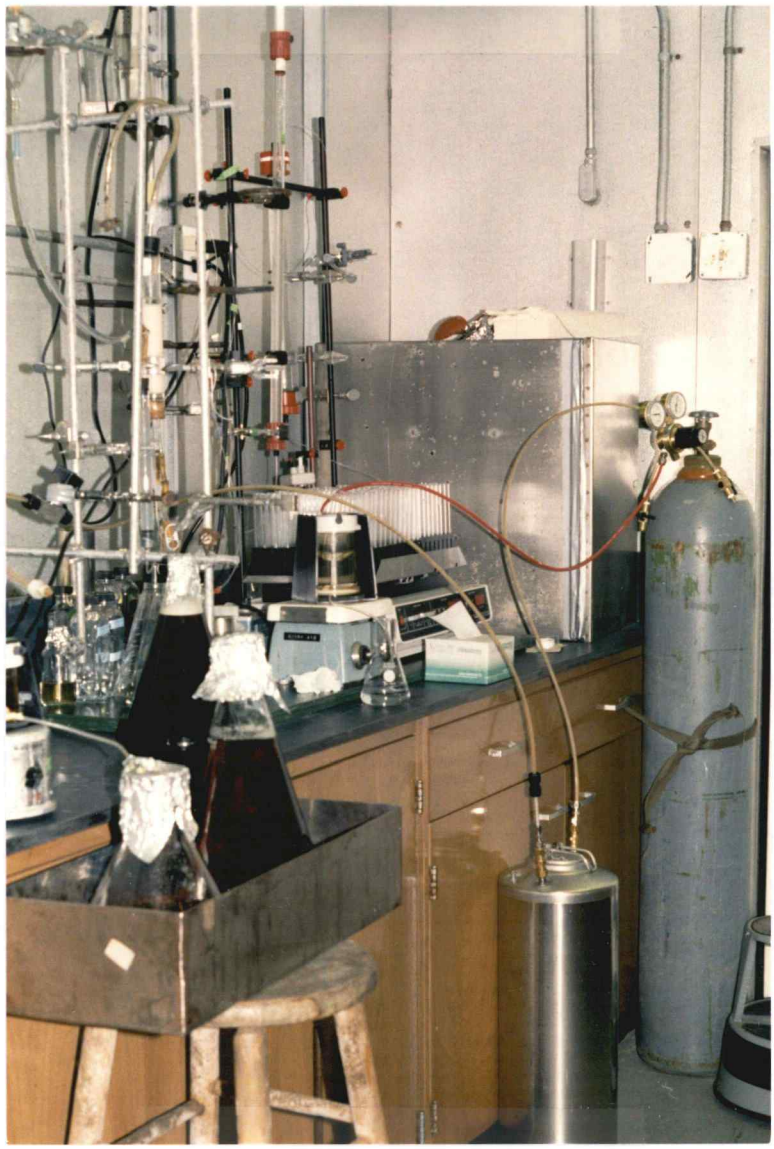


Figure 1.7 Laboratory constructed design of equipment used for beta-lactamase purification.

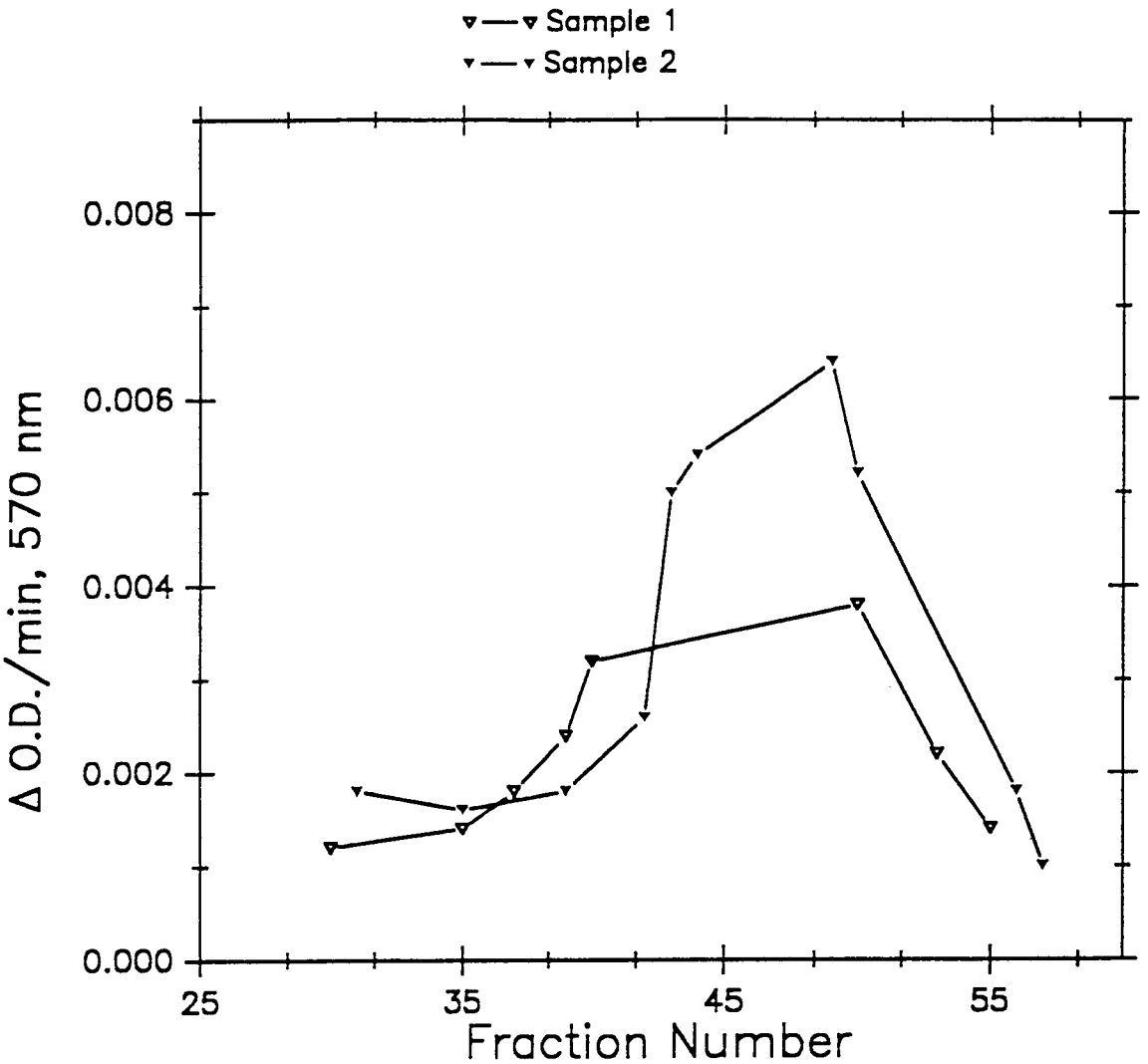


Figure 1.8 Optical density readings as measured at 570 nm of the successive steps in the chromatographic purification of beta-lactamase, from Lc. cremoris PR-108. Sample mixture from DEAE-Sephadex. Sample mixture contained 1 ml enzyme solution and 70 μl PADAC (125 μg/ml in .5 M tris-HCl buffer, pH 7.0).

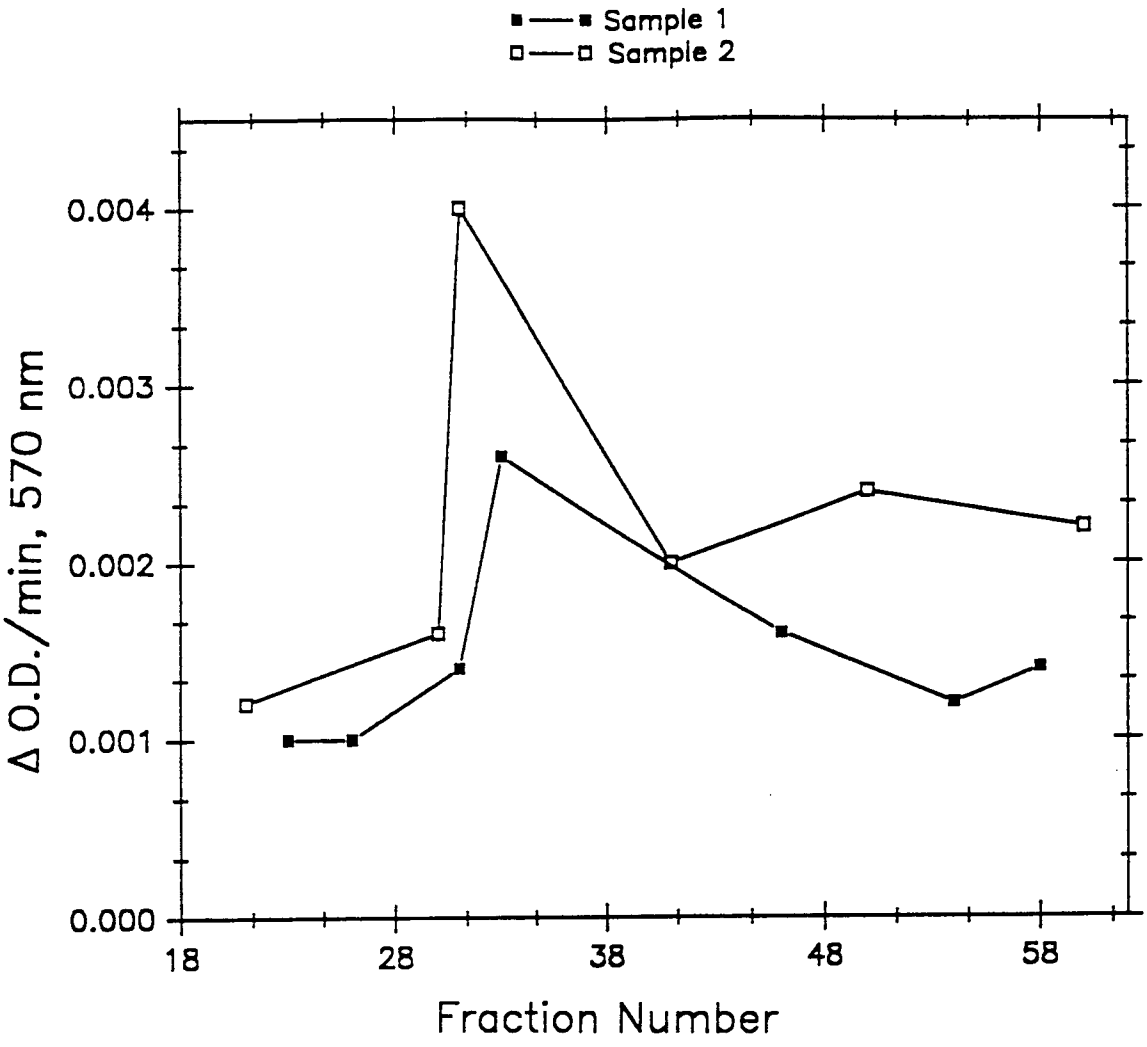


Figure 1.9 Optical density readings as measured at 570 nm of the successive steps in the chromatographic purification of beta-lactamase, from Lc. cremoris PR-108. Sample mixture from CM-Sephadex. Sample mixture contained 1 ml enzyme solution and 70 μl PADAC (125 μg/ml in .5 M sodium phosphate buffer, pH 6.5).

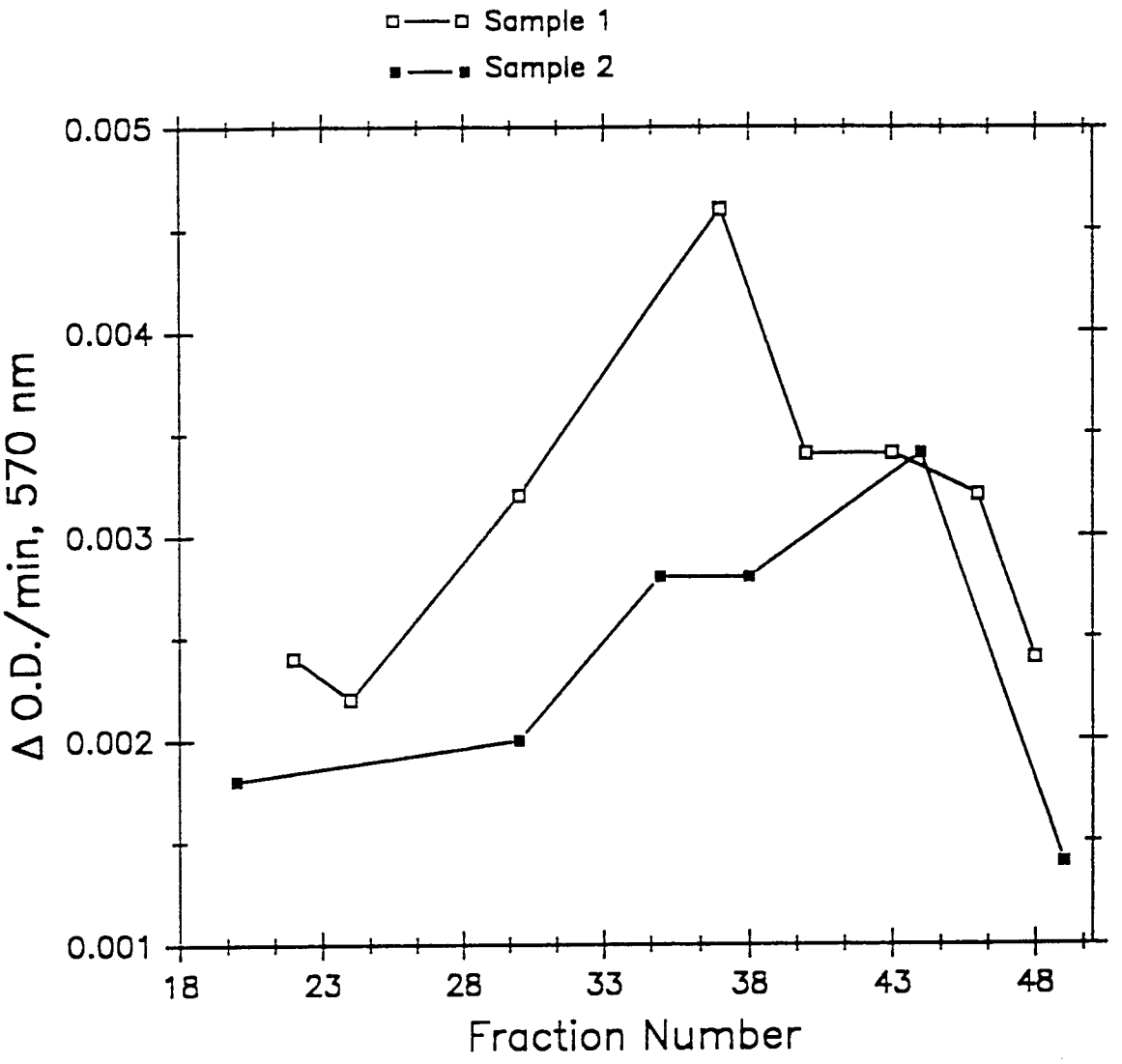


Figure 1.10 Optical density readings as measured at 570 nm of the successive steps in the chromatographic purification of beta-lactamase, from Lc. cremoris PR-108. Sample mixture from Gel-50. Sample mixture contained 1 ml enzyme solution and 70 μl PADAC (125 μg/ml in .5 M sodium phosphate buffer, pH 6.5).

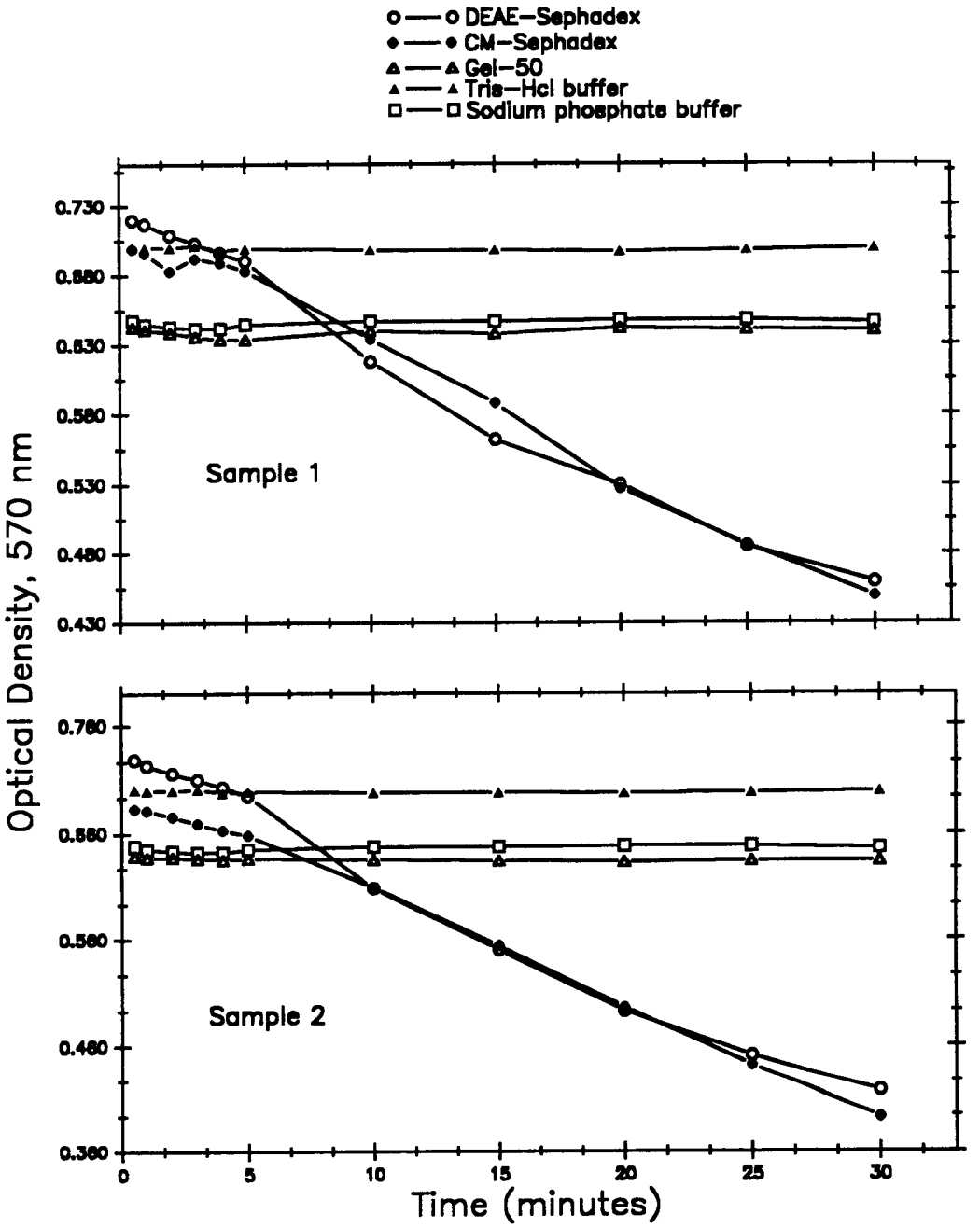


Figure 1.11 Time course of beta-lactamase action as measured at 570 nm, after purification through columns of DEAE and CM Sephadexes, and gel-50. Active fractions from samples 1 and 2, from each column, were concentrated in an Amikon ultrafiltration unit (YM-10 membrane filter). Sample mixtures contained 100 μl PADAC (128 μg/ml) and 1 ml concentrated active fraction from each respective column; controls .5 M tris-Hcl buffer, pH 7.0 and .5 M sodium phosphate buffer, pH 6.5.

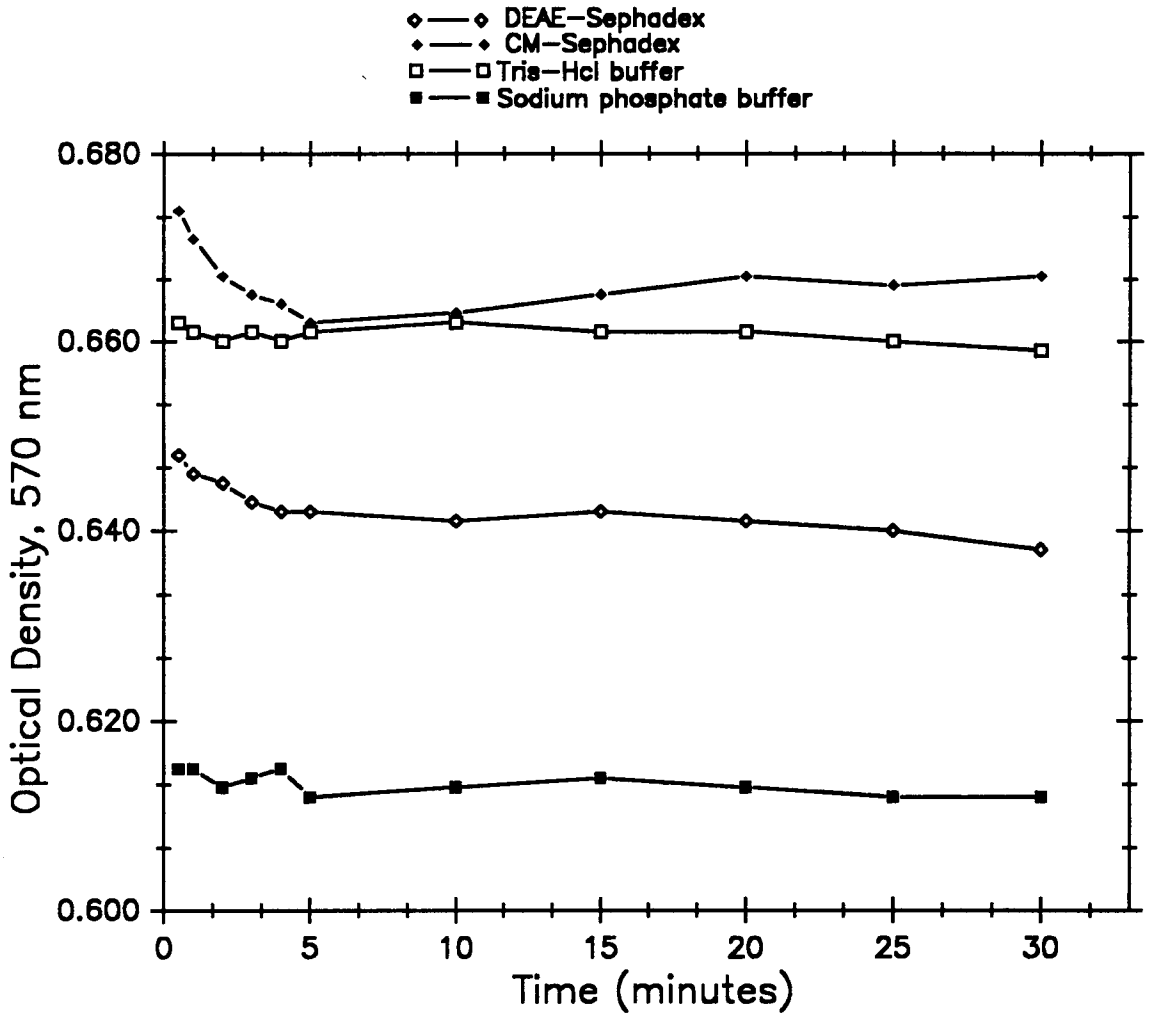


Figure 1.12 Optical density readings as measured at 570 nm of the degradation of 100 μl PADAC (128 μg/ml). Reaction mixtures composed of (1) 15 days old beta-lactamase solution, kept at 4°C in .5 M phosphate buffer, pH 6.5 after gone through CM-Sephadex column and PADAC; (2) 15 days old beta-lactamase solution, kept at 4°C in tris-Hcl buffer, pH 7.0 after gone through DEAE-Sephadex column and PADAC; (3) 15 days old tris-Hcl buffer/PADAC and (4) 15 days old sodium phosphate buffer/PADAC.

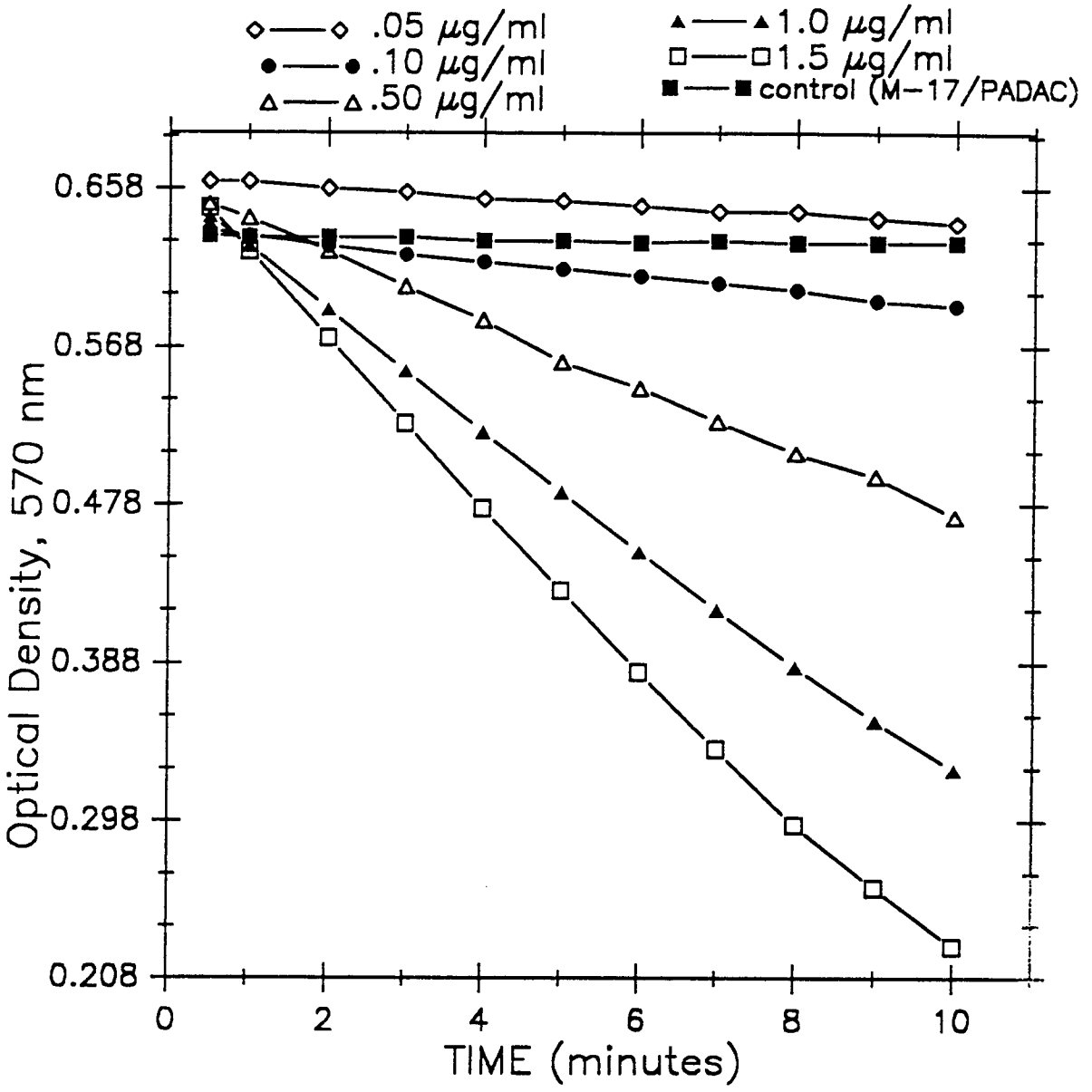


Figure 1.13 Degradation of PADAC (125 μg/ml in M-17 broth as measured at 570 nm by different concentrations of 75% pure beta-lactamase from Bacillus cereus (Sigma Chemical Company) in M-17 broth; 70 μl PADAC added to 1 ml sample; control M-17 broth/PADAC.

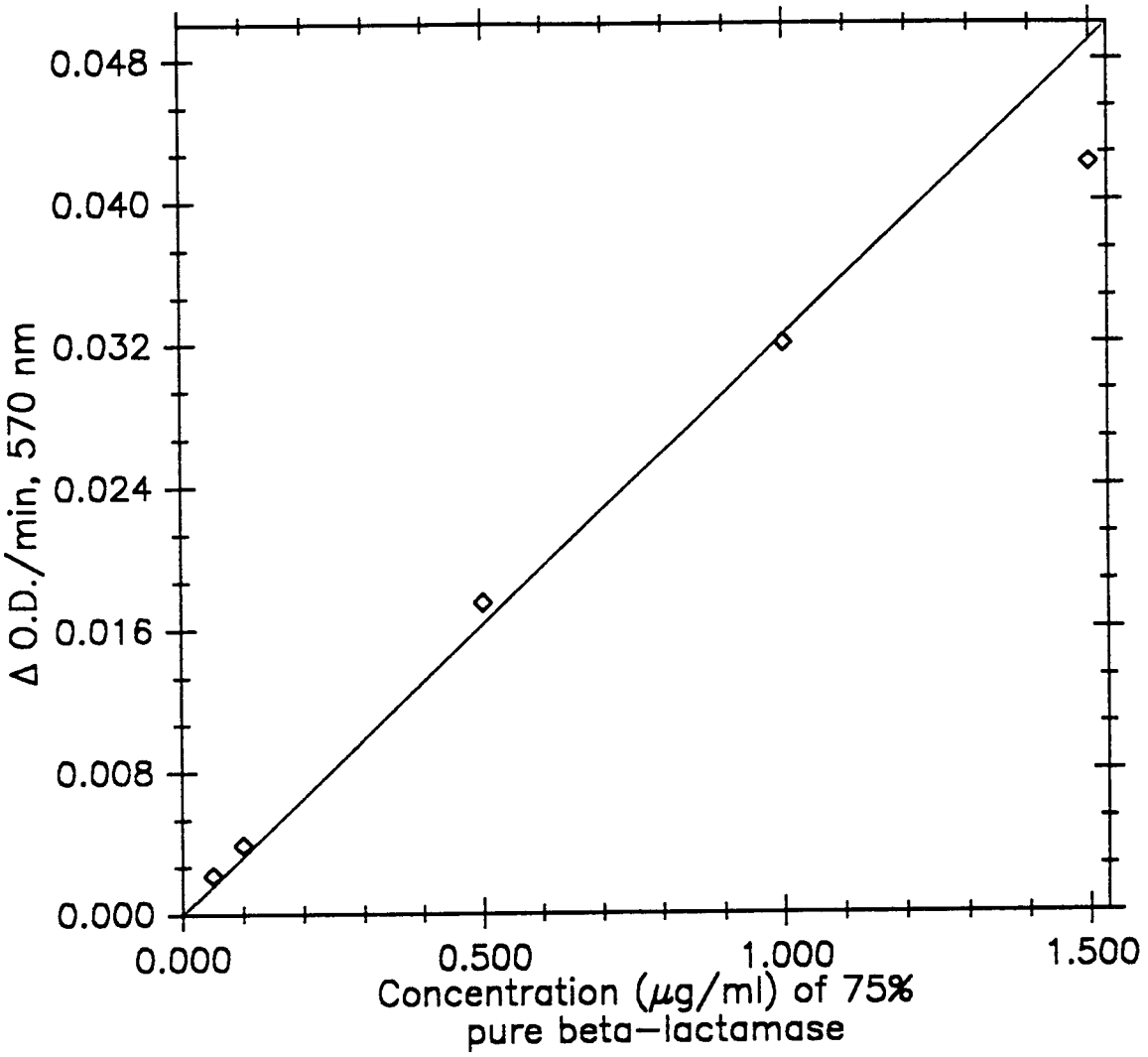


Figure 1.14 Standard curve of 75% pure beta-lactamase type II from Bacillus cereus (Sigma Chemical Company) showing different enzyme concentrations (μg/ml) and change in optical density readings at 570 nm, per minute (total 10 minutes) in M-17 broth; 70 μl PADAC (125 μg/ml in .05 M tris-Hcl buffer, pH 7.0) added to 1 ml sample.

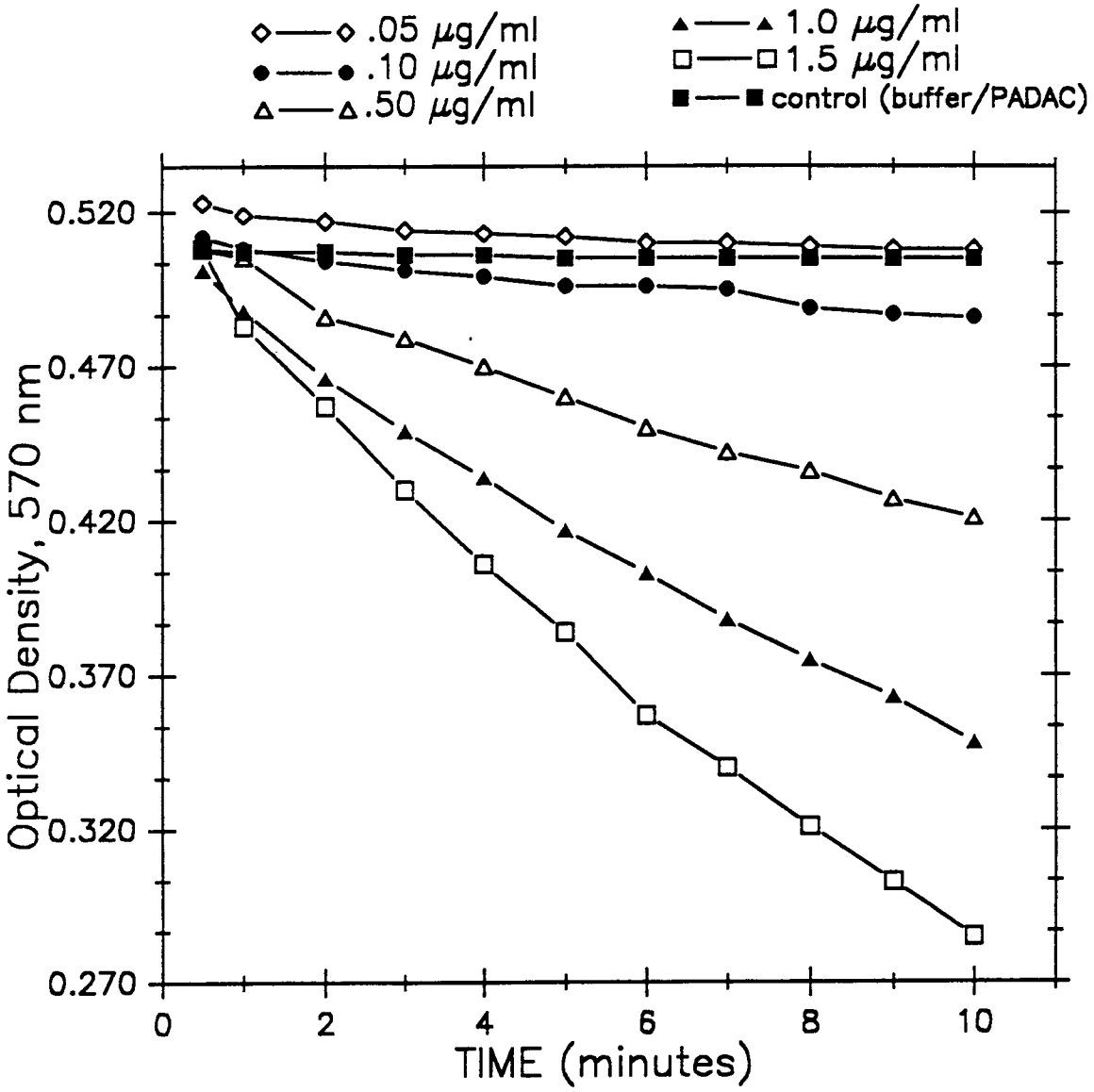


Figure 1.15 Degradation of PADAC (125 μg/ml in .05 M tris-HCl buffer, pH 7.0) as measured at 570 nm by different concentrations of 75% pure beta-lactamase from Bacillus cereus (Sigma Chemical Company) in .5M tris-HCl buffer, pH 7.0; 70 μl PADAC added to 1 ml sample; control tris-HCl buffer/PADAC.

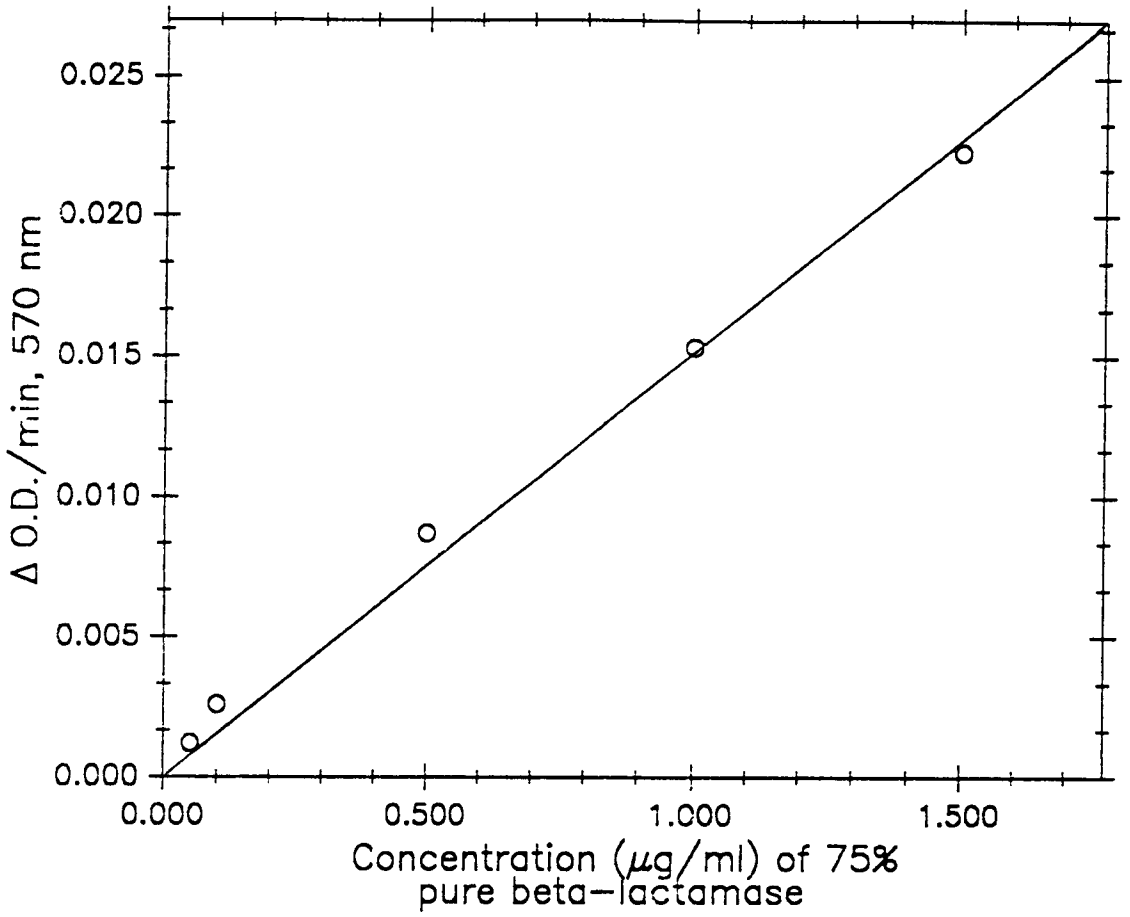


Figure 1.16 Standard curve of 75% pure beta-lactamase type II from Bacillus cereus (Sigma Chemical Company) showing different enzyme concentrations (μg/ml) and change in optical density readings at 570 nm, per minute (total 10 minutes) in .5 M tris-Hcl buffer, pH 7.0; 70 μl PADAC (125 μg/ml in .05 M tris-Hcl buffer, pH 7.0) added to 1 ml sample.

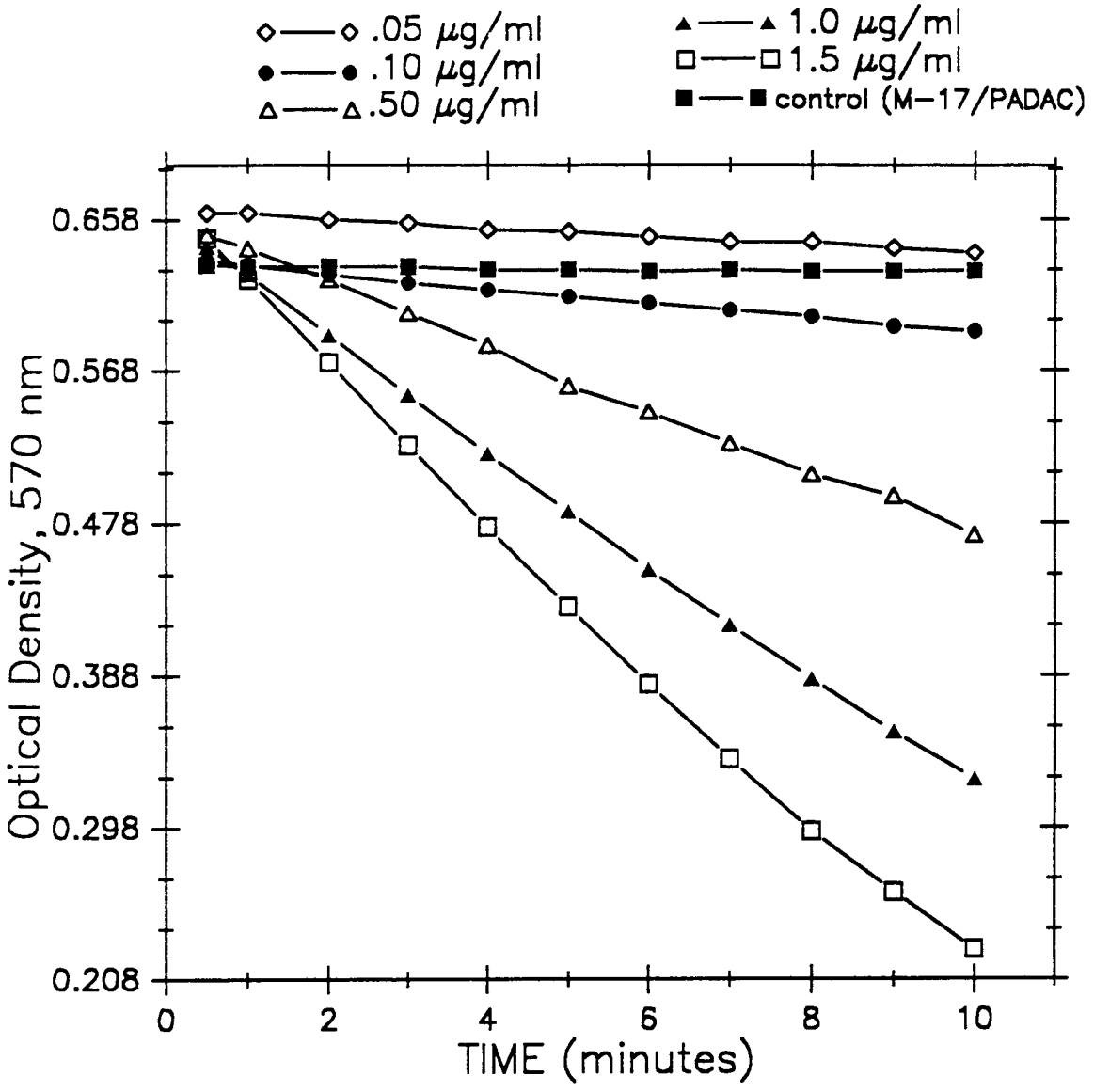


Figure 1.17 Degradation of PADAC (125 μg/ml in .05 M sodium phosphate buffer, pH 6.5) as measured at 570 nm by different concentrations of 75% pure beta-lactamase from Bacillus cereus (Sigma Chemical Company) in .5M sodium phosphate buffer, pH 6.5; 70 μl PADAC added to 1 ml sample; control sodium phosphate buffer/PADAC.

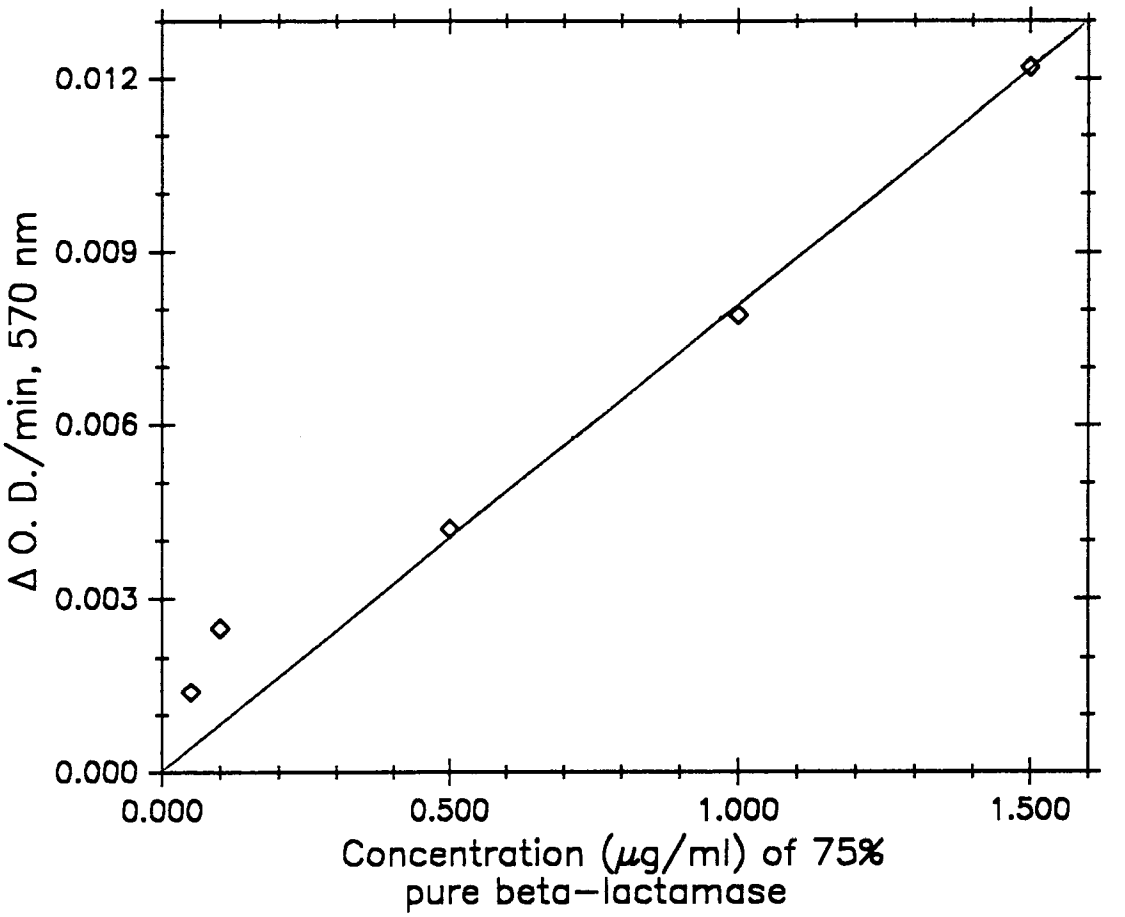


Figure 1.18 Standard curve of 75% pure beta-lactamase type II from Bacillus cereus (Sigma Chemical Company) showing different enzyme concentrations (μg/ml) and change in optical density readings at 570 nm, per minute (total 10 minutes) in .5 M sodium phosphate buffer, pH 6.5; 70 μl PADAC (125 μg/ml in .05 M sodium phosphate buffer, pH 7.0) added to 1 ml sample.

Table 1.7 Comparative values from the purification of exopenicillinase from Lc. cremoris PR-108 grown in 15 L of M-17 broth at 22°C for 18 hr (1% inoculum). Beta-lactamase hydrolysis was determined by spectrophotometric measurements at 570 nm and compared with 75% pure beta-lactamase (Sigma Chemical Co.). Reaction mixture made of 1.0 ml enzyme preparation and 70 µl PADAC (125 µg/ml) in .05M sodium phosphate or tris-Hcl buffer.

PROCEDURE	ENZYME RECOVERED[b] SAMPLE 1		SAMPLE 2		AVERAGE YIELD %
	mg/L	U/mg	mg/L	U/mg	
Culture fluid supernatant	50	.380	60	.400	-
DEAE-Sephadex A-50[a]	570	.286	690	.283	24
CM-Sephadex C-50	520	.300	670	.284	22
Sephadex G-50	120	.283	220	.309	8

[a] after enzyme concentration of 48 fold

[b] actual value X 10^{-3}

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CHAPTER 2

EFFECT OF VARIOUS ANTIBIOTICS ON LACTOCOCCAL CULTURES AS DETERMINED BY THE DISK ASSAY TECHNIQUE

ABSTRACT

Antibiotic resistance of 44 lactic starter culture strains currently used by the dairy industry have been examined in Mueller Hinton medium, supplemented with glucose (5 g/L) and yeast extract (5 g/L), by the agar disk diffusion assay. Results with antibiotics commonly used to treat cattle mastitis indicated that lactococcal cultures should be diluted 20% and incubated at 30°C for 16 to 18 hr in this medium for reliable results. The degree of antibiotic susceptibility was compared to standards established by the National Committee for Clinical Laboratory Standards (22). This procedure is recommended as a routine method for testing antibiotic resistance of lactococci.

INTRODUCTION

Several different factors can interfere with the normal acid producing activity of commercial lactic starters cultures, causing poor quality cheese, excess labor, and loss of cultured milk (16, 22). The bacteriophage susceptibility of most starter cultures is now well established (11, 18, 19), such that the advantages offered by phage inhibitory media such as Phase 4, which also provides a built-in control of acidity, and Marstar are essential to reasonable culture performance. In 1980, with the introduction of the Defined Strain Program (10), starter failure caused by phage infection was virtually eliminated, and in one case resulted in a revenue increase of over a million dollars for a cheese plant producing over 11.35 million kg of cheese per year (24).

Apart from bacteriophage, the principal inhibitory substances encountered in commercial milk are antibiotics. A wide variety of synthetic antibiotics are available to the farmer from both over the counter and prescription sources to treat mastitis and other diseases of dairy cows. Bovine mastitis is a common disease among U.S. dairy cows not under an effective control program (i.e. 10% of all US dairy cows show clinical symptoms and about 50% have subclinical

mastitis), and is the source of over 3 billion dollars loss each year for the 180,000 United States dairy producers.

Still, raw milk is examined routinely for the presence of antibiotics by conventional methods (3, 7, 17). Currently, the Bacillus stearothermophilus var. calidolactis disc assay is the official and accepted method for confirming the presence of antibiotics in raw milk (13, 20). Disks .5 mm in diameter are imbibed in milk then placed on the surface of agar plates seeded with the spore forming microorganism; after the proper incubation period, zones >14 mm surrounding the disks indicate the presence of an antibiotic substance (i.e. .008 IU penicillin/ml used as control gives a clear zone 17 to 20 mm). The Charm Screening Assay II (8, 9, 10, 21) is a faster and readily applicable method based on an irreversible binding of antibiotics to specific sites on the cell wall of Bacillus stearothermophilus; ^{14}C or ^{3}H labeled antibiotics and the antibiotic in the milk sample compete for the bacterial binding sites, so that the amount of bound ^{14}C or ^{3}H is counted and compared with the control to determine the presence or absence of antibiotic residues (i.e. beta-lactams, tetracyclines, streptomycins, erythromycins, chloramphenicols and sulfonamides). This qualitative assay detects beta-lactam antibiotics at concentrations from .01 IU Penicillin/ml. The most sensitive test is the standard Bromocresol Purple Test (15) in which the rapid growth and acid production by Bacillus stearothermophilus var. calidolactis changes the dye

from purple to yellow in the absence of Beta-lactam inhibitors. This test detects Beta-lactam antibiotics at concentrations >.005 IU/ml in processed fluid and raw milks. Despite all the different ways of testing for the presence of antibiotics in milk, Bacillus stearothermophilus is the control organism of choice. It is also important to consider the role of other possible inhibitors in milk which can interfere with a correct diagnosis; for instance, raw milk contains high levels of lactoperoxidase, lactose (48 g/l) and moderate levels of thiocyanate (.3 g/l); when lactose is hydrolyzed into glucose and galactose and further metabolized, hydrogen peroxide is produced. It has been demonstrated that lactoperoxidase, thiocyanate, and hydrogen peroxide form a bacteriocidal system capable of killing many gram negative and positive bacteria (6, 12, 15) due to inhibition of hexokinase and other glycolytic enzymes.

These findings suggest that when raw milk is tested for the presence of antibiotics in broth media, false positives can occur due to the bacteriocidal properties of natural inhibitors against Bacillus stearothermophilus (7). In addition, it is possible that some of these antibiotics can cause inhibition of lactic acid producing bacteria and still be undetectable by assays currently used. Some authors have suggested the use of starter cultures as indicator organisms in antibiotic assays (1, 2, 17).

Since many laboratories use various of these methods to detect antimicrobial inhibitors in milk, there is a need to reexamine the resistance pattern of starter cultures in the presence of different classes of antibiotics, with the disk agar diffusion method of susceptibility testing. The intent of this investigation was to evaluate the antibiotic resistance pattern of numerous strains of lactic acid bacteria from various areas of the world, which are used for the preparation of fermented milk products which may be used as potential indicator organisms for antimicrobial disc susceptibility testing. Also, since many new antibiotics appear on the market for possible use in treating mastitis, some recently introduced were included in this study. These were sulfathiazole, trimethoprim and nitrofurantoin.

MATERIALS AND METHODS

Media

Commercially available media from Difco laboratories, Detroit MI were prepared as follow:

1. Bacto Mueller Hinton broth (12, 20) was rehydrated (21 g/l) in distilled water, supplemented with .5% D+ glucose (Sigma Chemical Company) and .5% yeast extract (BBL), warmed gently to dissolve, dispensed in 10-ml aliquots and sterilized by autoclaving for 15 minutes at 15 pounds pressure (121°C). This broth was used as a suspension medium for preparing the inocula for both antimicrobial susceptibility testings;
2. Bacto Mueller Hinton II agar (12, 20) was reconstituted (38 g/l) in distilled water, supplemented with .5% D+ glucose (Sigma Chemical Company) and .5% yeast extract (BBL) and sterilized by autoclaving as before and cooled to 45°C in water bath for dispersion into petri plates (100 X 15 mm). The depth of each plate was approximately 3 mm (15 ml). The dispensed and solidified plates were kept inverted at 4°C in sealed plastic bags. These plates were satisfactory for use for up to two months. In addition, the Bacto Mueller Hinton II agar was prepared as described above, heated to boiling to dissolve completely; 9 ml of the molten agar were then dispersed into small capped tubes and autoclaved as before.

To ensure that the amount of medium in the petri dishes was the same for spread and overlay methods, 9 ml molten agar medium were added to the surface of pre-poured agar plate to be used in the spread technique;

3. M-17 broth (29) obtained from Difco Laboratories was reconstituted (37.25 g/l) in distilled water, autoclaved for 15 minutes at 15 pounds pressure (121°C), cooled to 45°C in a water bath, supplemented with .5% solution of filter-sterilized-lactose (Sigma Chemical Company), stored at 4°C until used. M-17 was also used for the propagation of strains used in this study;

4. M-17 constituents were prepared as above supplemented with 1% Bacto agar (Difco Laboratories) (29) and 15 ml quantities were added to sterile petri plates, which were allowed to solidify overnight; 9 ml of M-17 molten medium were dispersed onto the surface of some of these pre-poured plates for the spread technique; the plates were then placed inverted in sealed plastic bags and stored at 4°C for later use;

5. Instant Peake nonfat milk (Galloway West Co., Fond du Lac, WI) was reconstituted to 11% solids. Tubes containing 10 ml of nonfat milk were steamed for 45 minutes, cooled to room temperature and used to inoculate the strains in question. These tubes were stored at 4°C for up to five days before being discarded.

Bacterial Strains

Streptococcus faecalis 29212 was obtained from American Type Culture Collection, Rockville, MD, for use in determining the suitability of the Mueller Hinton II agar for sulfonamide and trimethoprim tests (22). The fourty-two strains used in this study were obtained from the culture collection maintained in the Dairy Microbiology laboratory, Department of Microbiology, Oregon State University and included: Lactococcus lactis ssp. lactis biovar. diacetylactis (Lc. diacetylactis) 18-16 and 26-2, originally isolated from mixed strain cheese starter cultures which produced slit open Cheddar cheese; Lactococcus lactis ssp. lactis (Lc. lactis) C2, C10, 01, 197 and F2D2. The former three strains were obtained from Australia; Lactococcus lactis ssp. cremoris (Lc. cremoris) 00, 107/6, 108, PR-108, 163, 178, 187, 188, 189, 190, 196, 203, 205, 211, 217, 220, 222, 223, 459, 799, 819, 852, 865, 990, BK5 (from New Zealand), C1, C3 and C11 (from Australia), C13, E8, EB2, EB4, EB9, HP and ML1 (all from New Zealand Dairy Research Institute). In addition Lc. cremoris was isolated from a starter (101) provided by Hansen's Laboratory Inc. (Cheddar Cheese Culture) and from a starter (MT) provided by Microlife Technics (Buttermilk Culture). All the above strains have been used to manufacture commercial fermented milk products in various parts of the world. The cultures were maintained in tubes containing 10 ml of litmus milk supplemented with 15% sterile glycerol, and stored in the unincubated condition (5% inoculum) at

-40°C. Before being inoculated into testing medium, cultures were initially thawed and grown overnight in litmus milk, then in M-17 broth (1% inoculum) at 30°C for 16 to 18 hours.

Citrate Utilization

Test tubes containing 10 ml of differential broth (24) were used to grow Lc. cremoris strains isolated from starters 101 and MT. These strains were characterized by their inability to ferment citrate and for lack of CO_2 production.

Arginine Hydrolysis

Lc. cremoris isolated from starters 101 and MT, were further identified by their inability to produce ammonia from the hydrolysis of arginine. The ammonia is detected by placing one drop of culture and one drop of Nessler's reagent (2) on a porcelain spot plate; a positive reaction is given in a few minutes by the development of a deep orange precipitate (23).

King's Test

Lc. cremoris isolated from starters 101 and MT were tested for production of C_4 compounds (i.e. diacetyl, acetylmethylcarbinol and 2,3 - butylene glycol) by the King's test (20). The approximate amount of C_4 compounds formed was

measured by mixing 2 ml of each culture grown in 11% NFM supplemented with .2% sodium citrate, 1 ml of reagent A (30% aqueous solution of KOH) and 1 ml of reagent B (4.0 g alpha-naphthol, 10 ml amyl alcohol and 90 ml ethyl alcohol). The cultures were placed in a 30°C water bath and vortexed 4 times in a 30 minutes period for aeration; the development of a red-liliac color is considered positive for the presence of C_4 compounds; Lc. cremoris is negative in this test.

Antimicrobial Susceptibility Testing - Standard Method

Each lactococcal strain was grown in 10 ml M-17 broth (2% inoculum from litmus milk) and incubated overnight at 30°C. Strains were then subcultured to 5 different tubes each containing fresh 10 ml Mueller Hinton broth (1% inoculum) and incubated for 16 to 18 hours at 30°C (Tables I through XLIV in the appendix). After incubation, cultures were diluted and a sterile non-toxic cotton swab, on a wooden applicator, was dipped into each culture suspension and used to inoculate the entire surface of Mueller Hinton II agar plates, each plate being streaked in two different directions by rotating the plate 180 degree after each streaking/dipping. The inoculum was allowed to dry and each susceptibility disk placed manually or with a Dispens-O-Disk dispenser (Difco laboratories, Michigan, MI) on the inoculated surface. After 15 minutes, the plates were inverted, and aerobically

incubated at 30°C for 16 to 18 hours. To check for the inhibitory role of phosphate (27) cultures also were subcultured to 5 different tubes containing fresh 10 ml M-17 broth (1% inoculum) and processed in the same fashion as for Mueller Hinton media. The diameters of zones of inhibition were measured to the nearest whole mm using a ruler. Each zone endpoint was defined as the area showing no visible growth when visually observed. Cultures were tested five times and the results reported as susceptible according to zone interpretations required by the National Committee for Clinical Laboratories Standards (NCCLS)(22).

Antimicrobial Susceptibility Testing - Agar Overlay Method

Each lactococcal strain was grown as described in "Antimicrobic Susceptibility Testing - Standard Method." After incubation in Mueller Hinton broth, cultures were diluted (Tables I through XLIV in the appendix) using the same broth, then 90 μl of well-mixed diluted broth culture was transferred to 9.0 ml Mueller Hinton II agar which had been cooled in a water bath to 45°C; unused tubes were discarded at the end of the day to avoid misleading results due to evaporation of the medium. The seeded agar was vortexed and then poured onto the surface of a plastic petri plate (100 X 15 mm) containing Mueller Hinton II agar (15 ml). The procedure was repeated using M-17 medium. Inoculated plates

were allowed to solidify and each susceptibility disk placed as described previously. After 15 minutes, plates were inverted and aerobically incubated at 30°C for 16 to 18 hours. Diameters of zones of inhibition were measured to the nearest whole mm with a ruler. Cultures were tested five times and results reported as susceptible according to the zone interpretation standards of NCCLS (22).

RESULTS AND DISCUSSION

The present study was undertaken to evaluate the resistance levels of several lactic starter bacteria against different types of antibiotics. In order to include the antibiotic resistance levels of 42 pre-defined starters with two fresh isolates, Lc. cremoris from Hansen 101 and Microlife Technics MT, Cheddar cheese and buttermilk mixed strain cultures were isolated.

Using Lc. diacetylactis 18-16, Lc. lactis 197 and Lc. cremoris 108 as positive controls, Lc. cremoris strains isolated from Hansen 101 and Microlife Technics MT cultures were identified by the inability to utilize citrate. As expected, both strains were unable to ferment citrate but produced lactic acid, turning the differential broth to a deep yellow color without producing any CO_2. The Hansen 101 strain completed the reactions in 18 hours while the Microlife Technics MT strain took 36 hours. Lc. diacetylactis 18-16 produced large amounts of CO_2 in 18 hours, turned the broth color first to yellow in 18 hours and later to purple due to liberation of NH_3 from arginine; Lc. lactis 197 gave similar reactions when compared to Lc. diacetylactis 18-16, but did

not produce any CO_2. The reactions in the broth are shown in Table 2.1. Lc. cremoris isolated from Hansen 101 and Microlife Technics MT were grown further in Niven's broth and showed a negative reaction when tested for arginine hydrolysis using Nessler's reagent. In addition, these strains gave negative reactions when tested for the production of acetoin and diacetyl, which is the basis for the King's test. These results were compared to the ones obtained from the negative control Lc. cremoris 108 and used to confirm the isolate's subspecies. As seen in Table 2.1 positive controls Lc. diacetylatics 18-16 and Lc. lactis 197 gave a positive reaction for arginine hydrolysis indicated by a deep-red precipitate with the addition of Nessler's reagent; furthermore, the results obtained from King's test, indicated a negative reaction for Lc. lactis 197 and a positive reaction for Lc. diacetylactis 18-16. These results were expected and are the basis for differentiating these two subspecies.

In order to show whether antibiotic resistance levels were or were not associated with medium composition, M-17 medium which is commonly used for the propagation of lactic acid bacteria, was chosen to determine if its phosphate buffering agents increase the resistance levels of lactococci against certain antibiotics, as compared to Mueller Hinton medium, under the same experimental conditions. The numerical values in Tables 2.2a and 2.3a were compared to Tables Ia

through XLVa in the appendix, by relating results to standards set by the National Committee for Clinical Laboratory Standards (NCCLS, 1989) and 1989 Difco antibiotic inserts. NCCLS standards are based on consensus involving clinicians working with infectious diseases, government projects, research laboratory professionals and the various industries (e.g. pharmacology) (22). The reference strain of choice has to show an intermediate antimicrobial susceptibility pattern that is stable genetically, and MIC values in between low and high concentrations of the antibiotic being analyzed.

In the case of the aminoglycoside, Amikacin, resistance levels for all strains tested were higher (average 17 to 29%) in seeded Mueller Hinton agar (cultures diluted 50%) than in M-17 medium (no culture dilution). These results confirm previous report by Sinha (27), suggesting that phosphates, by combining with aminoglycosides, enhance their antimicrobial effect. In this study, the aminoglycosides amikacin (5μg), tobramycin (10 μg) and streptomycin (10 μg) killed the lactococci strains at moderate percentages. Aminoglycosides act by binding to the 30 S subunit of the bacterial ribosomal RNA, causing the formation of a partially defective protein.

Tables Ia and Ib through XLIVa and XLIVb in the appendix show inhibition zones by the action of different antibiotics against 44 lactococci strains grown in Mueller Hinton agar.

Antibiotics seen on each of these tables were divided in groups according to similarities in their spectrum of activity against various prokaryotes tested (22). Streptococcus faecalis 29212 (ATCC) (Tables XLVa and XLVb) was used as the medium quality control organism. Among those various lactococcal strains, four namely Lc. cremoris 217 (Tables XXa and XXb), HP (Tables XXXIXa and XXXIXb), BK5 (Tables XXXa and XXXb) and Lc. lactis F_2D_2 (Tables VIIa and VIIb), showed significant higher resistance levels towards the following antibiotics: amikacin (30 μg), penicillin G (10 μg), streptomycin (10 μg), trimethoprim (5 μg), nitrofurantoin (300 μg), rifampin (5 μg), sulfathiazole (300 μg), lincomycin (2 μg) and neomycin (5 μg). In addition, Lc. diacetylactis 18-16 (Tables Ia and Ib) and Lc. lactis F_2D_2 (Tables VIIa and VIIb) did not show any inhibition zone when exposed to streptomycin while Lc. cremoris C3 was the most susceptible strain with a measured zone of 22 mm (overlay method) and 24 mm (spread method); Lc. cremoris HP was the most resistant strain [zones of 10 mm (overlay method and 10 mm (spread method)]. Strains with zones ≥ 15 mm are considered streptomycin susceptible, according to NCCLS (22). The results in this study are comparable to those of Wulf and Sandine (34) who observed wide variations in the activities of lactococci when grown in the presence of streptomycin.

The distribution of averaged zone sizes obtained with the antibiotics tested are depicted on Figures 2.1 through 2.3. In the overlay method, 31% of all strains showed high resistance levels against rifampin (5 μg) and 34% against nitrofurantoin (300 μg), a drug which inhibits nucleic acid synthesis by binding to the RNA polymerase; 7.1% showed low resistance levels against tobramycin (10 μg) and 17% against neomycin (5 μg). These aminoglycosides act by binding to the 30 S subunit of the microbial ribosome, resulting in the formation of a non-functional protein. While 26% of the strains were highly susceptible to chloramphenicol (30 μg), 24% were found sensitive to erythromycin (15 μg). The later binds to cell receptors, blocking peptidoglycan synthesis in the bacterial cell. Sulfathiazole (300 μg) and clindamycin (2 μg) are on the boardline in terms of efficiency against the strains studied in this paper. It is possible that at a lower concentration, lactococci would show definite resistance to these antibiotics.

Figures 2.4, 2.5 and 2.6 show how variations in the density of culture inoculum alter zone diameters. According to NCCLS (22), standard inoculum should have 50% of a culture containing approximately 10^8 CFU/ml. For example, for the overlay method, when Lc. cremoris EB2 is not diluted, a zone diameter of 28 mm is seen for penicillin G (zone diameter $\geq$ 28 mm is considered susceptible, Figure 2.4); however, at

50% dilution, the zone diameter is increased to 31 mm. A higher difference is shown for chlorotetracycline (≥ 19 mm range set for susceptibility), where at 0% dilution a zone of 30 mm is measured while at 50% the zone diameter is increased to 40 mm. Another factor which plays a major role in zone diameter, is the time of culture incubation. Figure 2.7 indicates that if the length of incubation time for Lc. cremoris 205 at 30 °C necessary to detect a positive result is extended, smaller zones of inhibition are measured, which is probably caused by antibiotic deterioration.

In conclusion, it is recommended that antimicrobial susceptibility testing for lactococci be performed using Mueller Hinton medium supplemented with 5 g/L glucose and 5 g/L yeast extract with a 20% diluted culture grown at 30°C for 16 to 18 hr. These parameters were based on the nutritional requirements and/or fastidious nature of lactococcal cultures, and the proximity of inoculum size/density in Cheddar cheese manufacturing.

The agar-overlay method is quite efficient due to the microaerophilic nature of these organisms and it was found to give more satisfactory results than the surface-swab method, largely because the zone edges are better defined and can be measured more precisely. Figures 2.8, 2.9 and 2.10 compares the seeded and spread methods for the following lacotococci:

Lc. diacetylacti 18-16, Lc. lactis F_2D_2, Lc. cremoris 190, Lc. lactis C10, Lc. cremoris 108 and PR-108 respectively.

Table 2.1 Comparison of biochemical reactions for representative lactococcal cultures.

Strains	Differential broth reactions			King's Test	Arginine Hydrolysis[e]
	Acid[c]	NH_3[d]	CO_2		
Lc. cremoris[a]	+	–	–	–	–
Lc. cremoris[b]	+	–	–	–	–
Lc. cremoris 108	+	–	–	–	–
Lc. diacetylactis 18-16	–	+	+	+	+
Lc. lactis 197	–	+	–	–	+

[a] Isolated from Hansen 101 Cheddar cheese culture.
[b] Isolated from Microlife Technique buttermilk culture.
[c] Yellow color.
[d] Reversal of yellow color to purple.
[e] Tested with Messler's reagent.

Table 2.2a Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of M-17 agar plates, seeded with lactococcal strains indicated below. The plates were incubated at 30°C for 16 hr. Average of 5 experiments.

Bacterial Strain	Zone diameter, nearest whole mm Means of 5 experiments					
	Amikacin 30 µg	Bacitracin 30 µg	Chloramphenicol 30 µg	Penicillin 10 µg	Streptomycin 10 µg	Tetracycline 30 µg
Lc. diacetylactis 18-16	14	27	32	33	9	35
Lc. diacetylactis 26-2	19	34	36	32	13	42
Lc. lactis 01	13	29	29	33	9	35
Lc. lactis 197	15	29	29	30	11	30
Lc. lactis C2	16	30	31	35	11	35
Lc. lactis C3	24	37	36	41	24	40
Lc. lactis C10	14	29	33	35	9	36
Lc. lactis F2D2	12	29	31	34	10	37
Lc. cremoris 00	22	34	37	38	21	42
Lc. cremoris 107/6	21	33	18	37	22	42
Lc. cremoris 163	28	33	33	36	20	40
Lc. cremoris 187	17	29	32	33	18	36
Lc. cremoris 189	22	35	35	45	23	44
Lc. cremoris 190	29	33	32	37	22	40
Lc. cremoris 196	25	33	35	40	21	41
Lc. cremoris 203	22	33	35	35	17	42
Lc. cremoris 205	21	31	34	33	19	38
Lc. cremoris 211	25	34	33	39	21	37
Lc. cremoris 220	24	34	34	39	19	41
Lc. cremoris 223	30	37	40	40	25	40
Lc. cremoris 459	27	36	39	38	21	41
Lc. cremoris 819	31	42	43	44	24	42

Table 2.2b Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of M-17 agar plates, seeded with lactococcal strains indicated below. The plates were incubated at 30°C for 16 hr.

Bacterial Strain	Zone diameter, nearest whole mm																													
	Trials																													
	Amikacin 30 µg					Bacitracin 30 µg					Chloramphenicol 30 µg					Penicillin 10 µg					Streptomycin 10 µg					Tetracycline 30 µg				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Lc. diacetylactis 18-16	13	14	14	13	14	26	28	28	26	27	27	34	34	33	34	31	34	30	34	34	13	8	8	9	9	35	34	34	35	35
Lc. diacetylactis 26-2	18	18	22	21	18	34	36	35	30	36	36	36	36	37	36	32	32	34	32	32	13	14	14	13	12	41	42	42	43	42
Lc. lactis 01	13	14	14	13	12	29	28	29	28	29	29	29	30	30	29	32	32	32	34	34	9	8	9	9	8	35	34	35	35	35
Lc. lactis 197	15	15	14	15	15	29	28	29	28	29	30	27	29	30	30	36	29	29	29	28	10	11	11	10	11	34	29	30	30	29
Lc. lactis C2	15	17	16	15	16	29	30	29	30	30	30	30	31	34	30	34	34	36	34	36	11	11	12	11	12	35	35	36	35	34
Lc. lactis C3	24	26	22	24	25	40	36	36	37	38	36	36	37	36	36	40	38	42	41	42	24	24	25	24	24	41	40	39	40	40
Lc. lactis C10	14	13	14	14	13	29	28	29	29	30	34	33	32	34	33	38	34	34	34	35	9	9	8	9	9	36	37	36	36	37
Lc. lactis F2D2	12	12	12	13	12	29	28	28	29	29	30	35	30	29	30	32	36	35	34	33	10	9	10	10	9	36	37	36	37	37
Lc. cremoris 00	21	22	22	21	22	32	34	35	34	36	36	37	36	38	36	38	39	37	38	37	21	22	21	21	21	41	44	42	43	42
Lc. cremoris 107/6	21	22	21	22	21	34	32	32	34	33	16	18	19	17	19	36	36	35	39	0	22	21	22	23	23	40	42	41	42	43
Lc. cremoris 163	29	24	29	29	28	34	33	32	33	32	32	34	32	33	34	38	36	37	34	36	20	19	20	19	20	40	38	40	39	41
Lc. cremoris 187	17	16	17	17	17	28	30	30	29	30	28	32	29	30	40	33	34	32	33	34	18	18	18	17	17	36	37	36	35	36
Lc. cremoris 189	22	21	22	22	21	36	36	36	35	34	34	36	34	35	36	46	44	46	44	45	22	24	23	24	24	44	43	42	44	45
Lc. cremoris 190	27	26	30	28	32	30	35	36	31	32	30	31	32	34	33	41	36	37	36	37	22	23	22	22	22	42	42	35	41	42
Lc. cremoris 196	26	24	25	26	25	33	32	34	32	33	36	34	35	36	34	41	40	39	39	41	21	22	21	21	21	42	41	42	40	41
Lc. cremoris 203	22	21	22	22	23	32	33	36	32	32	36	34	35	36	36	36	35	34	36	35	17	18	17	16	17	42	43	43	42	41
Lc. cremoris 205	21	22	20	20	21	32	31	30	30	31	32	37	33	34	32	34	32	32	32	33	20	18	17	18	20	38	39	38	37	38
Lc. cremoris 211	25	25	24	25	26	34	33	34	34	33	32	34	33	32	34	36	39	40	39	41	21	21	20	21	22	36	34	37	38	41
Lc. cremoris 220	25	21	25	24	25	34	33	34	35	32	34	34	36	33	34	38	38	40	39	39	19	20	20	18	20	40	41	40	41	41
Lc. cremoris 223	26	26	27	39	34	38	34	39	38	36	39	40	41	42	39	38	39	38	46	39	26	24	25	26	26	43	39	39	38	40
Lc. cremoris 459	27	27	26	27	28	38	38	31	37	36	38	39	38	41	38	34	38	43	38	37	22	22	19	20	21	36	42	43	43	41
Lc. cremoris 819	31	31	30	32	31	42	42	41	42	41	43	41	44	43	42	46	44	43	42	44	23	26	24	25	24	42	43	41	43	42

Table 2.3a Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of M-17 agar plates, spread with lactococcal strains indicated below. The plates were incubated at 30°C for 16 hr. Average of 5 experiments.

Bacterial Strain	Zone diameter, nearest whole mm Means of 5 experiments					
	Amikacin 30 µg	Bacitracin 30 µg	Chloramphenicol 30 µg	Penicillin 10 µg	Streptomycin 10 µg	Tetracycline 30 µg
Lc. diacetylactis 18-16	14	22	29	29	9	29
Lc. diacetylactis 26-2	16	30	33	30	12	36
Lc. lactis 01	12	21	21	24	7	25
Lc. lactis 197	14	23	25	24	11	28
Lc. lactis C2	14	25	27	29	11	29
Lc. lactis C3	12	20	15	21	13	21
Lc. lactis C10	8	22	20	25	0	22
Lc. lactis F2D2	8	19	21	25	8	27
Lc. cremoris 00	11	31	29	31	9	40
Lc. cremoris 107/6	15	19	24	21	15	29
Lc. cremoris 163	16	21	20	23	14	18
Lc. cremoris 187	16	22	23	28	16	23
Lc. cremoris 189	20	27	26	27	15	26
Lc. cremoris 190	16	29	26	32	12	34
Lc. cremoris 196	17	19	23	21	14	28
Lc. cremoris 203	12	24	24	24	12	28
Lc. cremoris 205	17	22	22	24	12	23
Lc. cremoris 211	15	20	27	25	17	23
Lc. cremoris 220	14	34	30	39	11	30
Lc. cremoris 223	13	29	25	37	17	37
Lc. cremoris 459	16	18	22	23	14	19
Lc. cremoris 819	9	21	18	22	12	21

Table 2.3b Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of M-17 agar plates, spread with lactococcal strains indicated below. The plates were incubated at 30°C for 16 hr.

Bacterial Strain	Zone diameter, nearest whole mm																													
	Trials																													
	Amikacin 30 µg					Bacitracin 30 µg					Chloramphenicol 30 µg					Penicillin 10 µg					Streptomycin 10 µg					Tetracycline 30 µg				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Lc. diacetylactis 18-16	14	14	14	13	14	23	22	22	22	23	29	29	30	29	29	29	29	28	28	29	10	9	9	9	10	30	28	30	27	30
Lc. diacetylactis 26-2	15	17	17	16	14	30	31	30	29	30	34	33	34	32	33	32	30	29	30	29	12	13	12	11	12	37	36	35	36	34
Lc. lactis 01	11	11	12	12	12	22	23	23	17	22	18	21	25	19	21	25	24	24	25	24	8	6	7	7	8	23	26	25	25	26
Lc. lactis 197	13	14	13	14	14	22	24	24	23	24	24	25	25	25	24	24	25	24	24	25	10	10	12	10	11	26	29	29	29	28
Lc. lactis C2	14	14	14	13	14	23	25	27	25	25	27	29	26	27	27	30	29	28	29	29	10	11	10	11	11	26	30	29	30	29
Lc. lactis C3	13	11	12	12	12	20	20	20	20	21	15	20	12	15	14	20	20	26	21	20	12	12	15	12	13	21	21	0	21	22
Lc. lactis C10	8	7	9	8	9	22	21	22	22	21	20	20	20	20	22	25	26	24	25	24	0	0	0	0	0	21	23	21	21	22
Lc. lactis F2D2	8	7	8	9	8	18	19	20	20	19	21	22	20	21	19	26	24	26	26	25	8	7	9	9	8	26	28	27	28	26
Lc. cremoris 00	10	11	12	10	11	29	30	30	30	34	29	30	29	28	30	32	32	30	31	32	9	8	8	9	9	38	39	43	40	39
Lc. cremoris 107/6	17	15	14	15	15	21	16	19	21	20	26	19	25	0	26	23	20	22	23	17	16	14	15	14	14	29	29	30	29	0
Lc. cremoris 163	15	16	16	17	16	21	22	20	22	20	20	21	20	20	20	25	24	22	22	24	15	14	14	15	14	19	14	20	19	19
Lc. cremoris 187	15	17	15	16	17	19	23	21	23	22	22	24	23	24	23	26	30	27	28	29	19	16	14	16	16	21	22	23	22	29
Lc. cremoris 189	21	19	20	20	19	28	26	27	28	28	26	27	26	23	27	26	26	30	27	26	14	16	14	14	15	27	19	28	28	27
Lc. cremoris 190	15	15	19	16	15	32	29	28	26	28	29	30	26	23	23	34	33	32	32	31	11	13	13	12	13	31	32	36	33	36
Lc. cremoris 196	18	17	18	17	17	24	23	0	24	24	23	22	22	24	23	19	22	20	21	22	14	15	14	13	15	29	27	0	29	28
Lc. cremoris 203	13	12	13	12	12	24	24	24	23	24	22	24	26	24	24	22	25	25	24	25	13	11	11	12	11	30	24	29	29	30
Lc. cremoris 205	18	16	14	17	18	23	23	24	17	24	24	21	22	21	22	23	25	20	25	25	11	15	11	12	11	25	22	20	25	24
Lc. cremoris 211	14	14	15	19	14	19	19	24	19	20	26	24	26	29	28	26	25	24	25	26	15	16	22	15	16	22	24	22	23	22
Lc. cremoris 220	15	14	13	14	13	34	33	33	34	35	26	27	42	26	28	38	41	38	41	38	10	12	11	12	10	30	29	30	29	30
Lc. cremoris 223	17	13	12	12	13	29	30	30	29	29	25	22	28	25	24	43	34	36	37	37	13	22	16	17	16	33	36	36	37	41
Lc. cremoris 459	15	17	15	15	16	17	19	18	19	19	24	17	23	24	24	24	20	24	25	24	14	13	14	14	13	20	19	17	19	20
Lc. cremoris 819	7	8	7	14	8	22	20	19	22	22	19	15	18	19	17	19	20	23	24	23	14	13	14	13	7	20	22	22	22	20

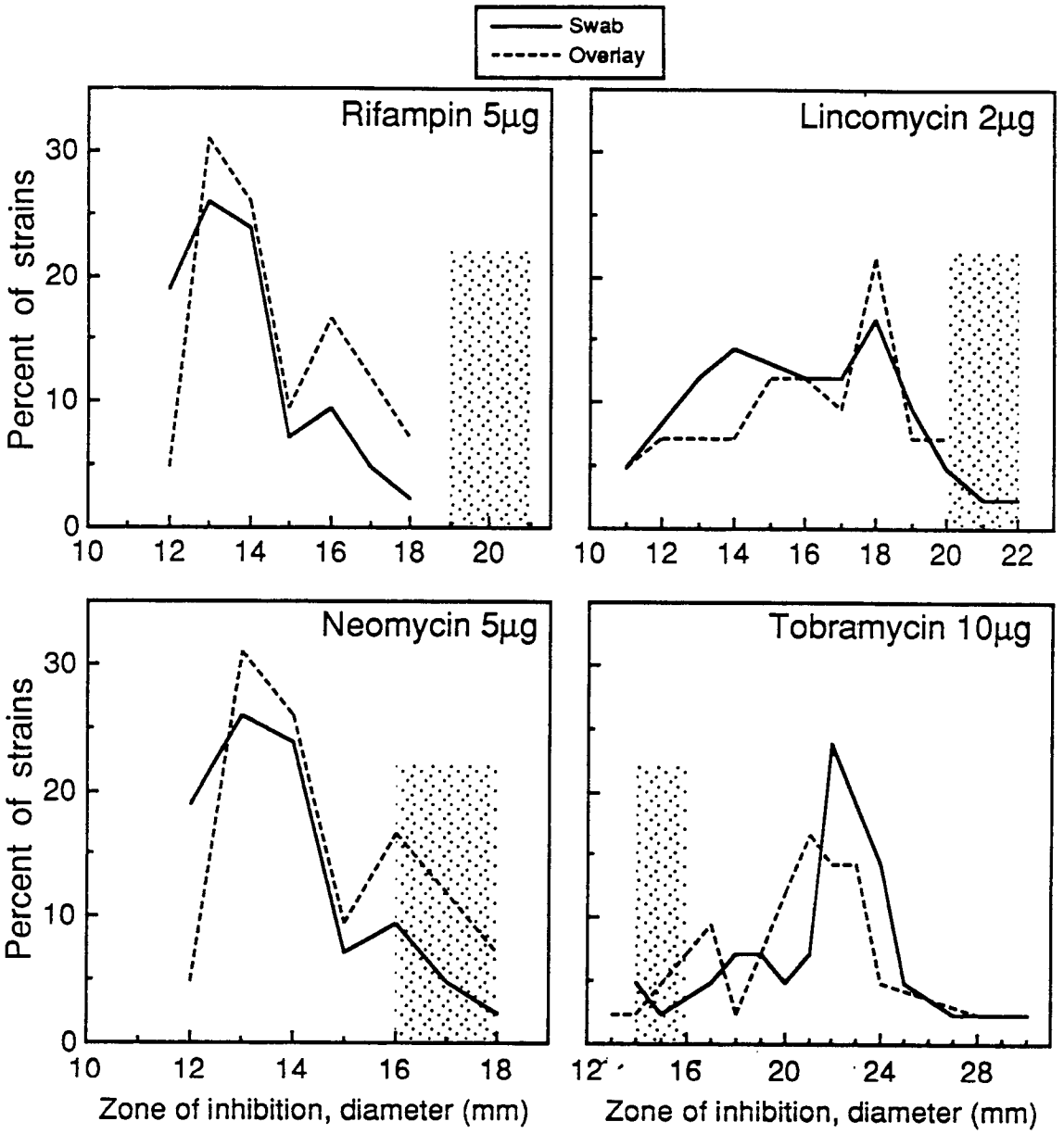

Figure 2.1 Distributions of averaged zone sizes obtained with 44 strains of lactic acid bacteria using the surface-swab and the agar overlay methods. Strains giving zones within the shaded area are reported to have intermediate susceptibility (±1), those with larger zones are sensitive and those with smaller zones resistant (NCCLS, 1989). Antibiotics purchased from Difco Laboratories.

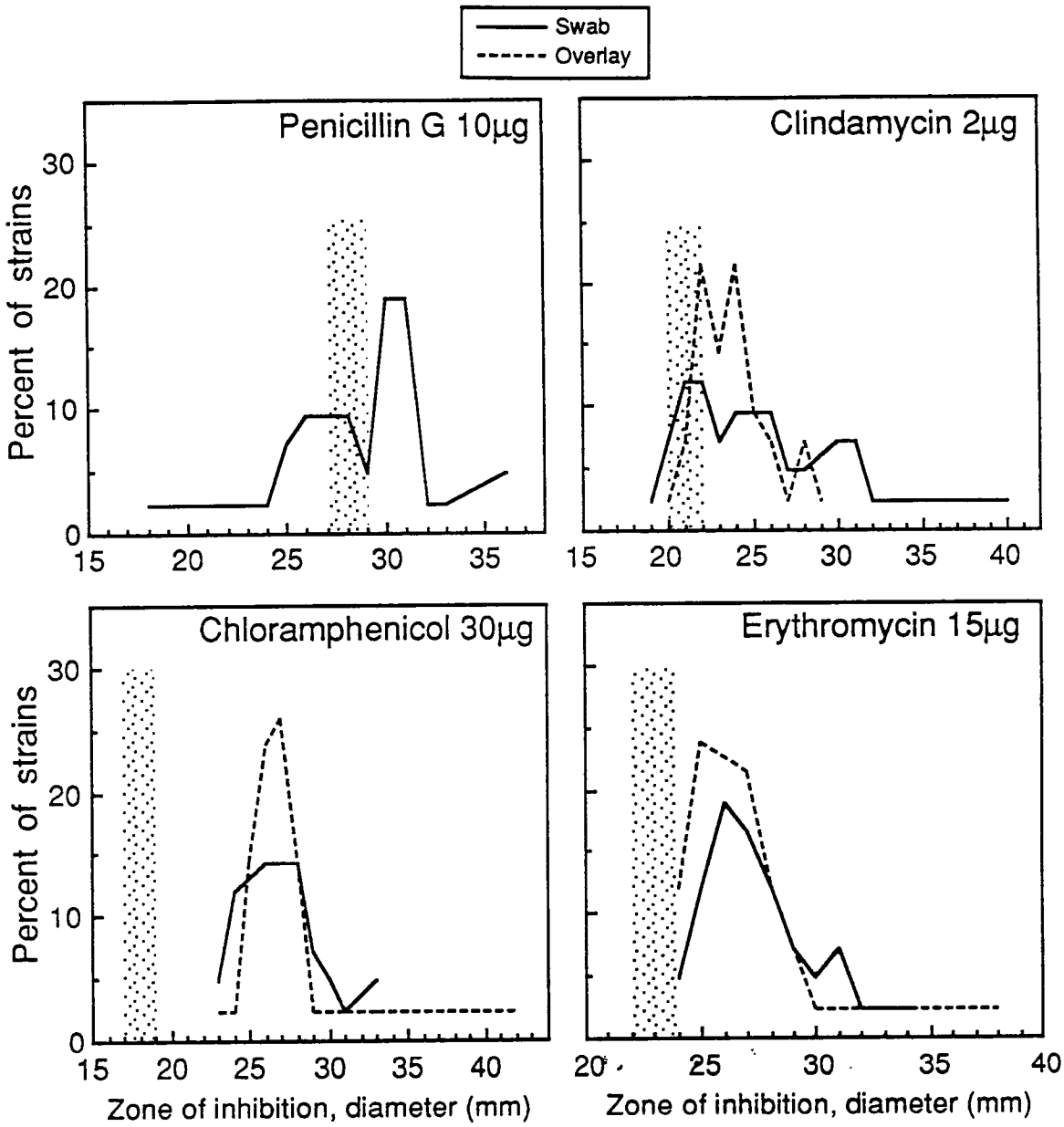

Figure 2.2 Distributions of averaged zone sizes obtained with 44 strains of lactic acid bacteria using the surface-swab and the agar overlay methods. Strains giving zones within the shaded area are reported to have intermediate susceptibility (±1), those with larger zones are sensitive and those with smaller zones resistant (NCCLS, 1989). Antibiotics purchased from Difco Laboratories.

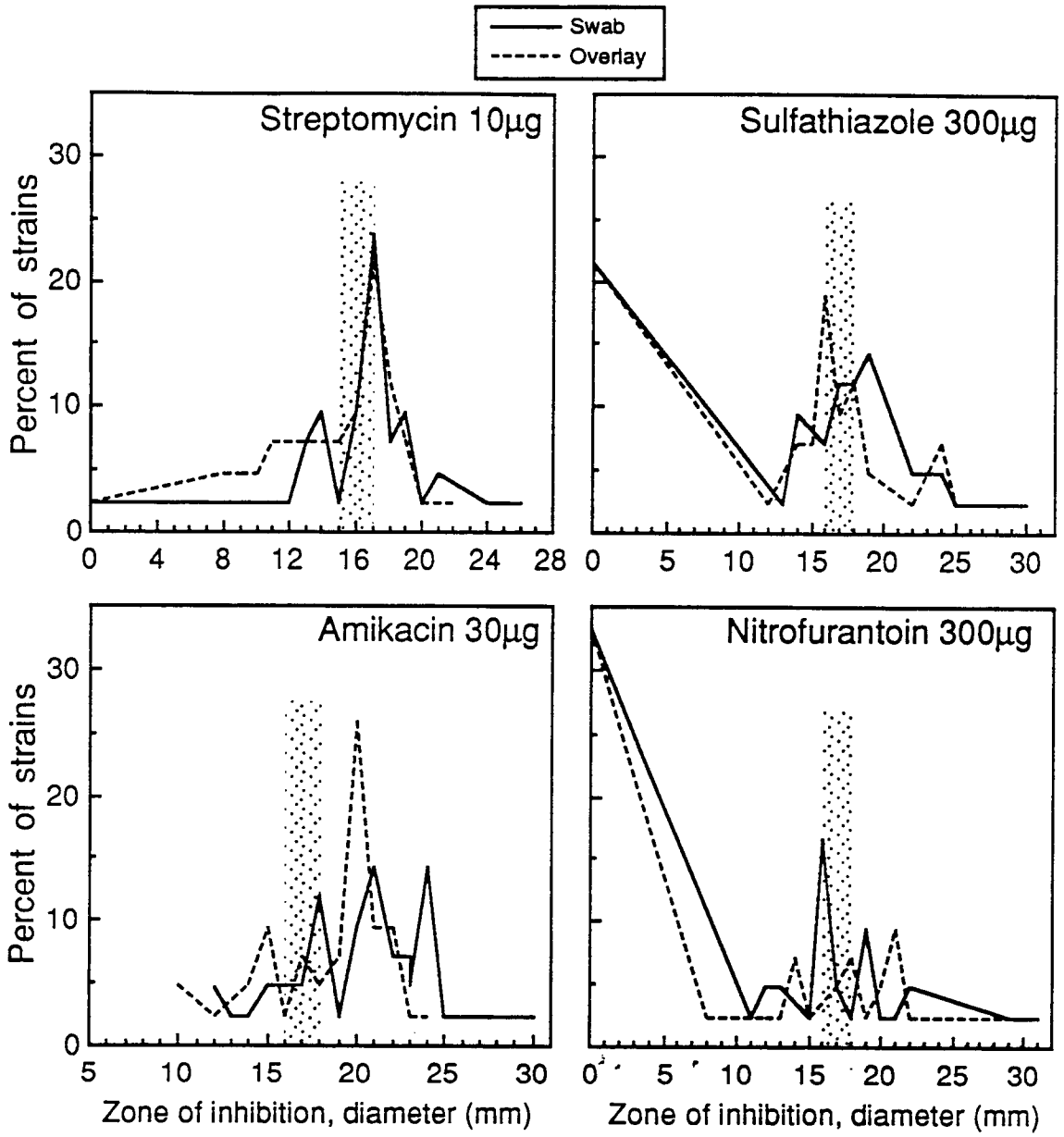

Figure 2.3 Distributions of averaged zone sizes obtained with 44 strains of lactic acid bacteria using the surface-swab and the agar overlay methods. Strains giving zones within the shaded area are reported to have intermediate susceptibility (±1), those with larger zones are sensitive and those with smaller zones resistant (NCCLS, 1989). Antibiotics purchased from Difco Laboratories.

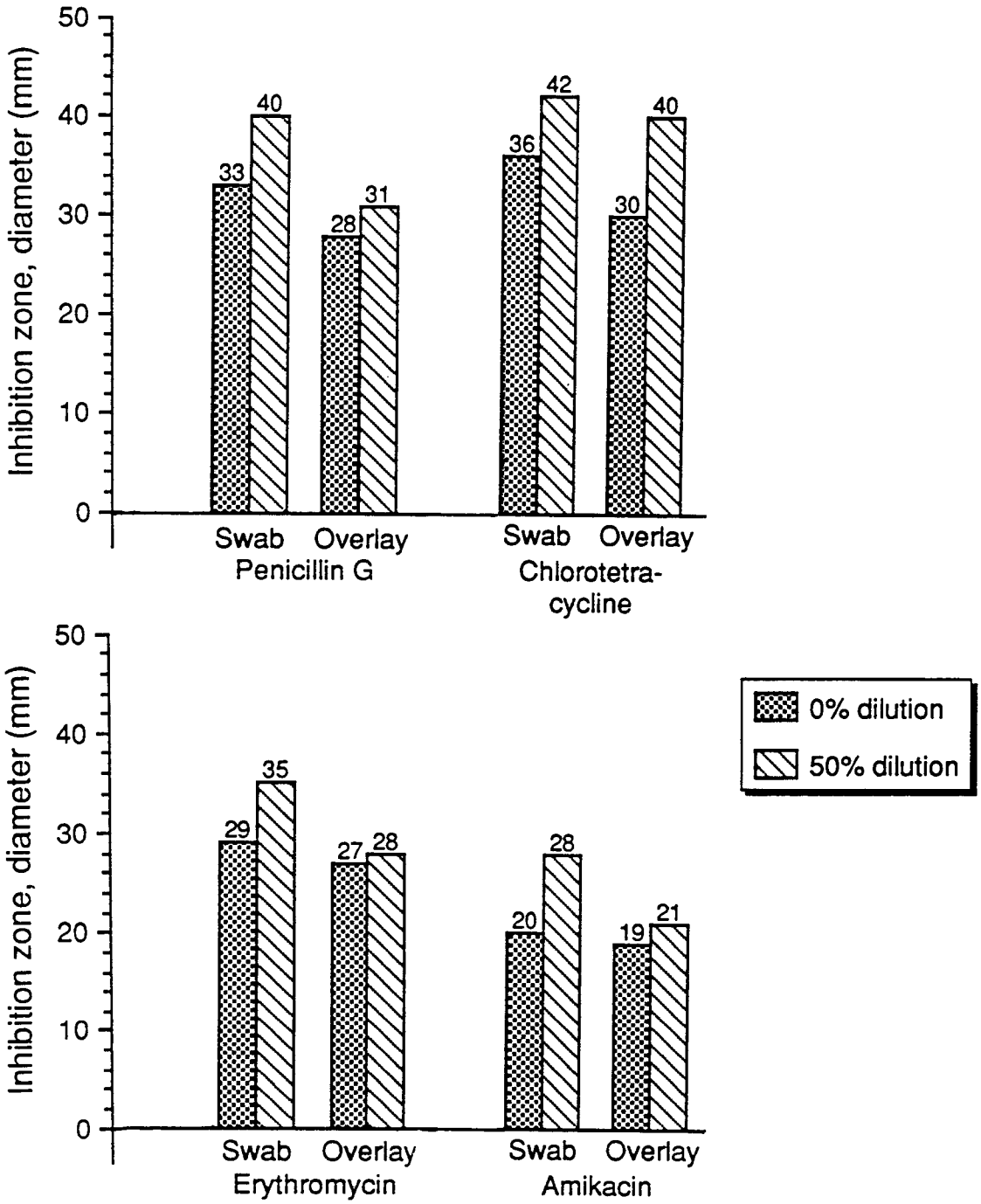

Figure 2.4 Relationship of antibiotic (Penicillin G, Chlortetracycline, erythromycin and amikacin) zone diameters and percent dilution obtained with Lc. cremoris EB2, using the surface-swab and agar overlay methods. Average of five experiments.

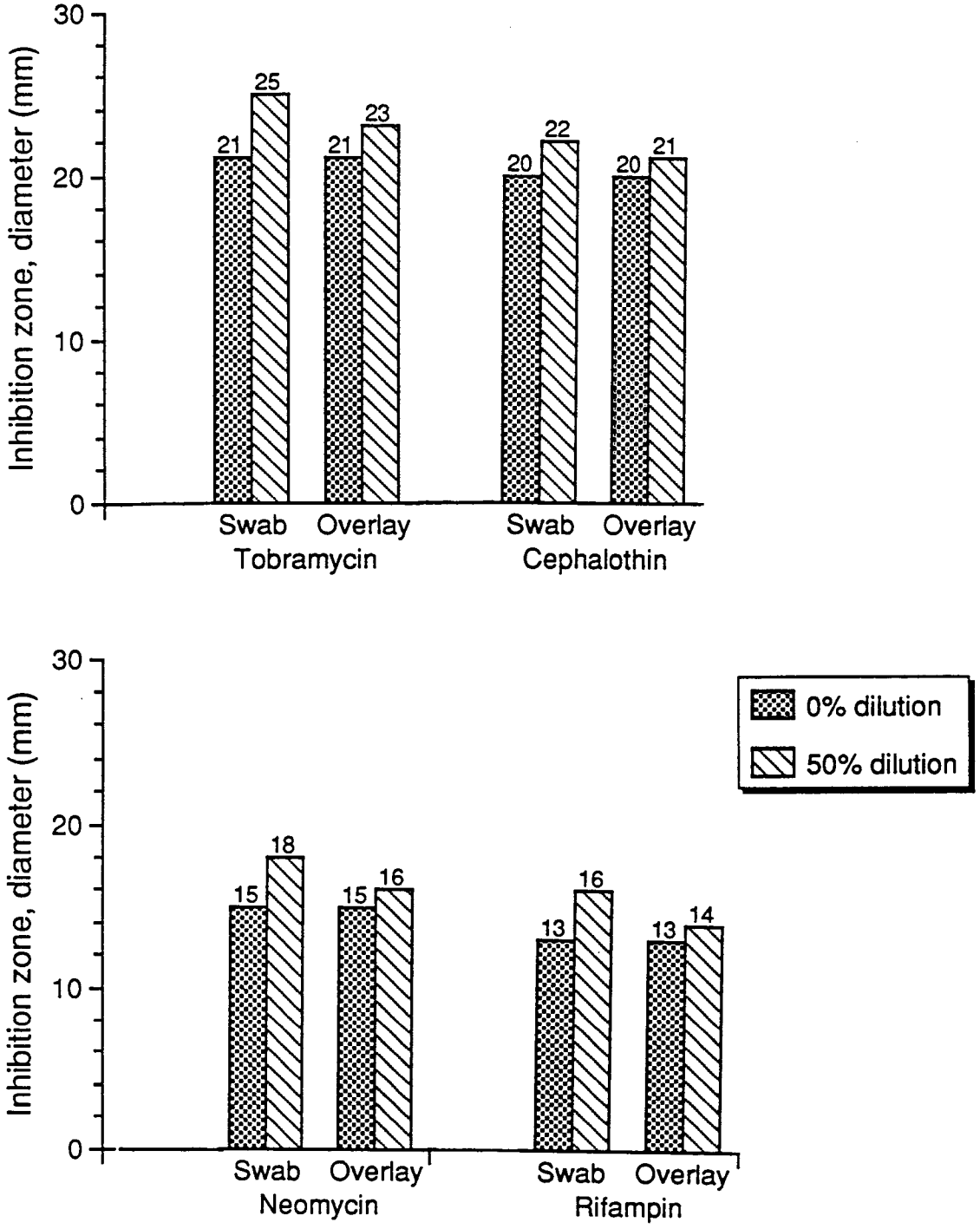

Figure 2.5 Relationship of antibiotic (tobramycin, cephalothin, neomycin and rifampin) zone diameter and percent dilution obtained with Lc. cremoris EB2, using the surface-swab and agar overlay methods. Average of five experiments.

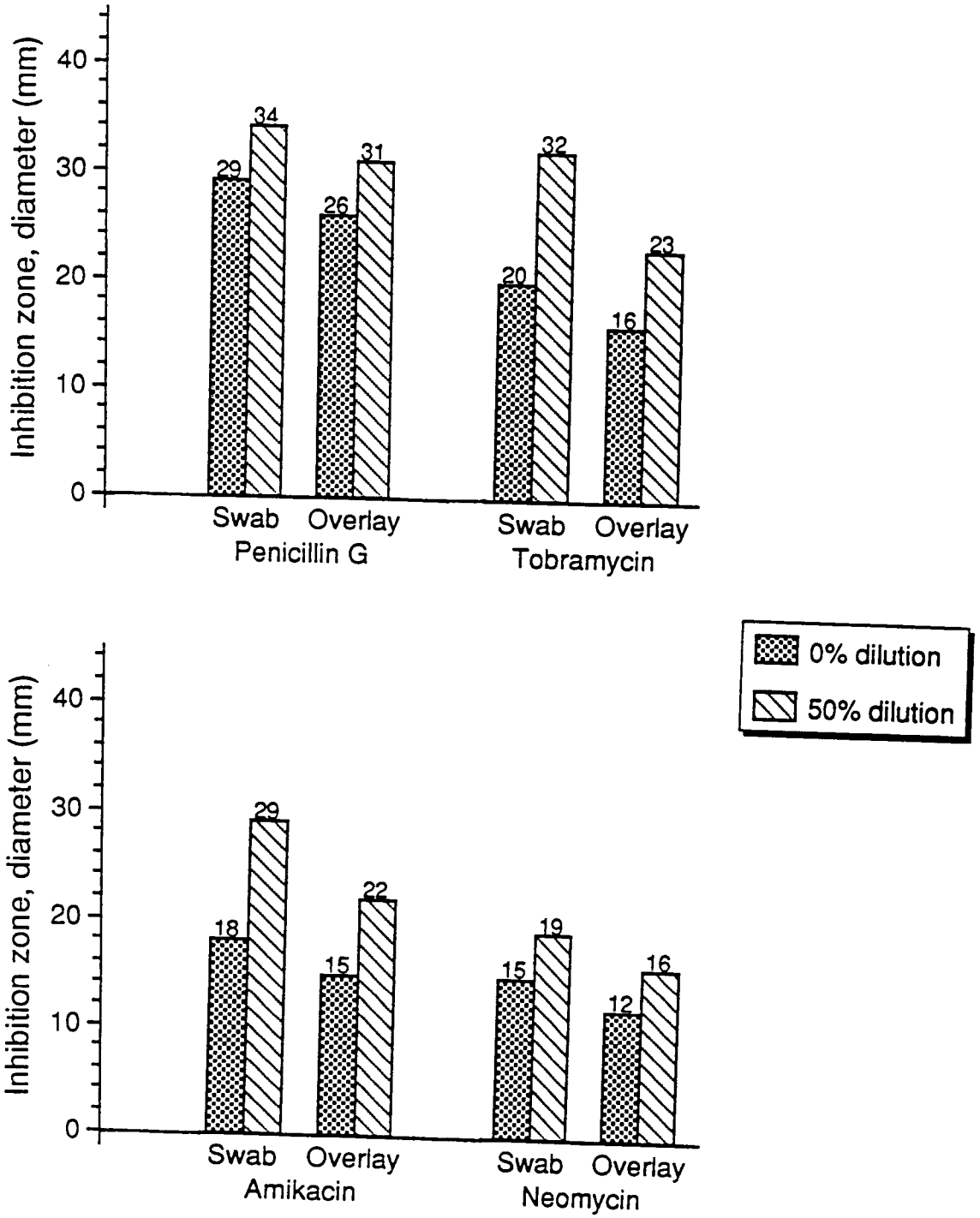

Figure 2.6 Relationship of antibiotic (penicillin G, tobramycin, amikacin and neomycin) zone diameter and percent dilution obtained with Lc. cremoris 178, using the surface-swab and agar overlay methods. Average of five experiments.

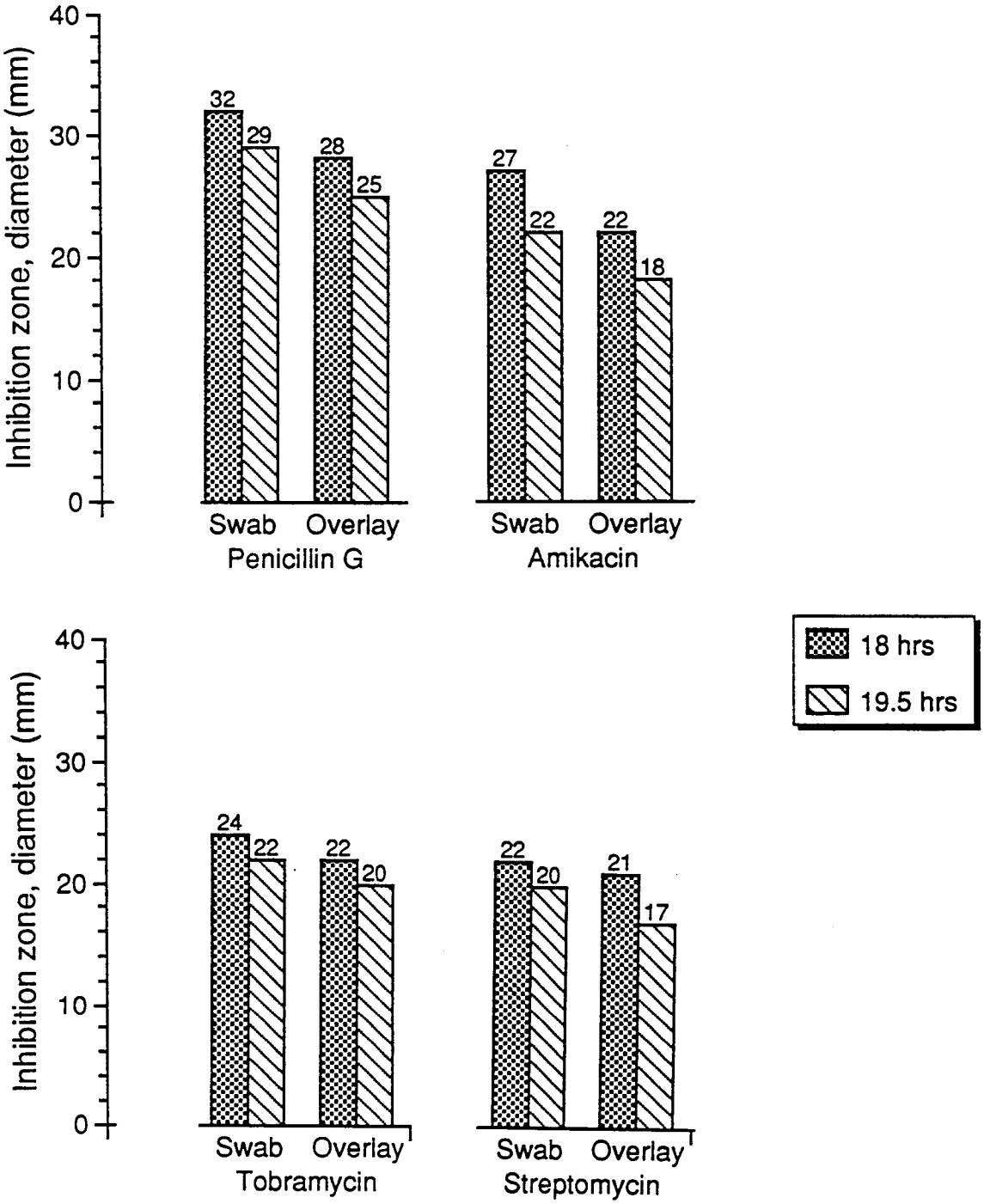

Figure 2.7 Relationship of antibiotic (Penicillin G, Amikacin, tobramycin and streptomycin) zone diameter and time of incubation at 30°C obtained with Lc. cremoris 205, using surface-swab and agar overlay methods. Average of five experiments.

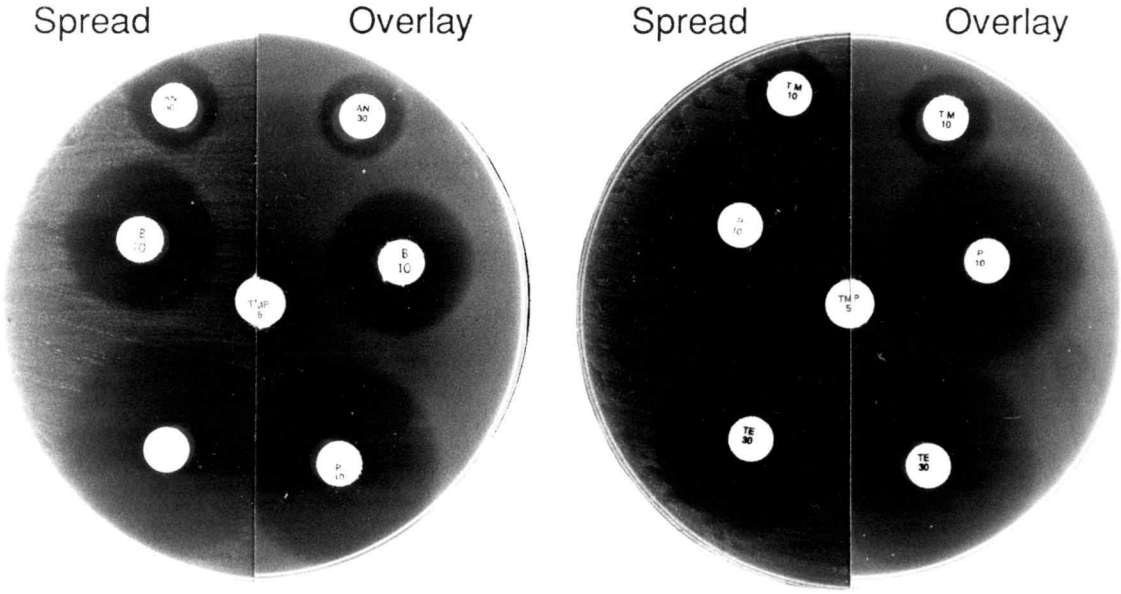

Figure 2.8 Different patterns of inhibitory zones obtained with cultures of Lc. diacetylactis 18-16 and Lc. lactis F_2D_2 on disk agar diffusion antimicrobial susceptibility test plates inoculated by the surface-swab and agar overlay method. The cultures were grown in Mueller Hinton broth, supplemented with 5 g/L glucose and 5 g/L yeast extract, at 30°C for 17 hr and further subjected to a 20% dilution. Discs contain different antibiotics at different concentrations.

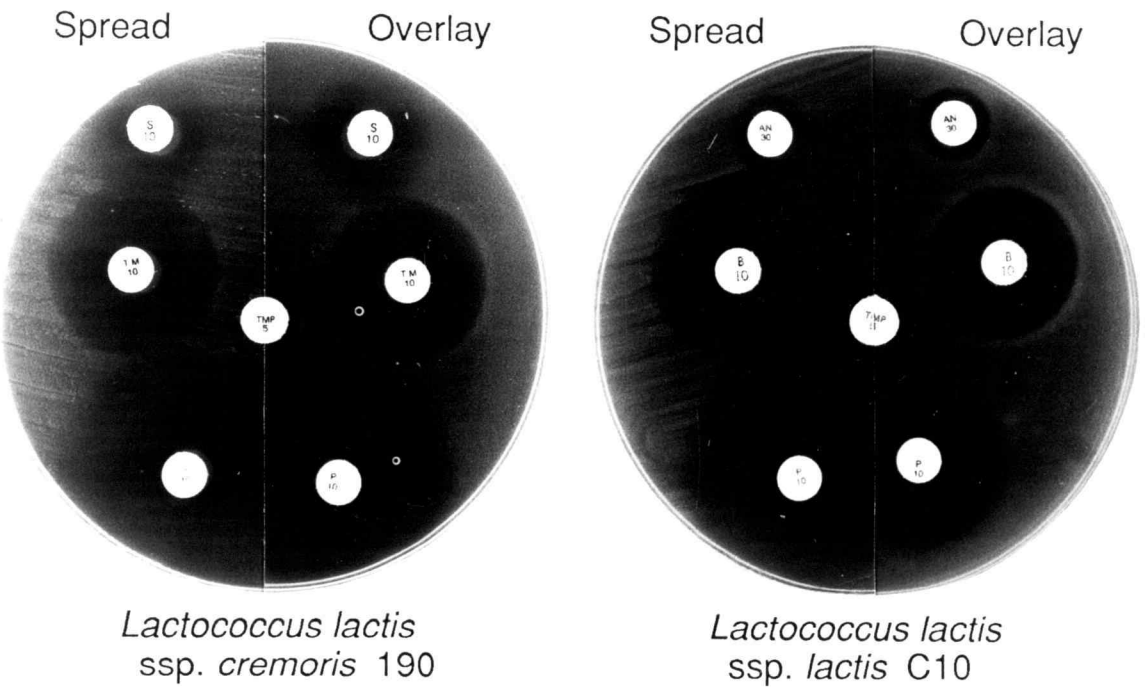

Figure 2.9 Different patterns of inhibitory zones obtained with cultures of Lc. cremoris 190 and Lc. lactis C10 on disk agar diffusion antimicrobial susceptibility test plates inoculated by the surface-swab and agar overlay method. The cultures were grown in Mueller Hinton broth, supplemented with 5 g/L glucose and 5 g/L yeast extract, at 30°C for 17 hr and further subjected to a 20% dilution. Discs contain different antibiotics at different concentrations.

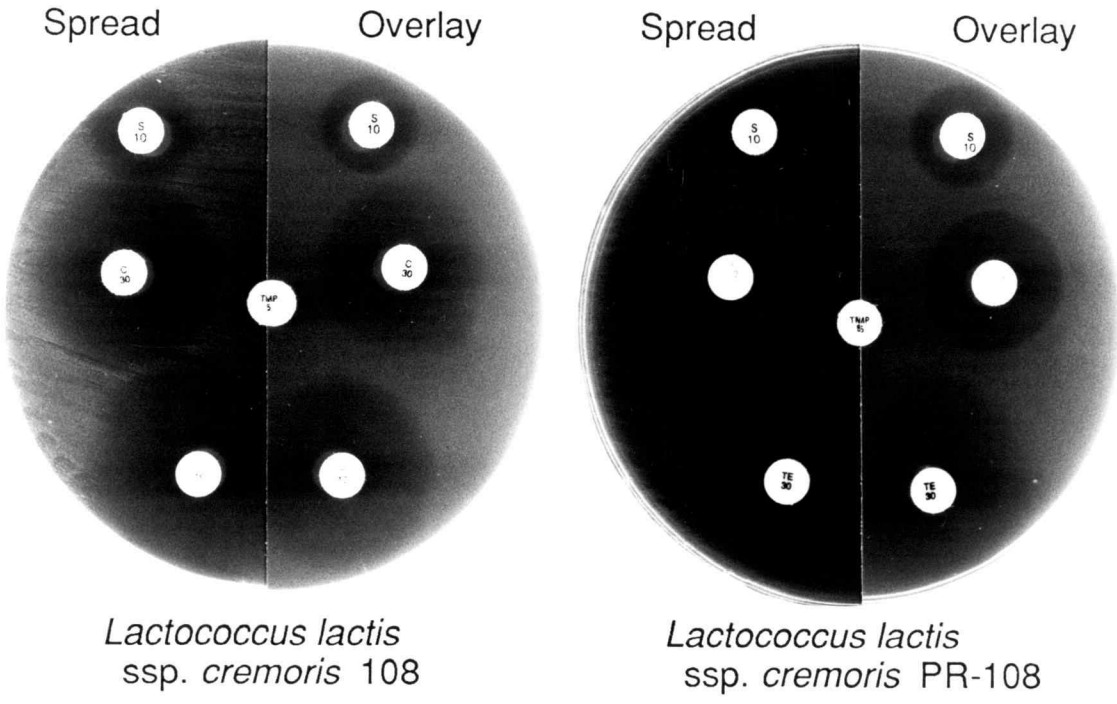

Figure 2.10 Different patterns of inhibitory zones obtained with cultures of Lc. cremoris 108 and PR-108 on disk agar diffusion antimicrobial susceptibility test plates inoculated by the surface-swab and agar overlay method. The cultures were grown in Mueller Hinton broth, supplemented with 5 g/L glucose and 5 g/L yeast extract, at 30°C for 17 hr and further subjected to a 20% dilution. Discs contain different antibiotics at different concentrations.

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CHAPTER 3

USE OF REVERSE-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TO DETECT QUATERNARY AMMONIUM COMPOUNDS IN MILK

ABSTRACT

A Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) method is described which permits analysis of quaternary ammonium compounds (QACS) in milk. The QAC used, Ster-bac, could not be efficiently extracted from milk using water-miscible solvents due to interference of milk fat molecules. Ster-bac was effectively separated using biological chromatographic matrix due to the retention on octadecylsilane-bonded stationary phase, which was commercially packed. A de-ashing pre-column packed with the same material provided an additional degree of separation and it was efficient in removing undesirable molecules still left after organic acid extraction of the milk samples. Ster-bac was selectively eluted from this column in the presence of organic solvent (acetonitrile) and ion-pairing agent (sodium perchlorate), then analyzed by spectrophotometric detection at 217 nm. Resolution of overlapping bands during separation was done by improving mobile phase and ion-pairing agent. QAC levels in the range of 2.7 to 200 ppm (20 to 200 ppm carried by the control samples) were analyzed clearly and precisely.

INTRODUCTION

Quaternary ammonium compounds (QACS) are commonly used as sanitizers in the food industry to control Listeria; as such they decontaminate food preparation equipment, utensils, floors, ceilings, walls, drains and any surface reachable by spray foams. The surfactant and bacteriocidal properties of QACS are related to both the quaternary ammonium cation and the length of the alkyl side chain, which is between 6 and 18 carbon atoms (13, 15). Of the QAC sanitizers, n-alkyl (50% C_{14}, 40% C_{12}, 10% C_{16}) dimethyl benzyl ammonium chloride (Ster-Bac) (Figure 3.1), manufactured by Klenzade Corporation, a Division of Ecolab Inc., Minneapolis, Minnesota, is used by many food industries and it was the sanitizer used in the present study. The recommended disinfecting dilution of this product is 1 oz. to 2 gallons of water, giving 400 ppm active quaternary. Other commercially available QACS are (15): Triton X-400, Hyamine 1622, Hyamine 10-X, Hyamine 3500, all from Rohm & Haas Co. (USA); Barquat LB-50 by Lonza Inc. (USA); Roccal by Sterwin Chemicals Inc. (USA); Vantoc CL by I.C.I. Ltd. (UK); Zephiran by Winthrop Laboratories (USA) and Bioquat 501 by Ivon-Watkins-Don Ltd. (NZ).

Residual amounts of QAC on equipment following food plant sanitation adversely affect the economics of food processing by altering product flavour, keeping quality or by interfering with fermentation starter culture activity.

Some chemicals, like QACS, can cause inhibition of acid production by lactic acid starter cultures (8, 16) by combining with or displacing specific constituents of the bacterial cell surface. The main structures of bacterial starter cells which are direct targets for attack, are the cell wall, cytoplasmic membrane and cytoplasm. The cell envelope of gram positive organisms such as those of the Lactococcus and Lactobacillus genera is made of a rigid peptidoglycan polymer of N-acetylglucosamine-N-acetylmuramic acid peptide side chain which can occur in layers connected by peptide bonds (21).

The interactions of QAC sanitizers and target sites on the cell surface leads to a change in charge on the bacterial cell, a fact which has been demonstrated by electrophoretic mobility studies. Reports also indicate that QACS enter bacterial cells and interfere with lipid metabolism (6, 23). Washam et al. (24) suggested that QAC resistant strains of the gram negative bacterium Pseudomonas aeruginosa had reduced esterase and lipase activity because they lost hydrolyzing capabilities for certain esters which accumulated in the

Tryptone-glucose-yeast extract broth used as growth medium.

Colorimetric methods have shown that cationic detergents are effective inhibitors of respiration in gram positive and gram negative organisms. For example, the effect of surface active agents on the cytochrome system is evident when the oxidation of p-phenylenediamine and glucose is followed spectrophotometrically (3). Also there are reports indicating that the effects of surface active substances on bacteria appear primarily to be at the cell surface (9, 19), where cationic detergents act by first disorganizing the cell membrane, denaturating essential proteins and finally by changing the permeability of the cell. Rapid release of phosphorus-containing compounds from Staphylococcus aureus occurred when cells were treated with QAC due to membrane damage (20), and free amino-acids were lost from intracellular pools (11). Armstrong (1) measured the total loss of phosphorus and the reduced production of acid and CO_2 from glucose in baker's yeast cells treated with QAC. Dunsmore (5) reviewed the effects of sanitizers on starter performance, indicating that thermophilic yoghurt starter cultures are less sensitive to QACS than mesophilic starter cultures; for yoghurt starters the sensitivity levels were 2.5 - 500 mg/L QAC, while for cheese cultures, the sensitivity levels were .5 - 200 mg/L. Guirguis et al. (12) reported sensitivity levels of .5 - 2 mg/L QAC for lactococci and lactobacilli.

Variations in culture growth conditions, fermentation procedures and strains used probably account for the different levels of sensitivity of starter cultures towards quaternaries.

The action of QAC sanitizers can be measured directly by a number of different laboratory tests. The phenol coefficient method was one of the first standard tests to be adopted by the food industry. In this test, a culture of Salmonella, Staphylococcus or Pseudomonas is exposed to various concentrations of the sanitizer being evaluated and results are compared to that of phenol. A phenol coefficient is determined by taking the number representing the highest sample dilution which gives complete killing of the microorganism and dividing it by the number representing the highest dilution of phenol showing the same results (2). The U.S. Public Health Service recommends Staphylococcus aureus as the best organism in this procedure since it is the most common cause of food poisoning. Escherichia coli is recommended for Weber and Black (25) or Chambers (4) procedures to evaluate food plant sanitizers. This later method exposes the bacterial culture to different strengths of the sanitizer being tested for different times; the action of the agent is then halted at a precise time by the addition

of a non-toxic neutralizer [e.g. Tween 80 Asolectin (lecithin) (4)]; finally, the presence or absence of colonies on agar plates determines the efficacy of the sanitizer.

In addition, the detection of QAC in milk can be based on a reaction between eosin and QAC, forming a red precipitate, followed by titration to the colorless endpoint with aerosol OT (dioctyl sodium sulfosuccinate) (17). This method is sensitive to 10 to 100 ppm QAC (10). The methylene blue method has undergone several modifications (2) and is the method of choice of Klenzade Corporation. Although several of these procedures have been used to detect QACS, the majority do not allow rapid determination and quantitation of QAC, especially in milk. In this study, the detection of Ster-bac QAC in raw milk samples using Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) was investigated.

RP-HPLC is a system first described by Howard and Martin (14), in which the eluent is more polar than the stationary phase. The combination of high resolution and peak sensitivity with detection of aromatic rings in the ultraviolet region has made RP-HPLC a powerful method for chromatographing amine-containing compounds (3). In this case, the sample can be analyzed directly without being derivatized first, as is required for gas-liquid chromatography.

MATERIALS AND METHODS

Medium

Instant Peak Nonfat Milk (NFM-Galloway West Co., Fond du Lac, WI) was reconstituted to 11% solids. Tubes containing 10 ml were steamed for 45 minutes, cooled to room temperature and used to inoculate the lactic strains. Strains were maintained at -20°C in litmus milk, autoclaved for 10 minutes at 15 pounds pressure (121°C), then supplemented with 15% sterile glycerol.

Cultures

Among the 44 strains of lactic acid bacteria obtained from the Microbiology Department, Oregon State University, 10 strains of 3 subspecies were used for QAC sensitivity threshold analyses and those used were: Lc. diacetylactis 18-16, Lc. lactis 197 and F_2D_2, Lc. cremoris 107/6, 178, 187, 190, 190, 203, 852 and BK5.

Inhibition Test

Each starter culture grown (0.1 ml inoculum) in 11% NFM (18 hr at 30°C) was added to duplicate test tubes containing 10 ml of 11% NFM holding varying concentrations of Ster-bac ranging from 0 to 300 ppm. These tubes were incubated in a 30°C circulating water-bath for 18 hr. The final pH of the contents of each tube was measured in a Beckman pH meter. In reporting results, concentrations of Ster-Bac are expressed as parts per million (ppm), which is the standard procedure used by food industries.

Apparatus

A Beckman DU spectrophotometer with 1 cm crystal cells was used. The chromatographic system consisted of a model 110 B pump, 420 microprocessor system controller, 340 organizer, 210 A sample injector valve and model 163 variable wavelength detector. A Hewlett Packard 18971-A input selector and 3390 A integrator were interfaced with the instrument. The controller initiated the HP-3390 A integrator upon 10 μl sample injection and was programmed to make an 18-minute run time per injection. It was also programmed for an initial flow rate of 2.0 ml/minute then changed to 3.0 ml/minute at time 10 minutes. Separation was obtained with a Bio-Rad Bio-Sil ODS-5S reverse phase column (250 X 4.0 mm I.D.; 5 μm particle size) with a Bio-Rad ODS-5S guard C_{18} (30 X 4.6 mm) de-ashing system as a precolumn. The column was held at room temperature and wrapped with .5 inch of foam.

Solvent Systems

HPLC grade acetonitrile, ethyl ether and sodium perchlorate (EM Science, a Division of EM Industries Inc.), 99.9% phosphoric acid (Aldrich Chemical Company Inc.) and 1-pentane sulfonic acid (Sigma Chemical Company), were reagent grade and used without further purification.

Freshly prepared eluents were composed of 82% C_2N in .1 M $NaClO_4$/distilled water, pH = 2.51 acidified with phosphoric acid, and 88% C_2N in .1 M $NaClO_4$/distilled water, pH = 2.46 acidified as before. Each newly made solvent was evaluated by running it at maximum sensitivity, .005 absorbing units full scale (AUFS) at 215 nm. A flat base line with a rise of no more than .03 absorbance units was considered normal (1).

QAC Standards

Benzylcetyldimethylammonium chloride monohydrate (95%), benzyldimethyldodecylammonium bromide (97%) and benzyldimethyltetradecylammonium chloride dihydrate (99%) from Aldrich Chemical Company, Inc., and Ster-Bac control samples were prepared in $C_2N/NaClO_4$ solution to final concentrations ranging from 20 to 200 ppm.

Preparation of Raw Milk Samples

A representative group of 22 raw milk producers was selected. Samples of raw milk were collected and placed in plastic vials, and the vials kept in a mixture of ice and water from the time of sampling until aliquots were taken for RP-HPLC analysis. The samples were each collected from separate milk producing farms by milk-hauler drivers from the Day Trucking Service of Corvallis, OR. Accuracy and suitability of the Mojonnier method (35) for fat extraction from the milk samples were evaluated, and modifications made in the procedure as follows: To 20-ml aliquots in duplicate of each sample, 1.5 ml of ammonium hydroxide (Ashland Chemical Company) were added. After mixing, 10 ml of ethanol (J.T. Baker Chemical Company) were added, vortexed and then 25 ml of ethyl ether were added followed by mixing. Each mixture was allowed to separate for 30 seconds, then the top clear ether layer was decanted. Then 5 ml of ethanol were added to the lower layer and mixed to prevent gel formation; then 15 ml of ethyl ether were added and mixed for 1 minute; samples were left undisturbed for 30 seconds at room temperature as before. This second top clear ether layer was mixed with the first one in an aluminum dish, from which solvents were evaporated using a hot plate at the low heat setting. Each dry sample was dissolved in 5 ml of eluent (82% or 88% C_2N in .1M $NaClO_4$/distilled water) and 10 μl portions applied directly to the ODS-5S RP-HPLC column.

RESULTS AND DISCUSSION

QACS such as Ster-bac have a strong ultraviolet absorption spectrum (Figure 3.2), and this product was the QAC of choice for the analysis of various raw milk samples. The RP-HPLC analysis of Ster-bac started by the addition of calculated concentrations of Ster-bac and twice as much 1-pentane sulfonic acid to raw milk samples. 1-pentane sulfonic acid appeared to compete with the various milk fat molecules for the positive nitrogen entity in QACS, minimizing sample loss in consecutive extraction procedures. Milk-Ster-bac samples were further treated with organic solvents, giving satisfactory yields. For example, 56% yield was calculated when sample # 15-33345 (area 905,870) carrying 120 ppm Ster-bac in 88% acetonitrile in .1 M sodium perchlorate, pH 2.46, was compared to the Ster-bac control sample under the same conditions. Although the extraction with organic acid was very complete, when Ster-bac had to be removed from the fat precipitate, it appeared to have formed a strong interaction with milk fat lessening the efficiency of further extraction.

In the RP-HPLC procedure, the combination of maximum resolution of components and minimum analysis time was

accomplished by using a mobile phase composed of acetonitrile/sodium perchlorate. Acetonitrile was the organic modifier of choice because it has lower viscosity which reduces the amount of back pressure during flow in the chromatographic column; it also has a low UV cut-off wavelength of 200 nm and a relative high boiling point (82°C), minimizing fire hazards. By increasing the concentration of acetonitrile in the mobile phase, there was an decrease in QAC retention time, mainly because of the addition of sodium perchlorate to the mobile phase which also contributed to a decrease in the retention time and an increase in resolution. This fact is explained by the fact that sodium ions compete with the positively charged QAC for binding sites on the silica matrix. The chromatographic matrix chosen for this experiment was made of silica particles with 80 A° pore size and 5 μm diameter. These small sized particles were filled with mobile phase components, being too small for the larger QAC molecules to enter. However, the effect of pore size exclusion against QAC was counterbalanced by the presence of octadecylsilanol groups attached to the silica surface. These lengthy alkyl groups appeared to decrease the access of small mobile phase molecules to the silica pores, in a sense acting like a cation exchanger, and therefore bringing about optimization to the stationary phase in the separation of QACS (8).

Chromatograms of raw milk samples containing different concentrations of Ster-bac added before milk treatment, are shown in Figures 3.3 through 3.21. Each figure was compared with a QAC blank. In general, contaminating milk fat eluted prior to elution of Ster-bac. Under the conditions stated on Figures 3.3 through 3.10, all with 82% acetonitrile in sodium perchlorate/water, Ster-bac eluted before a 14-minute period, and did not appear to change in intensity with changes in flow rate, probably due to the stable settings created by the mobile phase components. The last chromatographic peak seen in each figure (d) is part of the ster-bac composition, and comprises the benzyl dimethyldodecyl ammonium chloride, a fact confirmed by the control blank. On the other hand, Figures 3.11 through 3.21 show two consecutive QAC peaks which also make up the Ster-bac composition, benzyl dimethyldodecyl ammonium chloride and benzyl dimethyltetradecyl ammonium chloride (d), eluting at about 9 and 15 minutes, respectively. Therefore, by increasing the percentage of acetonitrile in the mobile phase, QACS carrying longer alkyl side chains elute more rapidly, resulting in the identification of well resolved peaks.

Ster-bac was quantitated in milk by comparison of calculated peak areas from a Hewlett Packard integrator unit, from samples containing known concentrations of QAC in the range of 20 to 200 ppm. For example, the lower detection

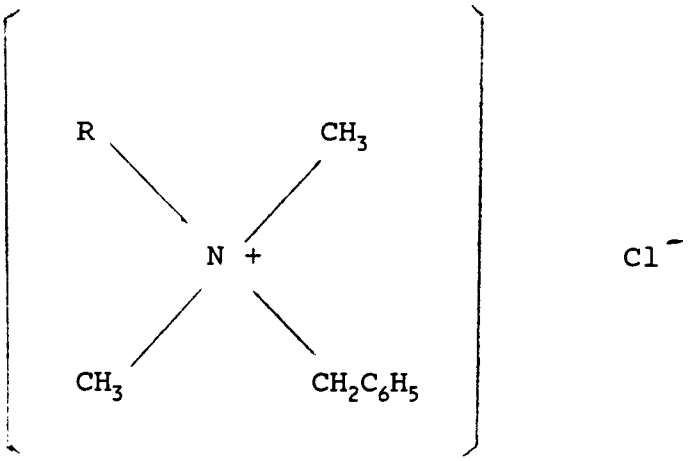
limit was seen for the raw milk sample # 15-35477 (area 8865), in mobile phase composed of 88% acetonitrile in .1 M sodium perchlorate/water at pH 2.46 (Figure 3.18). It was approximately 2.7 ppm QAC (80 ppm control 15-35477/QAC, providing an area of 261,570, when applied to the column in 10 μl injection volume). In Figure 3.8, sample # 15-35444, with mobile phase composed of 82% acetonitrile in .1 M sodium perchlorate/water, pH 2.51, had peak area of 24,531 and 6.3 ppm QAC contamination when compared to # 15-35444/QAC control sample carrying 40 ppm Ster-bac with peak area of 156,470.

Based upon results from the control samples 15-35477 / 80 ppm Ster-bac and 15-35444/40 ppm Ster-bac described above, a precision of 84% was calculated for the results obtained. However, a precision of 98% was calculated for the control samples 15-44880/100 ppm Ster-bac in 82% acetonitrile in .1 M sodium perchlorate/water, pH 2.51 (area 798,010), and 15-Silver Dome/120 ppm Ster-bac in 88% acetonitrile in .1 M sodium perchlorate/water, pH 2.46 (area 760,940). These results indicated that a control carrying a concentration of QAC close to the concentration of the sample analyzed, increased the percentage of method accuracy, even with variations in the percent composition of mobile phase components.

To complete this study, resistance of starters to QAC was examined. Figure 3.22 shows the resistance data for several strains to Ster-bac. Inhibitory effect on starter activity in the presence of 70 ppm QAC was evident at 30°C for Lc. cremoris 190, while 150 ppm was required for inhibition of Lc. cremoris BK5.

In summary, a rapid RP-HPLC method was developed for monitoring QACS in milk samples. Although only Ster-bac was investigated, the data presented in this study indicate that the method would be adaptable to other QACS as well, and it offers better detection of lower levels than required to inhibit acid production by lactic acid bacteria, a significant advantage over methods published to date.

Figure 3.1 Composition of 10% Ster-Bac {n-alkyl [50% C_{14} 40% C_{12}, 10% C_{16}] dimethyl benzyl ammonium chloride}; 90% inert ingredients.



R = $(CH_2)_{14}$

or

R = $(CH_2)_{12}$

or

R = $(CH_2)_{16}$

Figure 3.2 Absorption spectrum of 800 ppm Ster-Bac 10% n-alkyl [50% C_{14}, 40% C_{12}, 10% C_{16}] dimethyl benzyl ammonium chloride}; 90% inert ingredients in distilled water.

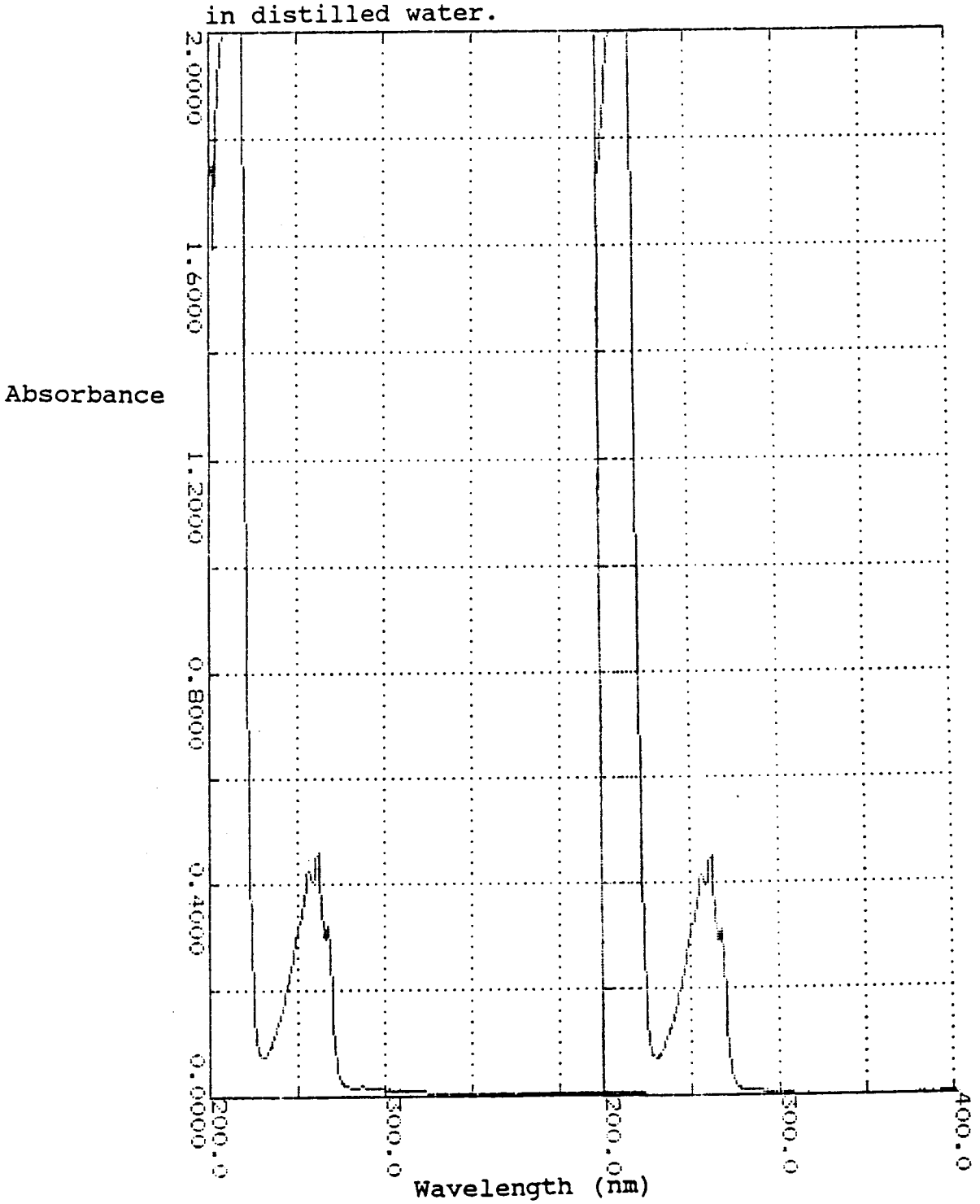


Figure 3.3 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 82% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.51

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-33530 + 160 ppm pentane sulfonate;

(b) Sample # 15-33530 + 80 ppm Ster-Bac + 160 ppm pentane sulfonate;

(c) 20 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent;

(d) 20 ppm benzyl dimethyldodecyl-ammonium chloride

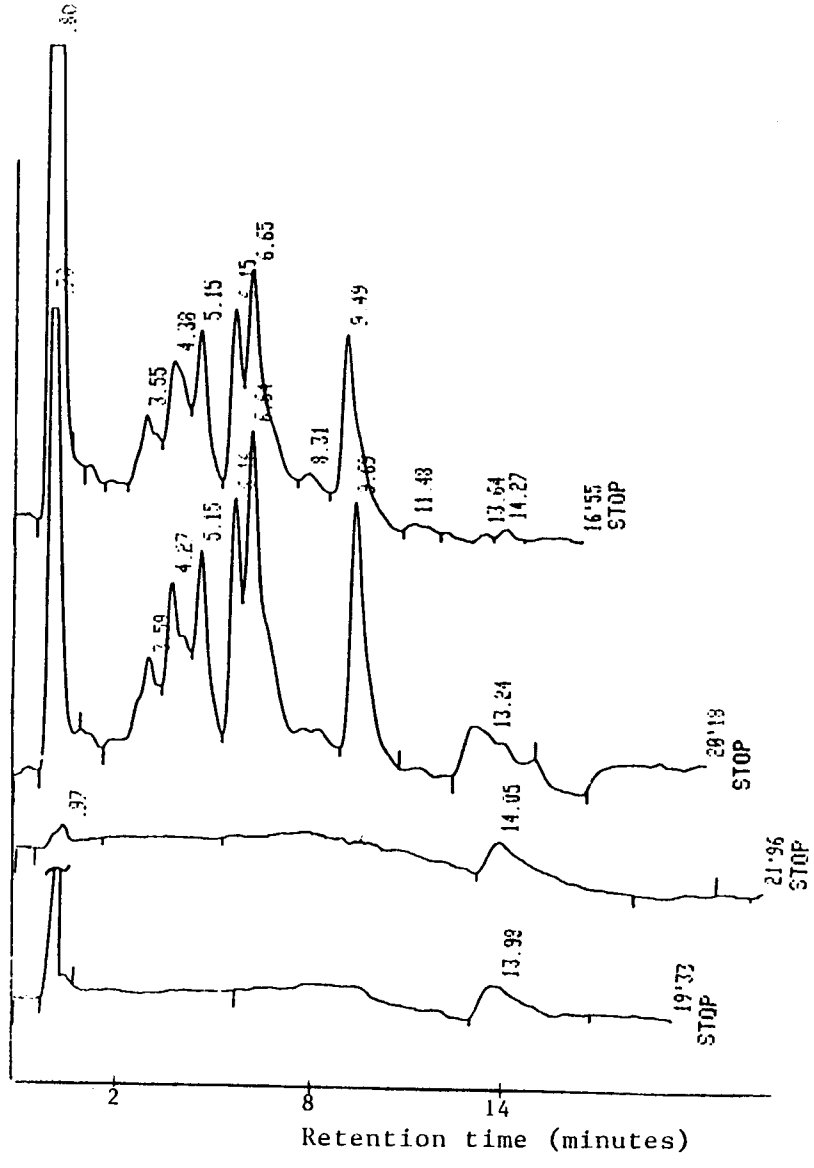


Figure 3.4 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 82% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.51

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-33746 + 160 ppm pentane sulfonate;

(b) Sample # 15-33746 + 80 ppm Ster-bac + 160 ppm pentane sulfonate;

(c) 20 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent

(d) 20 ppm benzyl dimethyldodecyl-ammonium chloride

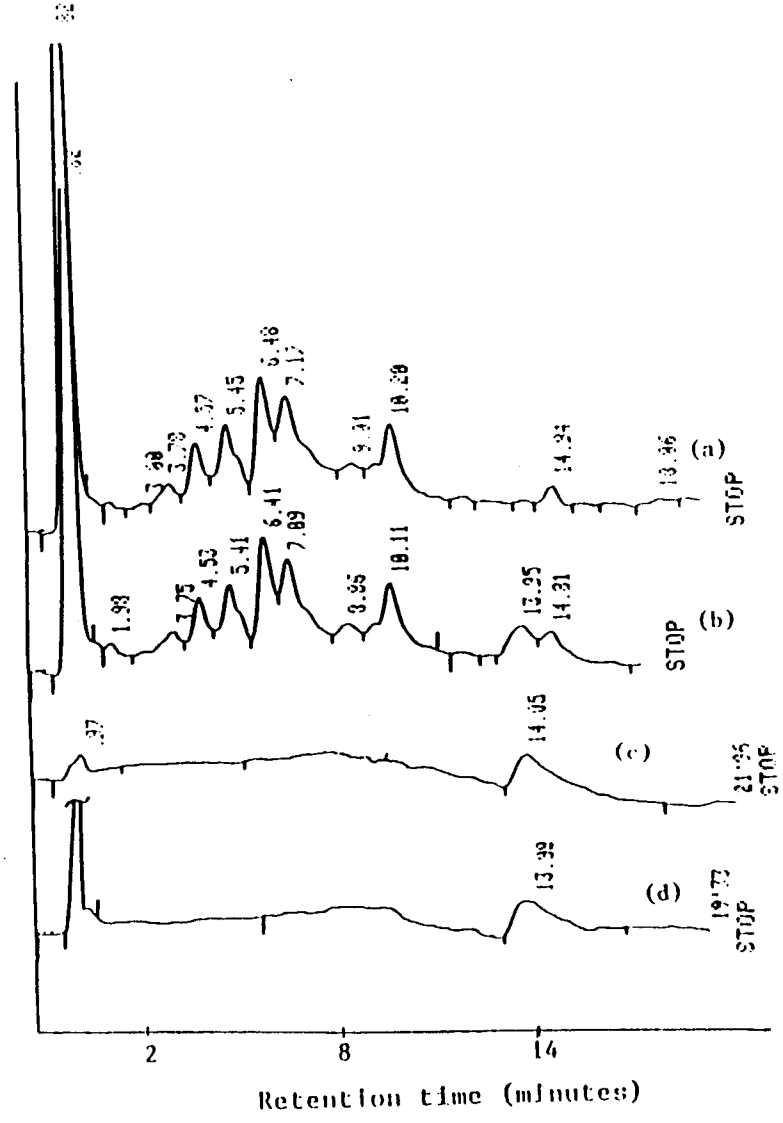


Figure 3.5 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-SIL ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 82% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.51

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-33787 + 400 ppm pentane sulfonate;

(b) Sample # 15-33787 +200 ppm Ster-bac + 400 ppm pentane sulfonate;

(c) 20 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent

(d) 20 ppm benzyl dimethyldodecyl-ammonium chloride

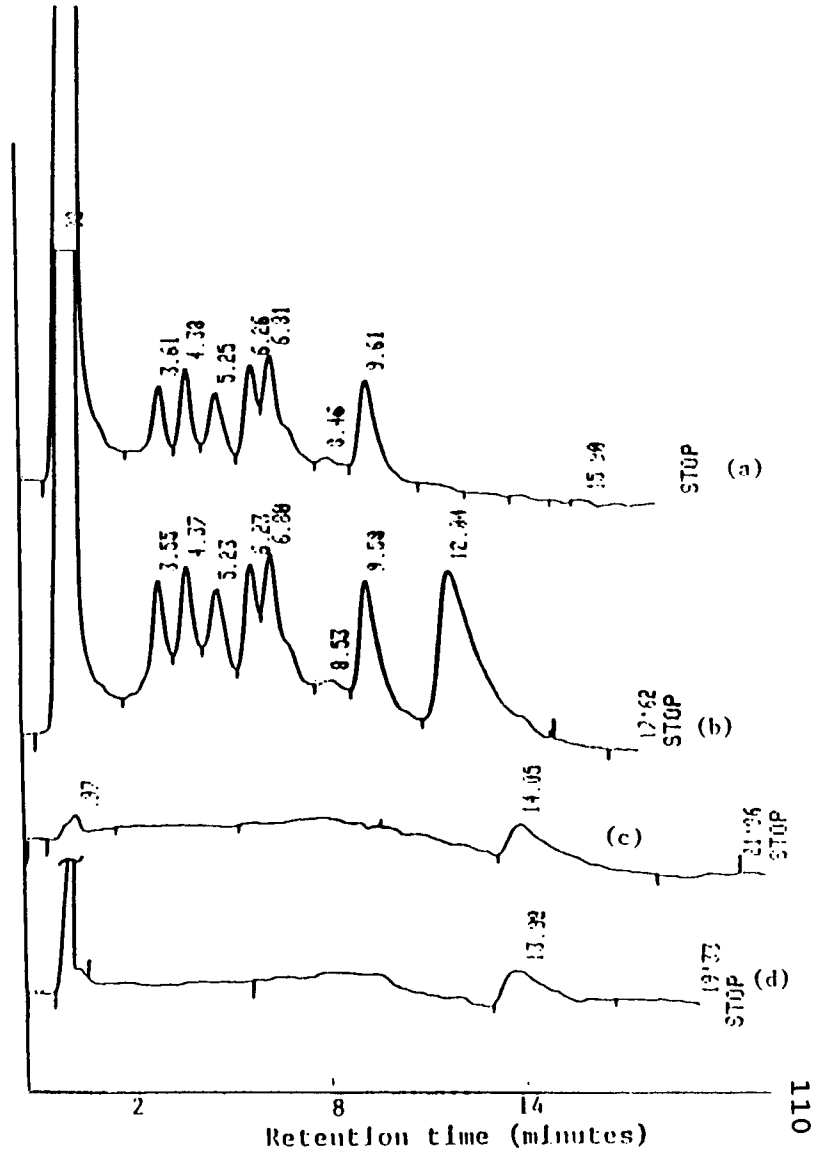


Figure 3.6 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 82% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-35402 + 160ppm pentane sulfonate;

(b) Sample # 15-35402 + 80 ppm Ster-bac + 160ppm pentane sulfonate;

(c) 20 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent

(d) 20 ppm benzyl dimethyldodecyl-ammonium chloride

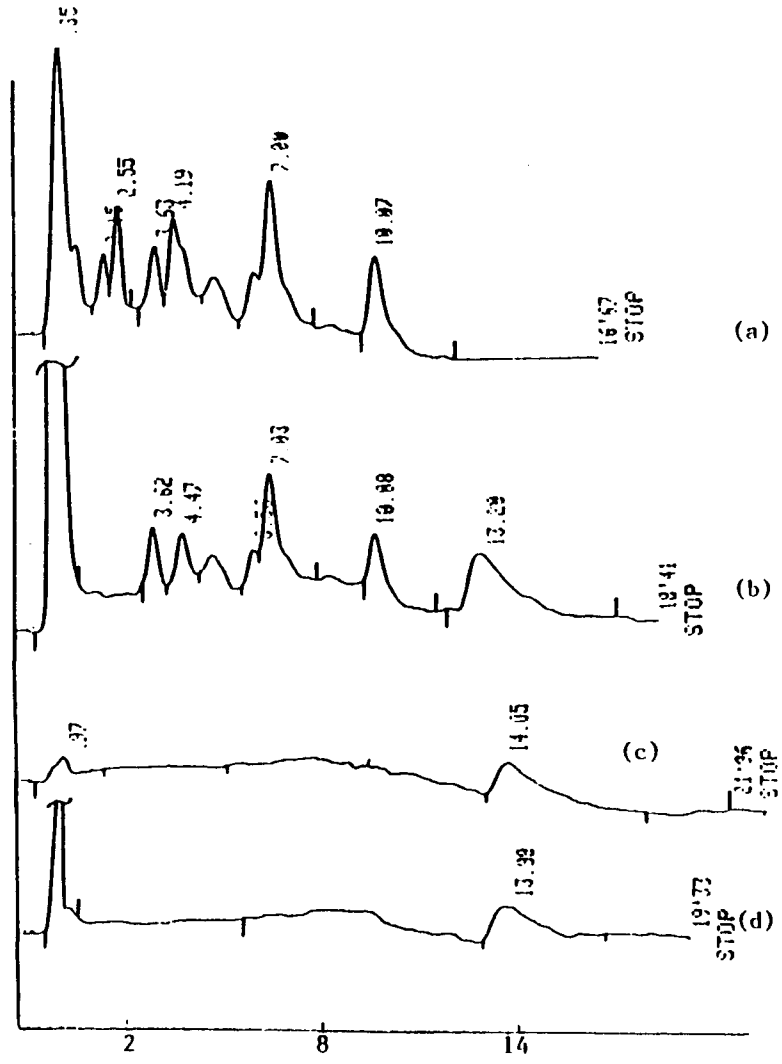


Figure 3.7 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 88% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.46

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-35444 + 80 ppm pentane sulfonate

(b) Sample # 15-35444 + 40 ppm Ster-bac + 80 ppm pentane sulfonate;

(c) 20 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent;

(d) 120 ppm benzyl dimethyltetradecyl-ammonium chloride and 120 ppm benzyl dimethyldodecyl-ammonium chloride

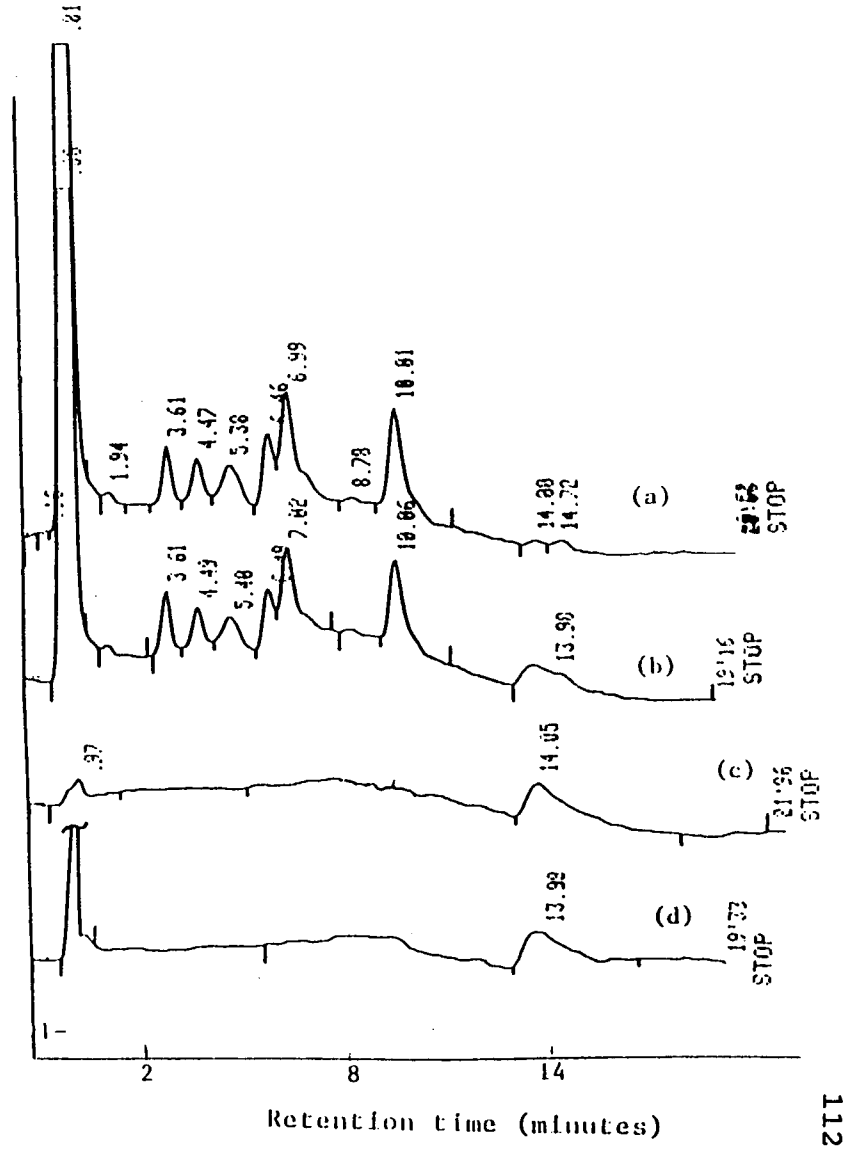


Figure 3.8 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 82% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.51

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-44880 + 200 ppm pentane sulfonate;

(b) Sample # 15-44880 + 100 ppm Ster-bac + 200 ppm pentane sulfonate;

(c) 20 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent ;

(d) 20 ppm benzyl dimethyldodecyl-ammonium chloride

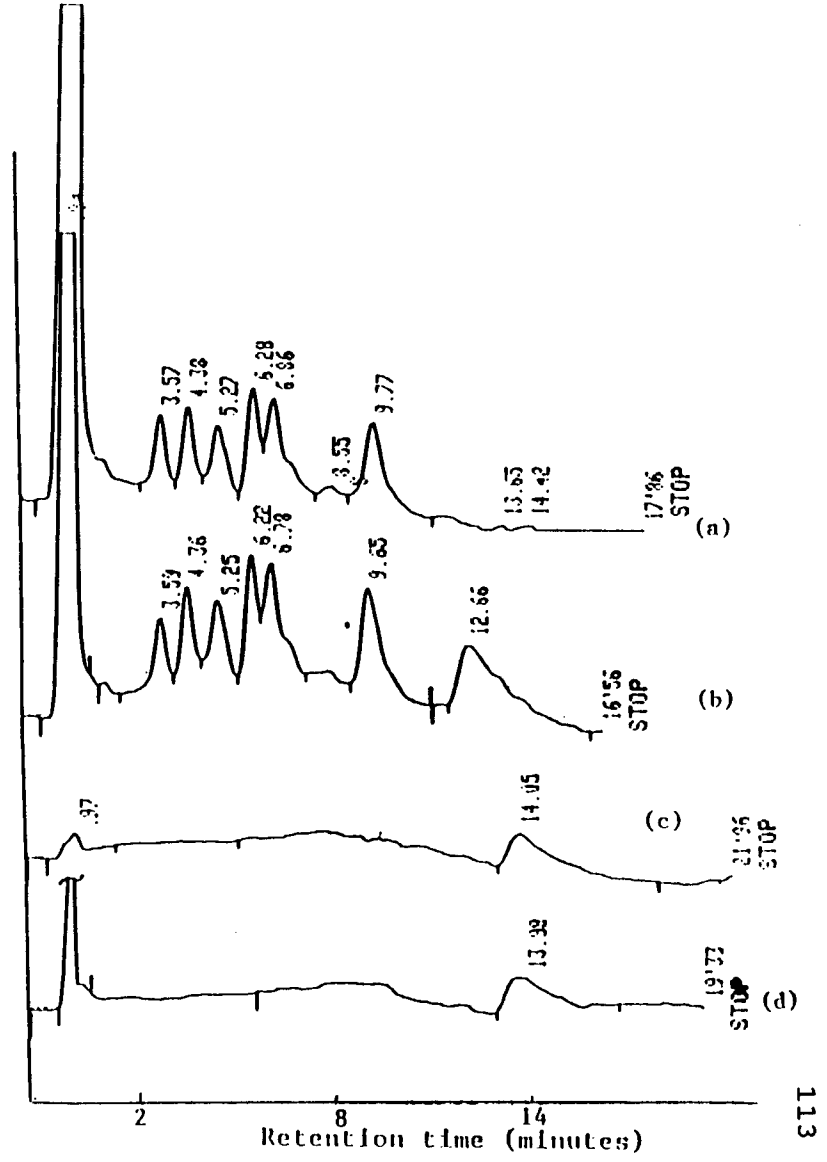


Figure 3.9 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 82% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.51

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15 - Manzi + 80 ppm pentane sulfonate;

(b) Sample # 15 - Manzi + 40 ppm Ster-bac + 80 ppm pentane sulfonate;

(c) 20 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent;

(d) 20 ppm benzyl dimethyldodecyl-ammonium chloride

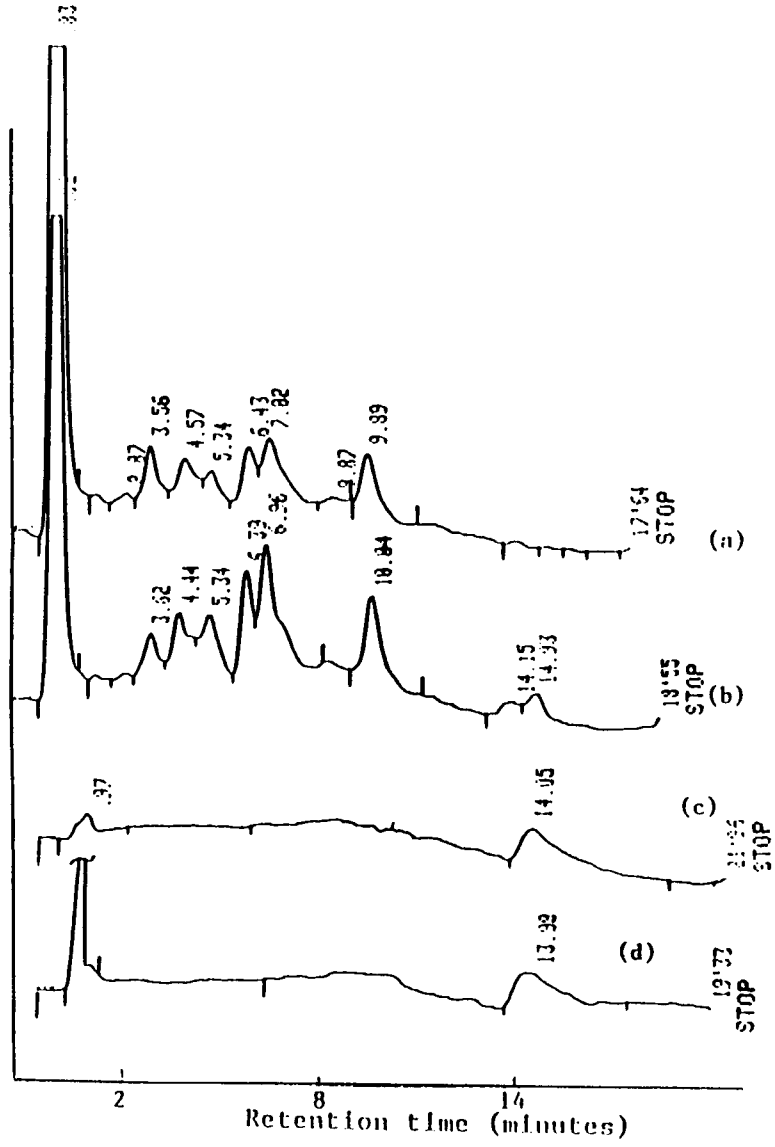


Figure 3.10 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 82% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.51

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # Miller's Dairy + 800 ppm pentane sulfonate;

(b) Sample # Miller's Dairy + 400 ppm Ster-bac + 800 ppm pentane sulfonate;

(c) 20 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent;

(d) 20 ppm benzyl dimethyldodecyl-ammonium chloride

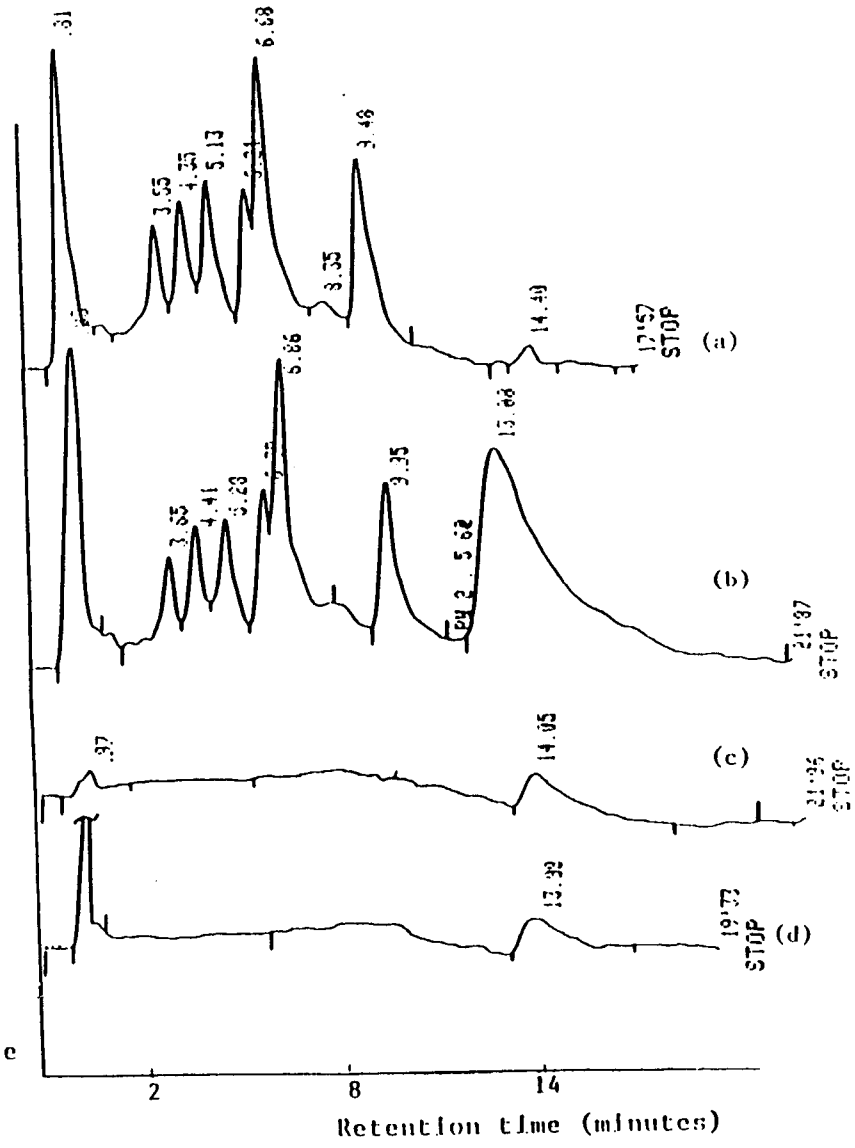


Figure 3.11 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 88% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.46

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-33345 + 240 ppm pentane sulfonate;

(b) Sample # 15-33345 + 120 ppm Ster-bac + 240 ppm pentane sulfonate;

(c) 80 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent

(d) 120 ppm benzyl dimethyltetradecyl-ammonium chloride and 120 ppm benzyl dimethyldodecyl-ammonium chloride

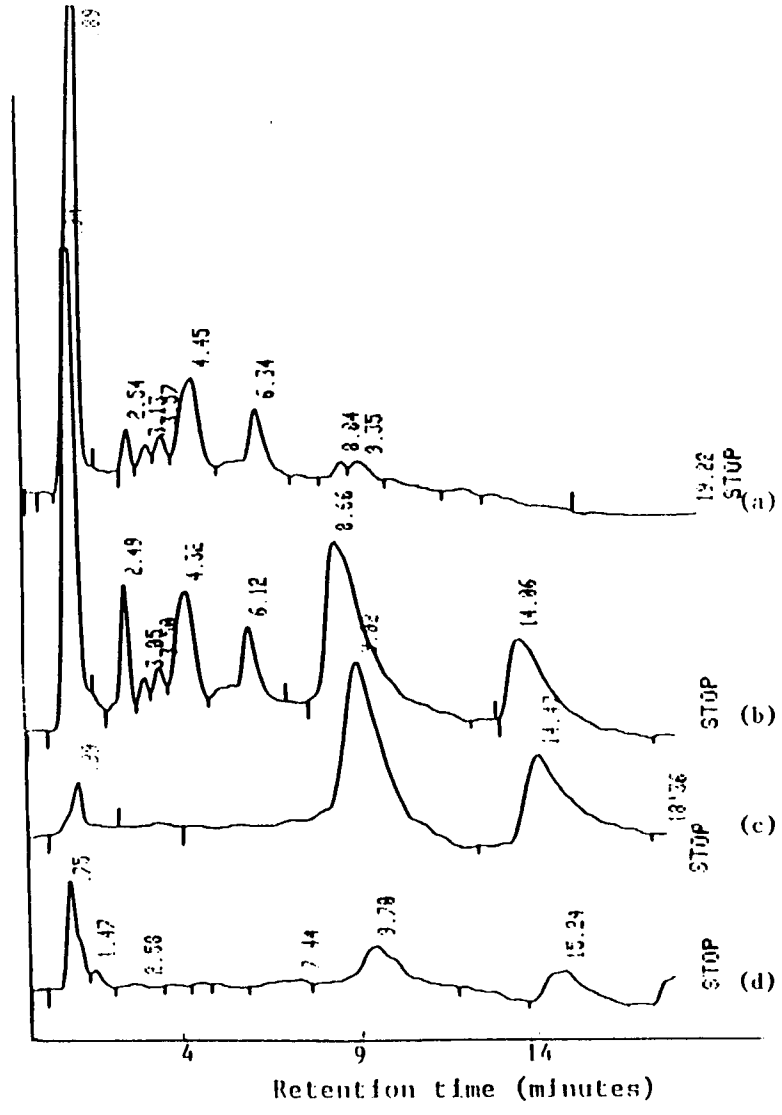


Figure 3.12 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-SIL ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 88% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.46

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-33548 + 240 ppm pentane sulfonate;

(b) Sample # 15-33548 + 120 ppm Ster-bac + 240 ppm pentane sulfonate;

(c) 80 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent

(d) 120 ppm benzyl dimethyltetradecyl-ammonium chloride and 120ppm benzyl dimethyldodecyl-ammonium chloride

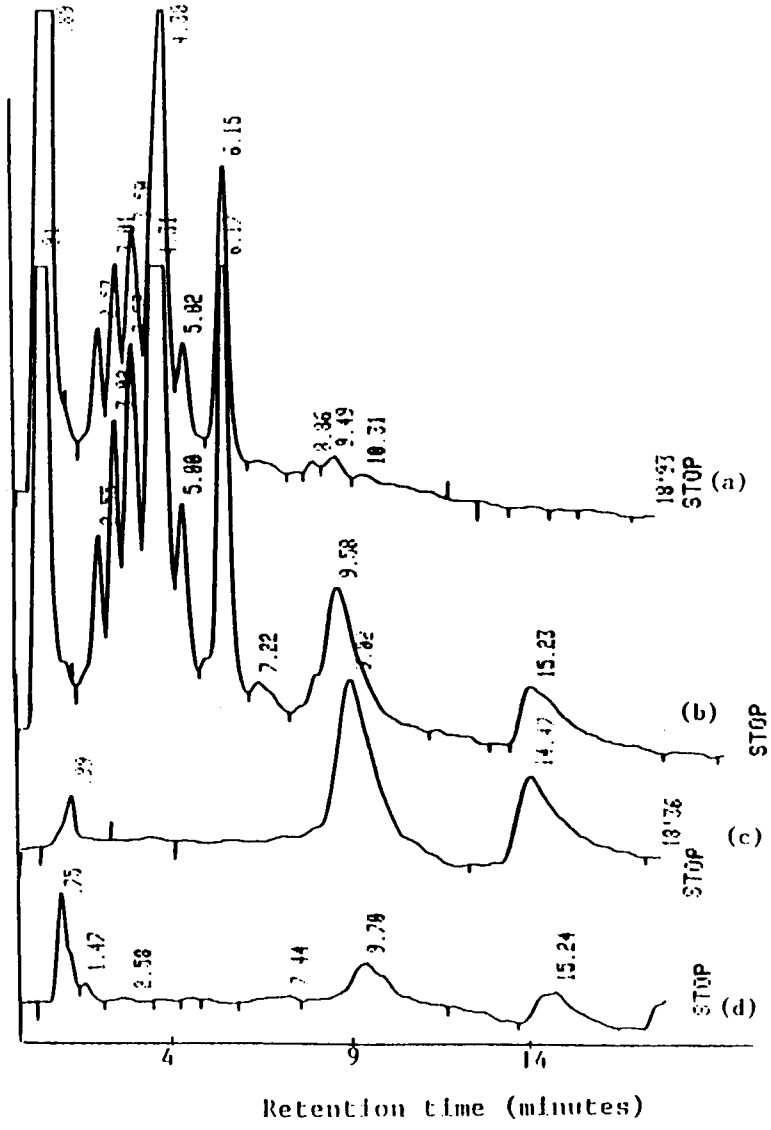


Figure 3.13 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 88% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.46

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-33605 + 160 ppm pentane sulfonate;

(b) Sample # 15-33605 + 80 ppm Ster-bac + 160 ppm pentane sulfonate;

(c) 80 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent;

(d) 120 ppm benzyl dimethyltetradecyl-ammonium chloride and 120 ppm benzyl dimethyldodecyl-ammonium chloride

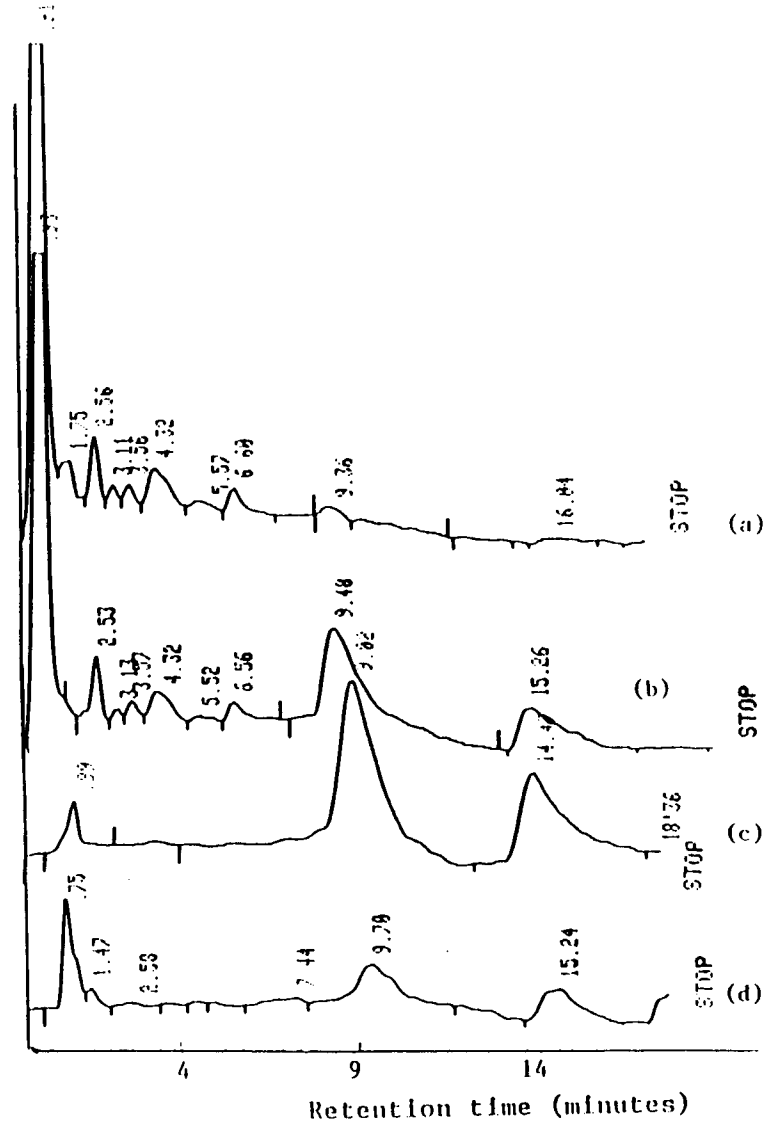


Figure 3.14 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 88% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.46

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-33779 + 200 ppm pentane sulfonate;

(b) Sample # 15-33779 + 100 ppm Ster-bac + 200 ppm pentane sulfonate;

(c) 80 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent;

(d) 120 ppm benzyl dimethyltetradecyl-ammonium chloride and 120 ppm benzyl dimethyldodecyl-ammonium chloride

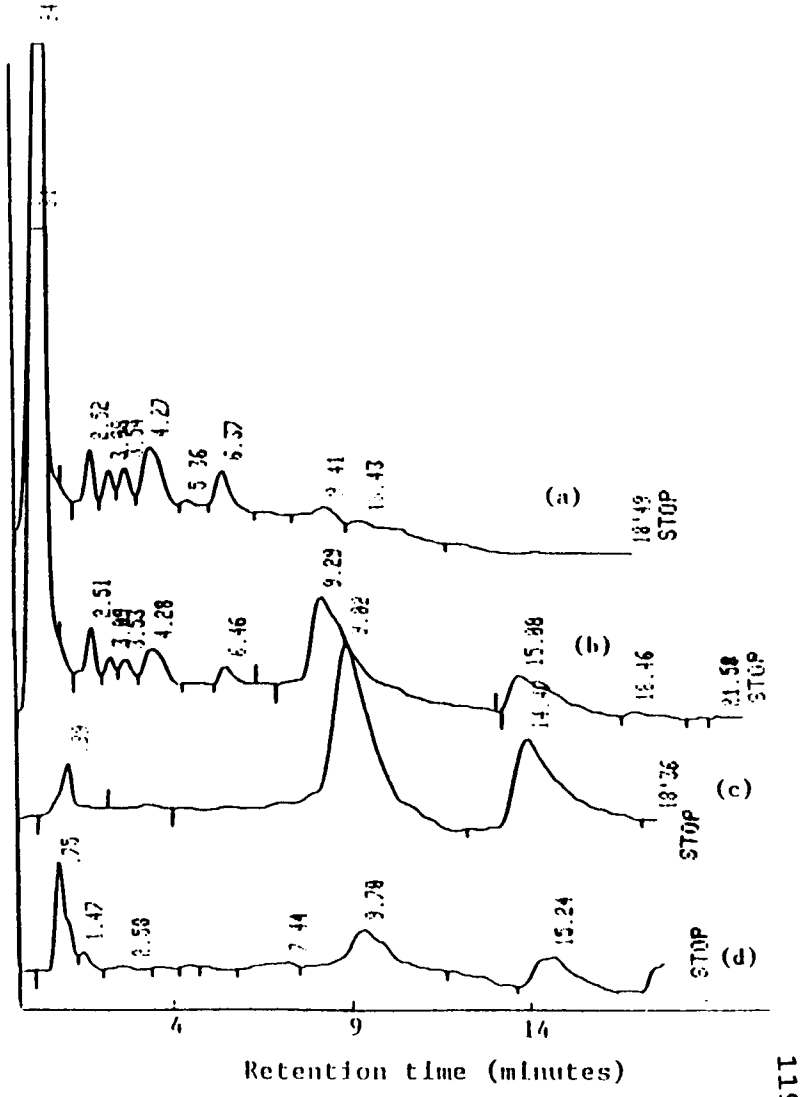


Figure 3.15 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 88% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.46

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-33886 + 160 ppm pentane sulfonate;

(b) Sample # 15-33886 + 80 ppm Ster-bac + 160 ppm pentane sulfonate;

(c) 80 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent;

(d) 120 ppm benzyl dimethyltetradecyl-ammonium chloride and 120 ppm benzyl dimethyldodecyl-ammonium chloride

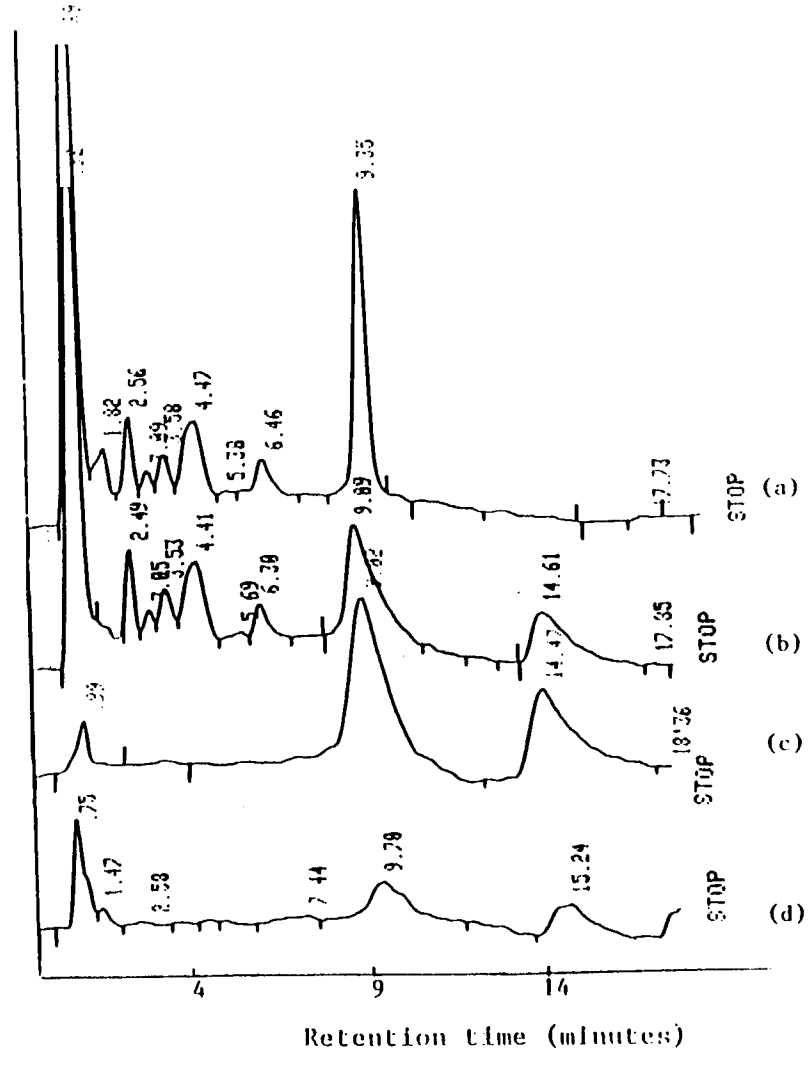


Figure 3.16 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 88% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.46

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-35451 + 160 ppm pentane sulfonate;

(b) Sample # 15-35451 + 80 ppm Ster-bac + 160 ppm pentane sulfonate;

(c) 80 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent ;

(d) 120 ppm benzyl dimethyltetradecyl-ammonium chloride and 120 ppm benzyl dimethyldodecyl-ammonium chloride

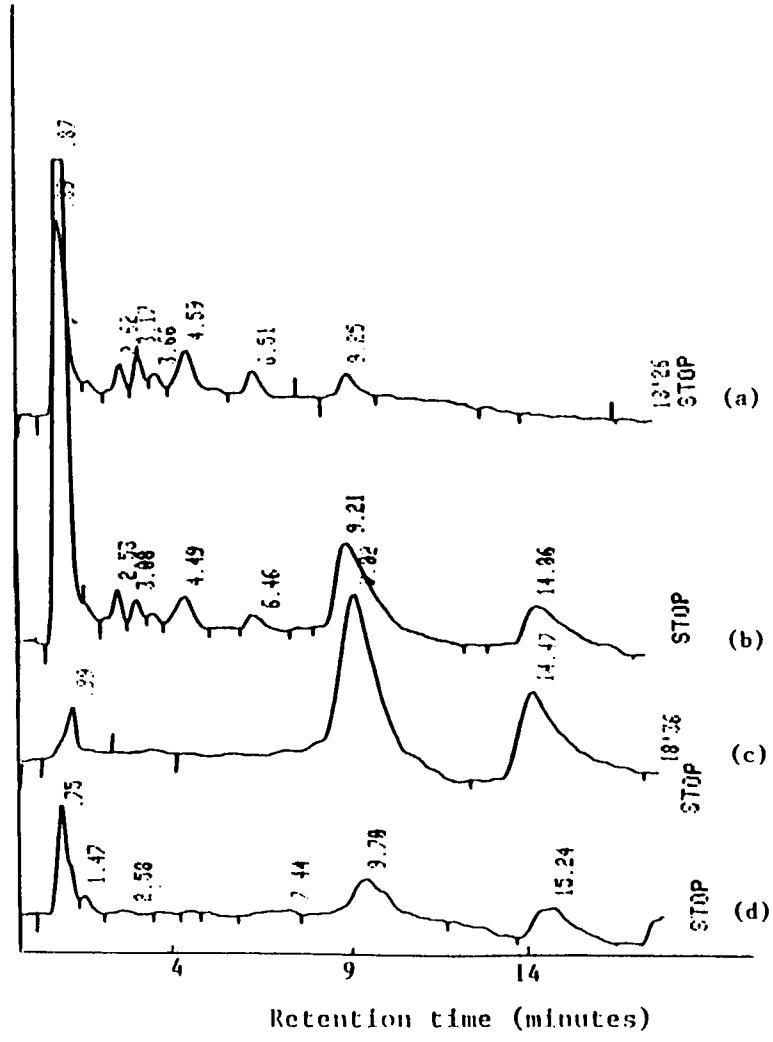


Figure 3.17 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 88% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.46

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-35469 + 160 ppm pentane sulfonate;

(b) Sample # 15-35469 + 80 ppm Ster-bac + 160 ppm pentane sulfonate;

(c) 80 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent;

(d) 120 ppm benzyl dimethyltetradecyl-ammonium chloride and 120 ppm benzyl dimethyldodecyl-ammonium chloride

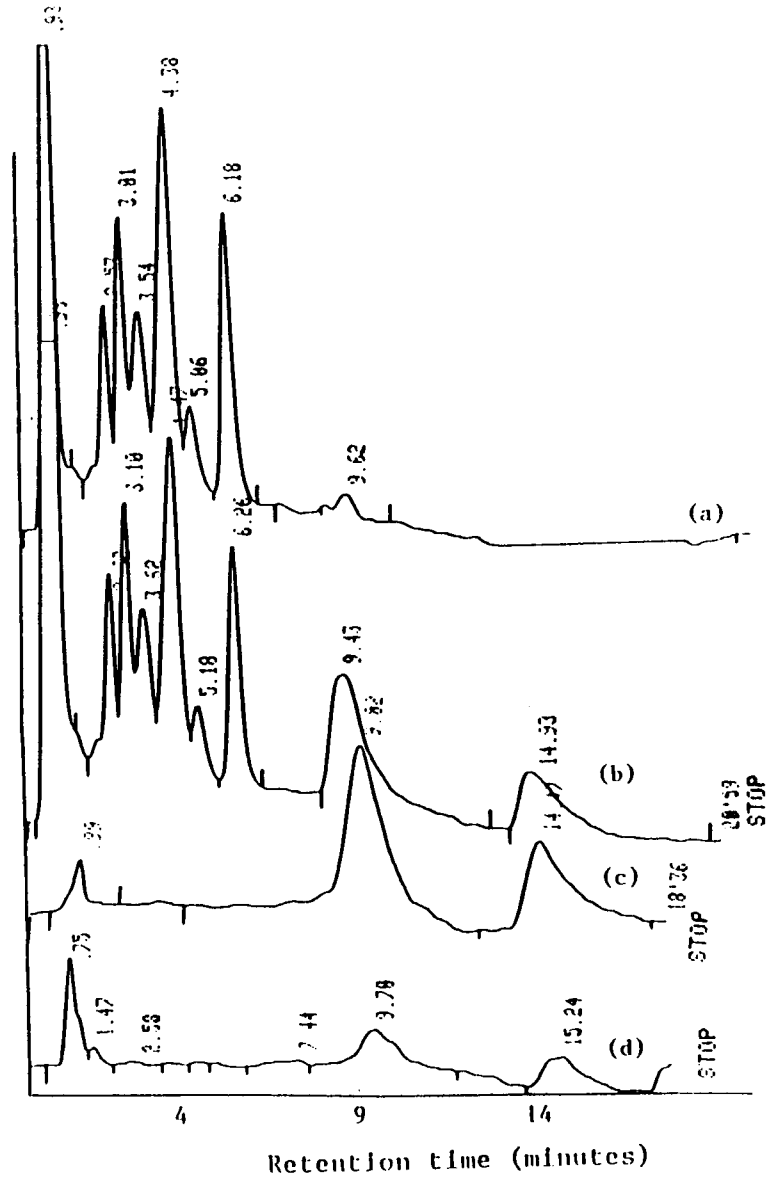


Figure 3.18 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 88% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.46

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-35477 + 160 ppm pentane sulfonate;

(b) Sample # 15-35477 + 80 ppm Ster-bac + 160 ppm pentane sulfonate;

(c) 80 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent ;

(d) 120 ppm benzyl dimethyltetradecyl-ammonium chloride and 120ppm benzyl dimethyldodecyl-ammonium chloride

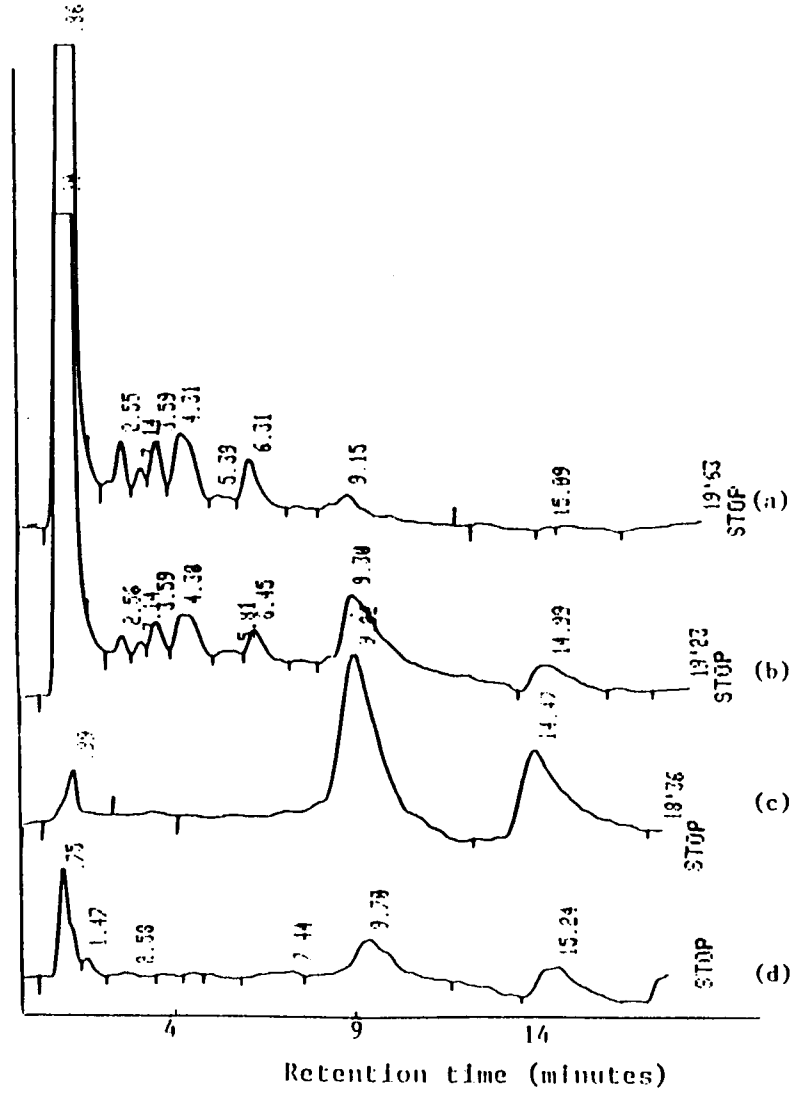


Figure 3.19 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 88% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.46

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-Manzi + 80 ppm pentane sulfonate;

(b) Sample # 15-Manzi + 40 ppm Ster-bac + 80 ppm pentane sulfonate;

(c) 80 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent ;

(d) 120 ppm benzyl dimethyltetradecyl-ammonium chloride and 120ppm benzyl dimethyldodecyl-ammonium chloride

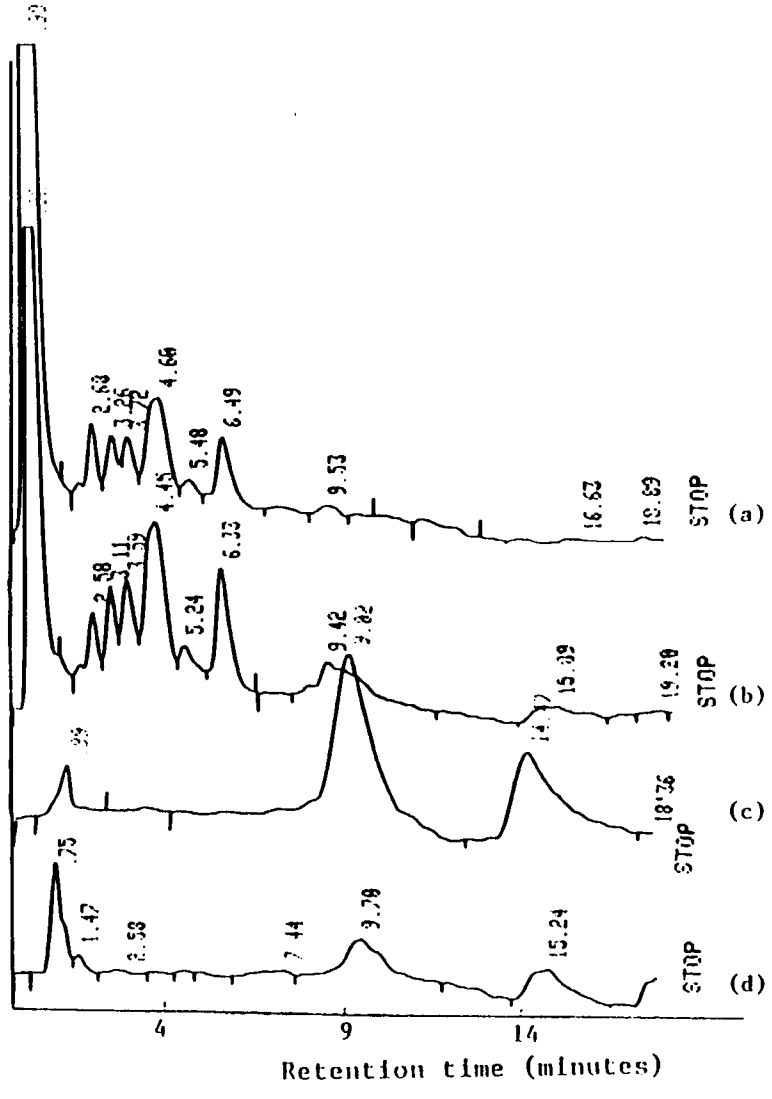


Figure 3.20 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 88% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.46

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

C H R O M A T O G R A M S

(a) Sample # 15-Platt + 160 ppm pentane sulfonate;

(b) Sample # 15-Platt + 80 ppm Ster-bac + 160 ppm pentane sulfonate;

(c) 80 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent ;

(d) 120 ppm benzyl dimethyltetradecyl-ammonium chloride and 120 ppm benzyl dimethyldodecyl-ammonium chloride

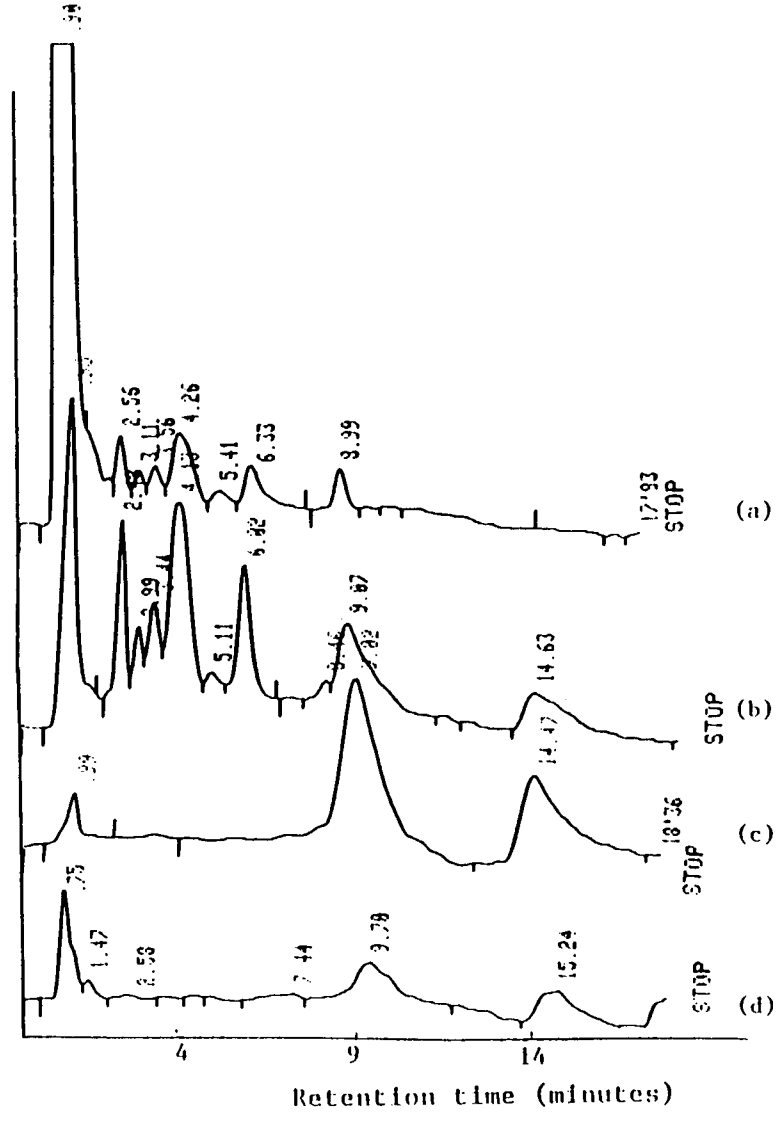


Figure 3.21 RP-HPLC analysis:

C O N D I T I O N S

COLUMN: Bio-Sil ODS-5S, 240 X 4.0 mm
ODS-5S Guard, C_{18}, 30 X 4.6 mm

ELUANT: 88% (V/V) acetonitrile in .1M sodium perchlorate/H_2O at pH = 2.46

FLOW RATE: 2 ml/minute up to 10 minutes then flow rate increased to 3 ml/minute

BACK PRESSURE: 1.9 - 2.8 X 1000 psi

TEMPERATURE: 25°C

DETECTION: UV @ 215 nm

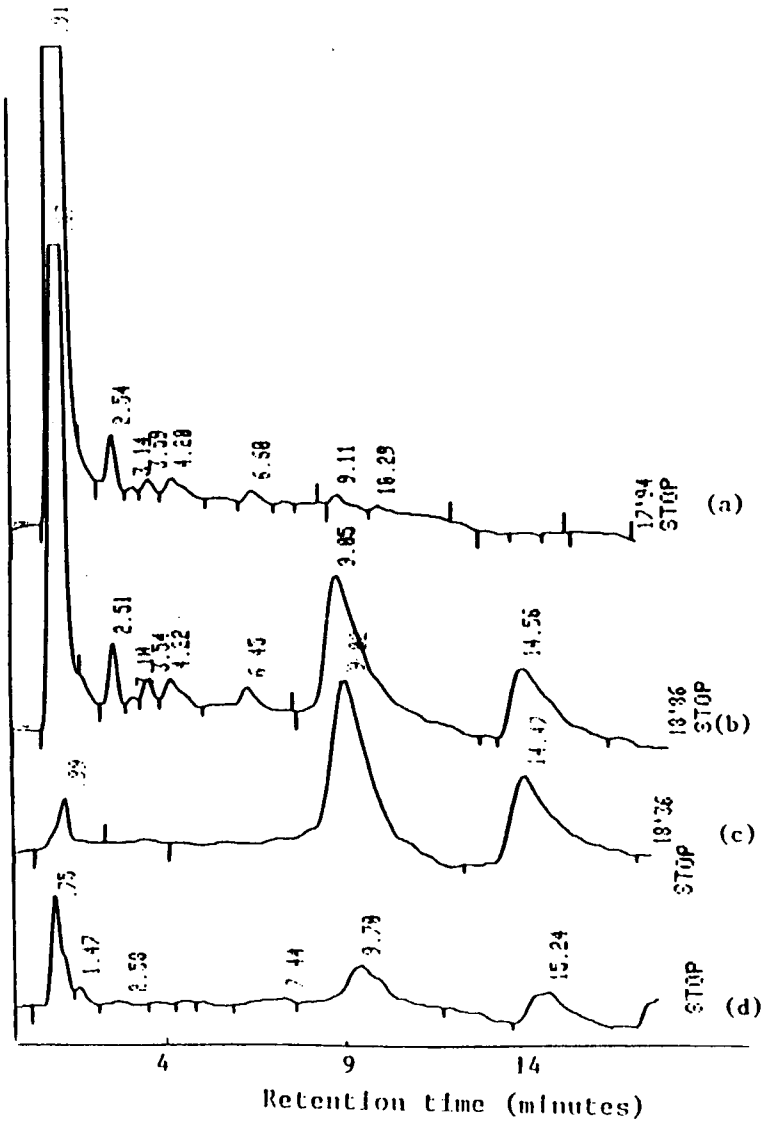
C H R O M A T O G R A M S

(a) Sample # 15-Silver Dome + 240 ppm pentane sulfonate;

(b) Sample # 15-Silver Dome + 120 ppm Ster-bac + 240 ppm pentane sulfonate;

(c) 80 ppm n-alkyl dimethyl benzyl ammonium chloride (ster-bac) in eluent ;

(d) 120 ppm benzyl dimethyltetradecyl-ammonium chloride and 120ppm benzyl dimethyldodecyl-ammonium chloride



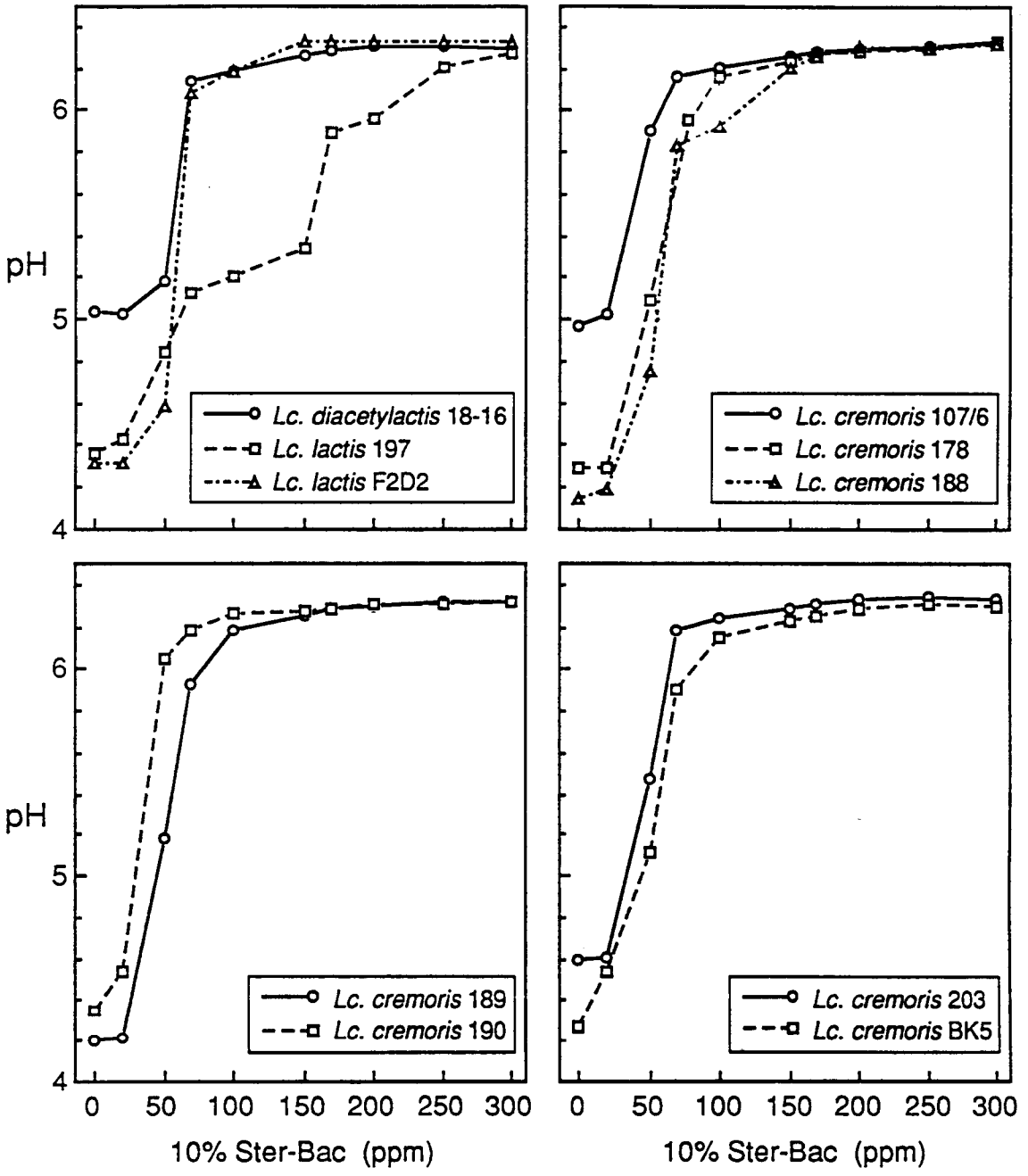


Figure 3.22 The effect of 10% ster-Bac {n-alkyl [50% C_{12}, 40% C_{14}, 10% C_{16}] dimethyl benzyl ammonium chloride} on the growth of various strains of lactic acid bacteria in 11% NFM at 30°C after 18 hr.

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APPENDIX

APPENDIX

INHIBITION ZONE (DIAMETER MEASURED TO THE NEAREST WHOLE MM) BY THE ACTION OF DIFFERENT ANTIBIOTICS

Table Ia: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus diacetylactis 18-16***											Date: 6.13 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 17 hrs; pH 6.83 (broth pH = 7.27) OD @600 = 0.293 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. 50% dilution made (10 ml culture + 10 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	12	12	12	12	12	11	11	11	11	12
Penicillin G	P-10	10 µg	28	27	28	27	27	25	26	26	27	27
Streptomycin	S-10	10 µg	0	0	0	0	0	0	0	0	0	0
Tobramycin	TM-10	10 µg	15	15	15	15	15	15	14	15	15	15
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	25	24	25	25	24	23	23	23	24	24
Chloramphenicol	C-30	30 µg	27	24	27	27	28	25	27	27	28	28
Chlortetracycline	A-30	30 µg	25	25	26	25	26	29	30	30	30	30
Clindamycin	CC-2	2 µg	25	24	25	24	24	23	24	24	24	23
Erythromycin	E-15	15 µg	26	25	25	25	25	24	25	25	25	25
Nitrofurantoin	FD-300	300 µg	21	20	21	22	21	19	18	18	18	19
Rifampin	RA-5	5 µg	11	11	11	11	11	11	11	12	14	14
Sulfathiazole	ST-300	300 µg	16	17	16	16	17	18	19	18	18	18
Tetracycline	TE-30	30 µg	30	30	29	30	29	29	29	29	29	29
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	22	22	21	21	22	22	22	22	21	22
Lincomycin	L-2	2 µg	18	19	18	18	18	18	18	18	19	19
Neomycin	N-5	5 µg	8	8	8	8	8	8	8	8	9	8

Table Ib. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus diacetylactis 18-16***						Date: 6.13.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 17 hrs; pH 6.83 (broth pH = 7.27) OD @600 = 0.293 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. 50% dilution made (10 ml culture + 10 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	12	11	12-12	11-12	≥17
Penicillin G	P-10	10 µg	27	26	27-28	25-27	≥28
Streptomycin	S-10	10 µg	0	0	0	0	≥15
Tobramycin	TM-10	10 µg	15	15	15-15	14-15	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	24	23	24-25	23-24	≥18
Chloramphenicol	C-30	30 µg	27	27	24-28	25-28	≥18
Chlortetracycline	A-30	30 µg	25	30	25-26	29-30	≥19*
Clindamycin	CC-2	2 µg	24	24	24-25	23-24	≥21
Erythromycin	E-15	15 µg	25	25	25-26	24-25	≥23
Nitrofurantoin	FD-300	300 µg	21	18	20-22	18-19	≥17
Rifampin	RA-5	5 µg	11	12	11-11	11-14	≥20
Sulfathiazole	ST-300	300 µg	16	18	16-17	18-19	≥17
Tetracycline	TE-30	30 µg	30	29	29-30	30-30	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	22	22	21-22	21-22	≥13*
Lincomycin	L-2	2 µg	18	18	18-19	18-19	≥21*
Neomycin	N-5	5 µg	8	8	8-08	8-09	≥17*

* From Difco antibiotic insert, 1989

Table IIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus diacetylactis 26-2***										Date: 6.13 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 17 hrs; pH 6.52 (broth pH = 7.27) OD @600 = 0.213 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. 50% dilution made (10 ml culture + 10 ml Mueller broth)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	18	17	18	17	18	14	14	14	14	14
Penicillin G	P-10	10 µg	25	25	24	27	27	21	21	21	21	21
Streptomycin	S-10	10 µg	11	12	12	12	12	9	10	9	9	9
Tobramycin	TM-10	10 µg	19	20	19	19	19	19	18	18	17	18
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	27	26	25	26	27	26	25	26	25	26
Chloramphenicol	C-30	30 µg	26	25	25	26	25	26	26	27	27	26
Chlortetracycline	A-30	30 µg	25	26	25	26	26	32	31	31	31	30
Clindamycin	CC-2	2 µg	24	24	25	25	25	25	25	26	25	25
Erythromycin	E-15	15 µg	25	27	26	27	27	26	25	26	26	26
Nitrofurantoin	FD-300	300 µg	18	19	18	18	18	22	22	22	21	22
Rifampin	RA-5	5 µg	12	13	12	12	13	14	14	13	13	13
Sulfathiazole	ST-300	300 µg	13	14	14	13	13	14	13	14	14	14
Tetracycline	TE-30	30 µg	29	29	30	29	29	31	30	29	29	29
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	25	25	25	26	25	25	26	25	26	26
Lincomycin	L-2	2 µg	17	18	18	18	17	18	18	18	18	17
Neomycin	N-5	5 µg	12	13	13	13	13	12	12	12	12	12

Table IIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus diacetylactis 26-2***						Date: 6.13.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 17 hrs; pH 6.52 (broth pH = 7.27) OD @600 = 0.213 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. 50% dilution made (10 ml culture + 10 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	18	14	17-18	14-14	≥17
Penicillin G	P-10	10 µg	26	21	24-27	21-21	≥28
Streptomycin	S-10	10 µg	12	9	11-12	9-10	≥15
Tobramycin	TM-10	10 µg	19	18	19-20	17-19	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	26	26	25-27	25-26	≥18
Chloramphenicol	C-30	30 µg	25	26	25-26	26-27	≥18
Chlortetracycline	A-30	30 µg	26	31	25-26	30-32	≥19*
Clindamycin	CC-2	2 µg	25	25	24-25	25-26	≥21
Erythromycin	E-15	15 µg	27	26	25-27	25-26	≥23
Nitrofurantoin	FD-300	300 µg	18	22	18-19	21-22	≥17
Rifampin	RA-5	5 µg	12	13	12-13	13-14	≥20
Sulfathiazole	ST-300	300 µg	13	14	13-14	13-14	≥17
Tetracycline	TE-30	30 µg	29	30	29-30	29-31	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	25	26	25-26	25-26	≥13*
Lincomycin	L-2	2 µg	18	18	17-18	17-18	≥21*
Neomycin	N-5	5 µg	13	12	12-13	12-12	≥17*

* From Difco antibiotic insert, 1989

Table IIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus lactis 01***										Date: 6.22 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 7.18 (broth pH = 7.27) OD @600 = 0.156 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. 50% dilution made (10 ml culture + 10 ml Mueller broth)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	12	13	12	13	13	12	12	12	12	11
Penicillin G	P-10	10 µg	29	30	30	30	30	30	30	30	29	29
Streptomycin	S-10	10 µg	8	8	8	8	9	8	8	8	8	8
Tobramycin	TM-10	10 µg	16	15	16	15	15	15	15	16	16	16
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	27	28	28	28	28	27	27	27	27	27
Chloramphenicol	C-30	30 µg	27	29	26	29	27	27	27	27	27	27
Chlortetracycline	A-30	30 µg	27	32	32	36	28	34	33	34	34	34
Clindamycin	CC-2	2 µg	25	24	26	25	24	24	24	24	24	24
Erythromycin	E-15	15 µg	27	28	26	26	26	27	27	27	27	27
Nitrofurantoin	FD-300	300 µg	19	21	19	19	18	21	21	21	21	20
Rifampin	RA-5	5 µg	14	14	14	13	13	15	14	15	13	15
Sulfathiazole	ST-300	300 µg	25	24	24	24	24	24	24	24	24	24
Tetracycline	TE-30	30 µg	31	31	31	31	31	31	31	31	31	30
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	26	26	26	26	25	27	27	27	26	26
Lincomycin	L-2	2 µg	18	18	18	17	17	17	16	16	16	16
Neomycin	N-5	5 µg	9	9	9	9	9	8	8	8	8	8

Table IIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus lactis 01***				Date: 6.22.89		
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 7.18 (broth pH = 7.27) OD @600 = 0.156 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. 50% dilution made (10 ml culture + 10 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	13	12	12-13	11-12	≥17
Penicillin G	P-10	10 µg	30	30	29-30	29-30	≥28
Streptomycin	S-10	10 µg	8	8	8-9	8-8	≥15
Tobramycin	TM-10	10 µg	15	16	15-16	15-16	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	28	27	27-28	27-27	≥18
Chloramphenicol	C-30	30 µg	28	27	26-29	27-27	≥18
Chlortetracycline	A-30	30 µg	33	34	28-37	33-34	≥19*
Clindamycin	CC-2	2 µg	25	24	24-26	24-24	≥21
Erythromycin	E-15	15 µg	27	27	26-28	27-27	≥23
Nitrofurantoin	FD-300	300 µg	19	21	19-21	20-21	≥17
Rifampin	RA-5	5 µg	14	14	13-14	13-15	≥20
Sulfathiazole	ST-300	300 µg	24	24	24-25	24-24	≥17
Tetracycline	TE-30	30 µg	31	31	31-31	30-31	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	26	27	25-26	26-27	≥13*
Lincomycin	L-2	2 µg	18	16	17-18	16-17	≥21*
Neomycin	N-5	5 µg	9	8	9-09	8-08	≥17*

* From Difco antibiotic insert, 1989

Table IVa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus lactis 197***										Date: 6.22 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 6.46 (broth pH = 7.27) OD @600 = 0.440 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. 50% dilution made (10 ml culture + 10 ml Mueller broth)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	15	15	16	15	15	15	15	14	15	15
Penicillin G	P-10	10 µg	28	28	28	27	28	25	24	27	26	25
Streptomycin	S-10	10 µg	11	12	13	12	12	9	9	9	9	9
Tobramycin	TM-10	10 µg	18	18	17	17	17	17	17	17	17	17
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	29	24	25	26	25	24	24	24	24	23
Chloramphenicol	C-30	30 µg	27	25	25	26	26	27	28	27	29	29
Chlortetracycline	A-30	30 µg	29	29	24	26	29	31	31	31	29	29
Clindamycin	CC-2	2 µg	21	21	21	21	21	21	21	21	21	21
Erythromycin	E-15	15 µg	26	26	25	26	26	26	26	26	26	26
Nitrofurantoin	FD-300	300 µg	20	20	21	19	20	22	19	19	19	19
Rifampin	RA-5	5 µg	12	13	12	12	12	13	13	13	13	13
Sulfathiazole	ST-300	300 µg	18	18	18	18	18	17	17	17	17	17
Tetracycline	TE-30	30 µg	28	29	26	28	28	30	30	30	30	30
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	24	25	25	25	26	26	26	26	26	25
Lincomycin	L-2	2 µg	12	15	14	13	13	11	11	11	11	11
Neomycin	N-5	5 µg	11	12	12	12	12	11	12	11	11	11

Table IVb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus lactis 197***						Date: 6.22.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 6.46 (broth pH = 7.27) OD @600 = 0.440 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. 50% dilution made (10 ml culture + 10 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	15	15	15-16	14-15	≥17
Penicillin G	P-10	10 µg	28	25	27-28	24-27	≥28
Streptomycin	S-10	10 µg	12	9	11-13	9-9	≥15
Tobramycin	TM-10	10 µg	17	17	17-18	17-17	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	26	24	24-29	23-24	≥18
Chloramphenicol	C-30	30 µg	26	28	25-27	27-29	≥18
Chlortetracycline	A-30	30 µg	27	30	24-29	29-31	≥19*
Clindamycin	CC-2	2 µg	21	21	21-21	21-21	≥21
Erythromycin	E-15	15 µg	26	26	25-26	26-26	≥23
Nitrofurantoin	FD-300	300 µg	20	20	19-21	19-22	≥17
Rifampin	RA-5	5 µg	12	13	12-13	13-13	≥20
Sulfathiazole	ST-300	300 µg	18	17	18-18	17-17	≥17
Tetracycline	TE-30	30 µg	28	30	26-29	30-30	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	25	26	24-26	25-26	13*
Lincomycin	L-2	2 µg	13	11	12-15	11-11	21*
Neomycin	N-5	5 µg	12	11	11-12	11-12	17*

* From Difco antibiotic insert, 1989

Table Va: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus lactis C2***										Date: 6.13 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 21 hrs; pH 7.04 (broth pH = 7.27) OD @600 = 0.261 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. No dilution made										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	15	14	14	14	14	14	14	14	15	14
Penicillin G	P-10	10 µg	26	27	28	28	27	25	26	26	27	26
Streptomycin	S-10	10 µg	11	11	11	11	10	9	9	9	9	9
Tobramycin	TM-10	10 µg	17	16	17	17	17	17	17	17	17	17
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	25	25	24	25	24	23	24	24	24	25
Chloramphenicol	C-30	30 µg	26	25	25	24	27	26	25	26	27	27
Chlortetracycline	A-30	30 µg	32	29	27	27	26	29	30	30	29	28
Clindamycin	CC-2	2 µg	21	21	21	21	21	21	21	21	21	21
Erythromycin	E-15	15 µg	25	25	25	26	26	25	25	26	25	26
Nitrofurantoin	FD-300	300 µg	19	19	20	21	18	19	21	19	19	20
Rifampin	RA-5	5 µg	12	13	13	13	13	13	14	15	13	13
Sulfathiazole	ST-300	300 µg	16	17	17	17	16	16	16	16	16	16
Tetracycline	TE-30	30 µg	29	28	29	29	29	29	29	29	29	28
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	24	25	24	23	24	25	25	26	26	26
Lincomycin	L-2	2 µg	12	12	14	12	13	11	12	11	11	11
Neomycin	N-5	5 µg	11	11	11	11	11	11	11	11	11	11

Table Vb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus lactis C2***					Date: 6.13.89	
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 21 hrs; pH 7.04 (broth pH = 7.27) OD @600 = 0.261 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm				
			Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989
			Swab	Overlay	Swab	Overlay	Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	14	14	14-15	14-15	≥17
Penicillin G	P-10	10 µg	27	26	26-28	25-27	≥28
Streptomycin	S-10	10 µg	11	9	10-11	9-9	≥15
Tobramycin	TM-10	10 µg	17	17	16-17	17-17	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	25	24	24-25	23-25	≥18
Chloramphenicol	C-30	30 µg	25	26	24-27	25-27	≥18
Chlortetracycline	A-30	30 µg	28	29	26-32	28-30	≥19*
Clindamycin	CC-2	2 µg	21	21	21-21	21-21	≥21
Erythromycin	E-15	15 µg	25	25	25-26	25-26	≥23
Nitrofurantoin	FD-300	300 µg	19	20	18-21	19-21	≥17
Rifampin	RA-5	5 µg	13	14	12-13	13-15	≥20
Sulfathiazole	ST-300	300 µg	17	16	16-17	16-16	≥17
Tetracycline	TE-30	30 µg	29	29	28-29	28-29	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	24	26	23-25	25-26	≥13*
Lincomycin	L-2	2 µg	13	11	12-14	11-12	≥21*
Neomycin	N-5	5 µg	11	11	11-11	11-11	≥17*

* From Difco antibiotic insert, 1989

Table VIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus lactis C10***										Date: 6.30 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 21 hrs; pH 6.89 (broth pH = 7.34) OD @600 = 0.260 Plates (made on 6/6/89) incubated at 30°C for 17 hrs. No dilution made										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	12	12	12	11	12	11	10	11	10	10
Penicillin G	P-10	10 µg	29	25	26	29	29	27	27	27	27	27
Streptomycin	S-10	10 µg	8	6	8	6	8	8	9	8	8	8
Tobramycin	TM-10	10 µg	14	14	14	15	15	14	14	14	15	15
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	27	26	27	28	27	29	27	27	29	29
Chloramphenicol	C-30	30 µg	28	27	28	26	28	27	27	26	26	26
Chlortetracycline	A-30	30 µg	25	25	25	30	22	31	32	31	31	31
Clindamycin	CC-2	2 µg	23	19	23	23	23	24	24	24	24	24
Erythromycin	E-15	15 µg	27	26	26	26	25	26	26	26	26	26
Nitrofurantoin	FD-300	300 µg	18	18	18	16	18	18	18	18	18	18
Rifampin	RA-5	5 µg	13	11	12	11	14	14	15	14	12	14
Sulfathiazole	ST-300	300 µg	19	19	19	20	19	18	19	18	18	19
Tetracycline	TE-30	30 µg	30	29	29	29	29	30	29	30	30	30
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	24	24	24	23	24	26	26	25	26	26
Lincomycin	L-2	2 µg	16	16	17	17	16	16	16	16	16	16
Neomycin	N-5	5 µg	8	8	9	8	9	8	8	7	8	8

Table VIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus lactis C10***					Date: 6.30.89	
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 21 hrs; pH 6.89 (broth pH = 7.34) OD @600 = 0.260 Plates (made on 6/6/89) incubated at 30°C for 17 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm				
			Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989
			Swab	Overlay	Swab	Overlay	Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	12	10	11-12	10-11	≥17
Penicillin G	P-10	10 µg	28	27	25-29	27-27	≥28
Streptomycin	S-10	10 µg	7	8	6-8	8-9	≥15
Tobramycin	TM-10	10 µg	14	14	14-15	14-15	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	27	28	26-28	27-29	≥18
Chloramphenicol	C-30	30 µg	27	26	26-28	26-27	≥18
Chlortetracycline	A-30	30 µg	25	31	22-30	31-32	≥19*
Clindamycin	CC-2	2 µg	22	24	19-23	24-24	≥21
Erythromycin	E-15	15 µg	26	26	25-27	26-26	≥23
Nitrofurantoin	FD-300	300 µg	18	18	16-18	18-18	≥17
Rifampin	RA-5	5 µg	12	14	11-14	12-14	≥20
Sulfathiazole	ST-300	300 µg	19	18	19-20	18-19	≥17
Tetracycline	TE-30	30 µg	29	30	29-30	29-30	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	24	26	23-24	25-26	≥13*
Lincomycin	L-2	2 µg	16	16	16-17	16-16	≥21*
Neomycin	N-5	5 µg	8	8-9	8	7-8	≥17*

* From Difco antibiotic insert, 1989

Table VIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus lactis F2 D2***										Date: 6.30 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 21 hrs; pH 7.04 (broth pH = 7.37) OD @600 = 0.262 Plates (made on 6/6/89) incubated at 30°C for 17 hrs. No dilution made										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	12	12	12	12	12	10	10	10	10	10
Penicillin G	P-10	10 µg	27	26	27	26	27	26	26	26	26	26
Streptomycin	S-10	10 µg	0	0	0	0	0	0	0	0	0	0
Tobramycin	TM-10	10 µg	14	14	14	14	15	13	13	13	13	13
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	27	27	30	27	27	27	27	26	27	27
Chloramphenicol	C-30	30 µg	27	27	30	27	27	27	27	27	27	27
Chlortetracycline	A-30	30 µg	29	28	29	27	29	27	30	30	30	30
Clindamycin	CC-2	2 µg	24	24	29	25	24	29	24	24	24	29
Erythromycin	E-15	15 µg	27	26	27	26	27	26	26	26	26	26
Nitrofurantoin	FD-300	300 µg	16	17	16	16	16	17	17	15	17	17
Rifampin	RA-5	5 µg	12	12	12	12	14	13	13	13	13	13
Sulfathiazole	ST-300	300 µg	16	16	16	15	16	16	16	16	16	16
Tetracycline	TE-30	30 µg	30	30	29	30	30	30	30	30	30	30
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	29	25	25	25	25	26	26	26	26	26
Lincomycin	L-2	2 µg	17	17	17	17	17	15	15	15	15	15
Neomycin	N-5	5 µg	0	0	0	0	0	0	0	0	0	0

Table VIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus lactis F2 D2***						Date: 6.30.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 21 hrs; pH 7.04 (broth pH = 7.37) OD @600 = 0.262 Plates (made on 6/6/89) incubated at 30°C for 17 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989 Susceptible
			Swab	Overlay	Swab	Overlay	
Primary Grouping:							
Amikacin	AN-30	30 µg	12	10	12-12	10-10	≥17
Penicillin G	P-10	10 µg	27	26	26-27	26-26	≥28
Streptomycin	S-10	10 µg	0	0	0	0	≥15
Tobramycin	TM-10	10 µg	14	13	14-15	13-13	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	28	27	27-30	26-27	≥18
Chloramphenicol	C-30	30 µg	28	27	27-30	27-27	≥18
Chlortetracycline	A-30	30 µg	28	28	27-29	27-30	≥19*
Clindamycin	CC-2	2 µg	25	26	24-29	24-29	≥21
Erythromycin	E-15	15 µg	27	26	26-27	26-26	≥23
Nitrofurantoin	FD-300	300 µg	16	16	16-17	15-17	≥17
Rifampin	RA-5	5 µg	13	13	12-14	13-13	≥20
Sulfathiazole	ST-300	300 µg	16	16	15-16	16-16	≥17
Tetracycline	TE-30	30 µg	30	30	29-30	30-30	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	26	26	25-29	26-26	≥13*
Lincomycin	L-2	2 µg	17	15	17-17	15-15	≥21*
Neomycin	N-5	5 µg	0	0	0	0	≥17*

* From Difco antibiotic insert, 1989

Table VIIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 00***											Date: 6.30 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 21 hrs; pH 7.34 (broth pH = 7.17) OD @600 = 0.034 Plates (made on 6/6/89) incubated at 30°C for 17 hrs. No dilution made (not good lawn)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	24	24	24	24	24	30	30	30	30	30
Penicillin G	P-10	10 µg	42	42	42	42	42	36	37	36	37	36
Streptomycin	S-10	10 µg	26	25	26	26	26	14	16	14	14	14
Tobramycin	TM-10	10 µg	30	30	30	30	30	23	25	24	25	25
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	38	38	36	38	36	40	41	40	40	40
Chloramphenicol	C-30	30 µg	36	36	34	36	34	41	43	41	41	42
Chlortetracycline	A-30	30 µg	40	40	38	40	40	41	40	41	40	40
Clindamycin	CC-2	2 µg	40	40	39	40	39	25	26	24	26	26
Erythromycin	E-15	15 µg	38	34	36	34	34	38	38	38	38	38
Nitrofurantoin	FD-300	300 µg	29	29	29	29	29	28	29	28	29	28
Rifampin	RA-5	5 µg	14	15	14	14	14	13	14	12	13	13
Sulfathiazole	ST-300	300 µg	30	30	30	30	30	24	24	23	24	23
Tetracycline	TE-30	30 µg	42	42	40	41	42	37	39	38	38	38
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	34	36	32	36	36	34	36	34	34	34
Lincomycin	L-2	2 µg	14	14	14	14	14	12	12	11	12	12
Neomycin	N-5	5 µg	16	16	15	15	16	15	15	15	14	15

Table VIIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 00***						Date: 6.30.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 21 hrs; pH 7.34 (broth pH = 7.17) OD @600 = 0.034 Plates (made on 6/6/89) incubated at 30°C for 17 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989 Susceptible
			Swab	Overlay	Swab	Overlay	
Primary Grouping:							
Amikacin	AN-30	30 µg	24	30	24-24	30-30	≥17
Penicillin G	P-10	10 µg	42	36	42-42	36-37	≥28
Streptomycin	S-10	10 µg	26	15	25-26	14-16	≥15
Tobramycin	TM-10	10 µg	30	24	30-30	23-25	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	37	40	36-38	40-41	≥18
Chloramphenicol	C-30	30 µg	35	42	34-36	41-43	≥18
Chlortetracycline	A-30	30 µg	40	40	38-40	40-41	≥19*
Clindamycin	CC-2	2 µg	40	25	39-40	24-26	≥21
Erythromycin	E-15	15 µg	35	38	34-38	38-38	≥23
Nitrofurantoin	FD-300	300 µg	29	28	29-29	28-29	≥17
Rifampin	RA-5	5 µg	14	13	14-15	12-14	≥20
Sulfathiazole	ST-300	300 µg	30	24	30-30	23-24	≥17
Tetracycline	TE-30	30 µg	41	38	40-42	37-39	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	35	34	32-36	34-36	≥13*
Lincomycin	L-2	2 µg	14	12	14-14	11-12	≥21*
Neomycin	N-5	5 µg	16	15	15-16	14-15	≥17*

* From Difco antibiotic insert, 1989

Table IXa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 107/6***										Date: 7.14 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 19.5 hrs; pH 4.06 (broth pH = 6.70) OD @600 = 0.648 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. 50% dilution made for overlay only (5 ml culture + 5 ml Mueller broth)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	20	19	21	21	22	21	20	20	19	21
Penicillin G	P-10	10 µg	33	32	31	31	31	29	31	31	31	32
Streptomycin	S-10	10 µg	18	17	17	17	18	17	18	17	18	17
Tobramycin	TM-10	10 µg	24	25	24	24	26	23	23	24	23	23
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	30	31	30	32	31	28	29	29	29	28
Chloramphenicol	C-30	30 µg	29	28	29	28	27	28	27	28	28	27
Chlortetracycline	A-30	30 µg	38	39	40	39	41	38	38	37	39	38
Clindamycin	CC-2	2 µg	28	29	28	28	27	27	27	30	27	27
Erythromycin	E-15	15 µg	30	31	30	30	30	28	27	27	28	28
Nitrofurantoin	FD-300	300 µg	32	31	32	31	31	33	32	31	31	31
Rifampin	RA-5	5 µg	17	17	18	19	18	17	17	15	16	17
Sulfathiazole	ST-300	300 µg	0	0	0	0	0	0	0	0	0	0
Tetracycline	TE-30	30 µg	36	35	36	37	35	36	36	36	34	36
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	28	28	27	28	28	27	26	26	26	26
Lincomycin	L-2	2 µg	18	19	19	18	19	17	18	18	18	18
Neomycin	N-5	5 µg	16	16	16	17	16	16	16	16	16	17

Table IXb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 107/6***						Date: 7.14.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 19.5 hrs; pH 4.06 (broth pH = 6.70) OD @600=0.648 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	21	20	19-22	19-21	≥17
Penicillin G	P-10	10 µg	32	31	31-33	29-32	≥28
Streptomycin	S-10	10 µg	17	17	17-18	17-18	≥15
Tobramycin	TM-10	10 µg	25	23	24-26	23-24	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	31	29	30-32	28-29	≥18
Chloramphenicol	C-30	30 µg	28	28	27-29	27-28	≥18
Chlortetracycline	A-30	30 µg	39	38	38-41	37-39	≥19*
Clindamycin	CC-2	2 µg	28	28	27-29	27-30	≥21
Erythromycin	E-15	15 µg	30	28	30-31	27-28	≥23
Nitrofurantoin	FD-300	300 µg	31	32	31-32	31-33	≥17
Rifampin	RA-5	5 µg	18	16	17-19	15-17	≥20
Sulfathiazole	ST-300	300 µg	0	0	0	0	≥17
Tetracycline	TE-30	30 µg	36	35	35-37	34-36	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	28	26	27-28	26-27	≥13*
Lincomycin	L-2	2 µg	19	18	18-19	17-18	≥21*
Neomycin	N-5	5 µg	16	16	16-17	16-17	≥17*

* From Difco antibiotic insert, 1989

Table Xa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 163***											Date: 7.12 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 17.5 hrs; pH 4.18 (broth pH = 6.73) OD @600 = 0.627 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	23	24	24	25	24	20	21	22	22	22
Penicillin G	P-10	10 µg	33	33	33	32	34	24	28	27	27	27
Streptomycin	S-10	10 µg	18	19	17	18	19	17	17	16	17	16
Tobramycin	TM-10	10 µg	23	23	22	22	22	21	22	21	21	21
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	29	30	30	29	29	27	26	26	26	25
Chloramphenicol	C-30	30 µg	30	29	28	30	29	25	23	26	27	26
Chlortetracycline	A-30	30 µg	31	33	31	31	31	31	31	30	31	31
Clindamycin	CC-2	2 µg	29	29	29	28	26	23	24	23	23	23
Erythromycin	E-15	15 µg	29	29	29	29	30	25	26	25	25	24
Nitrofurantoin	FD-300	300 µg	0	0	0	0	0	0	0	0	0	0
Rifampin	RA-5	5 µg	13	13	13	12	12	12	14	14	13	14
Sulfathiazole	ST-300	300 µg	13	12	13	14	13	12	12	12	12	11
Tetracycline	TE-30	30 µg	30	30	31	31	31	30	29	30	29	29
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	30	30	32	32	33	26	26	25	26	26
Lincomycin	L-2	2 µg	19	17	17	19	19	17	17	17	17	17
Neomycin	N-5	5 µg	17	16	16	17	17	16	16	16	15	16

Table Xb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 163***						Date: 7.12.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 17.5 hrs; pH 4.18 (broth pH = 6.73) OD @600=0.627 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	24	22	23-25	20-22	≥17
Penicillin G	P-10	10 µg	33	27	32-34	24-28	≥28
Streptomycin	S-10	10 µg	18	17	17-19	16-17	≥15
Tobramycin	TM-10	10 µg	22	21	22-23	21-22	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	29	26	29-30	25-27	≥18
Chloramphenicol	C-30	30 µg	29	25	28-30	23-27	≥18
Chlortetracycline	A-30	30 µg	32	31	31-33	30-31	≥19*
Clindamycin	CC-2	2 µg	28	23	26-29	23-24	≥21
Erythromycin	E-15	15 µg	29	25	29-30	24-26	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	13	13	12-13	12-14	≥20
Sulfathiazole	ST-300	300 µg	13	12	12-14	11-12	≥17
Tetracycline	TE-30	30 µg	31	29	30-31	29-30	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	31	26	30-33	25-26	≥13*
Lincomycin	L-2	2 µg	18	17	17-19	17-17	≥21*
Neomycin	N-5	5 µg	17	16	16-17	15-16	≥17*

* From Difco antibiotic insert, 1989

Table XIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 178***										Date: 7.31 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.05 (broth pH = 6.73) OD @600 = 0.675 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. No dilution made										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm								
			Trials								
			Swab					Overlay			
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	18	18	21	17	18	15	15	15	16	15
Penicillin G	P-10	10 µg	30	30	30	28	28	25	26	26	26	26
Streptomycin	S-10	10 µg	15	14	15	15	14	11	11	10	10	10
Tobramycin	TM-10	10 µg	20	20	20	19	20	16	16	16	17	17
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	25	23	25	25	23	24	24	24	24	24
Chloramphenicol	C-30	30 µg	27	28	28	26	26	25	25	25	25	25
Chlortetracycline	A-30	30 µg	29	29	29	30	29	29	29	29	28	29
Clindamycin	CC-2	2 µg	23	22	22	22	22	22	22	22	22	22
Erythromycin	E-15	15 µg	25	24	24	25	25	25	25	25	25	25
Nitrofurantoin	FD-300	300 µg	12	11	12	11	12	12	12	12	12	12
Rifampin	RA-5	5 µg	15	14	16	15	15	15	15	15	15	17
Sulfathiazole	ST-300	300 µg	14	15	15	15	16	16	15	15	16	15
Tetracycline	TE-30	30 µg	29	29	29	28	28	29	29	29	28	29
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	26	25	25	25	25	25	25	25	25	25
Lincomycin	L-2	2 µg	15	14	15	14	14	12	12	12	13	12
Neomycin	N-5	5 µg	15	15	15	14	15	11	12	12	12	12

Table XIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 178***						Date: 7.31.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.05 (broth pH = 6.73) OD @600 = 0.675 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	18	15	18-21	15-16	≥17
Penicillin G	P-10	10 µg	29	26	28-30	25-26	≥28
Streptomycin	S-10	10 µg	15	10	14-15	10-11	≥15
Tobramycin	TM-10	10 µg	20	16	19-20	16-17	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	24	24	23-25	24-24	≥18
Chloramphenicol	C-30	30 µg	27	25	26-28	25-25	≥18
Chlortetracycline	A-30	30 µg	29	29	29-30	28-30	≥19*
Clindamycin	CC-2	2 µg	22	22	22-23	22-22	≥21
Erythromycin	E-15	15 µg	25	25	24-25	25-25	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	15	15	14-16	15-17	≥20
Sulfathiazole	ST-300	300 µg	15	15	14-16	15-16	≥17
Tetracycline	TE-30	30 µg	29	29	28-29	28-29	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	25	25	25-26	25-25	≥13*
Lincomycin	L-2	2 µg	14	12	14-15	12-13	≥21*
Neomycin	N-5	5 µg	15	12	14-15	11-12	≥17*

* From Difco antibiotic insert, 1989

Table XIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 187***										Date: 7.10 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 16 hrs; pH 4.11 (broth pH = 6.71) OD @600 = 0.638 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	26	25	25	26	25	21	21	22	21	21
Penicillin G	P-10	10 µg	31	30	32	32	31	31	30	31	30	31
Streptomycin	S-10	10 µg	20	21	18	20	19	20	18	18	19	18
Tobramycin	TM-10	10 µg	23	24	23	24	24	23	23	23	23	25
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	29	28	29	28	27	28	27	27	28	27
Chloramphenicol	C-30	30 µg	27	28	29	29	28	28	27	28	30	28
Chlortetracycline	A-30	30 µg	32	33	32	34	32	28	32	32	32	32
Clindamycin	CC-2	2 µg	26	25	26	26	25	25	24	26	25	25
Erythromycin	E-15	15 µg	30	31	29	29	30	27	27	27	28	29
Nitrofurantoin	FD-300	300 µg	22	23	21	22	23	21	21	21	20	21
Rifampin	RA-5	5 µg	13	14	13	12	14	14	14	14	14	14
Sulfathiazole	ST-300	300 µg	18	19	18	17	18	17	17	17	17	17
Tetracycline	TE-30	30 µg	32	32	31	32	33	33	31	31	33	33
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	28	28	28	29	29	28	28	28	28	28
Lincomycin	L-2	2 µg	18	18	18	18	18	18	18	18	18	18
Neomycin	N-5	5 µg	17	17	17	17	17	17	17	17	17	17

Table XIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 187***						Date: 7.10.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 161 hrs; pH 4.11 (broth pH = 6.71) OD @600 = 0.638 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	25	21	25-26	21-22	≥17
Penicillin G	P-10	10 µg	31	31	30-32	30-31	≥28
Streptomycin	S-10	10 µg	20	19	18-20	18-20	≥15
Tobramycin	TM-10	10 µg	24	23	23-24	23-25	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	28	27	27-29	27-28	≥18
Chloramphenicol	C-30	30 µg	28	28	27-29	27-30	≥18
Chlortetracycline	A-30	30 µg	33	31	32-34	28-32	≥19*
Clindamycin	CC-2	2 µg	26	25	25-26	24-26	≥21
Erythromycin	E-15	15 µg	30	28	29-31	27-29	≥23
Nitrofurantoin	FD-300	300 µg	22	21	21-23	20-21	≥17
Rifampin	RA-5	5 µg	13	14	12-14	14-14	≥20
Sulfathiazole	ST-300	300 µg	18	17	17-19	17-17	≥17
Tetracycline	TE-30	30 µg	32	32	31-33	31-33	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	28	28	28-29	28-28	≥13*
Lincomycin	L-2	2 µg	18	18	18-18	18-18	≥21*
Neomycin	N-5	5 µg	17	17	17-17	17-17	≥17*

* From Difco antibiotic insert, 1989

Table XIIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 188***										Date: 7.10 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 16 hrs; pH 4.11 (broth pH = 6.71) OD @600 = 0.750 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	26	26	26	26	27	21	21	21	22	22
Penicillin G	P-10	10 µg	36	37	36	37	37	33	33	33	33	32
Streptomycin	S-10	10 µg	20	21	20	21	21	19	21	19	21	20
Tobramycin	TM-10	10 µg	27	26	28	28	28	23	23	22	23	23
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	38	38	37	38	38	31	31	31	31	30
Chloramphenicol	C-30	30 µg	30	30	30	29	29	28	28	28	27	29
Chlortetracycline	A-30	30 µg	34	34	34	34	32	33	33	33	33	33
Clindamycin	CC-2	2 µg	29	30	29	28	29	24	24	24	24	25
Erythromycin	E-15	15 µg	32	32	32	30	31	27	27	27	27	28
Nitrofurantoin	FD-300	300 µg	0	0	0	0	0	0	0	0	0	0
Rifampin	RA-5	5 µg	14	13	13	13	13	14	13	13	13	14
Sulfathiazole	ST-300	300 µg	14	14	14	14	14	17	13	15	17	15
Tetracycline	TE-30	30 µg	36	32	34	35	35	32	32	35	32	32
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	33	33	33	33	33	28	28	28	28	27
Lincomycin	L-2	2 µg	14	14	14	14	14	15	14	15	15	14
Neomycin	N-5	5 µg	18	14	18	18	18	16	16	16	16	16

Table XIIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 188***					Date: 7.10.89	
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 16 hrs; pH 4.11 (broth pH = 6.71) OD @600 = 0.750 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989
			Swab	Overlay	Swab	Overlay	Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	26	21	26-27	21-22	≥17
Penicillin G	P-10	10 µg	37	33	36-37	32-33	≥28
Streptomycin	S-10	10 µg	21	20	20-21	19-21	≥15
Tobramycin	TM-10	10 µg	27	23	26-28	22-23	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	38	31	37-38	30-31	≥18
Chloramphenicol	C-30	30 µg	30	28	29-30	27-29	≥18
Chlortetracycline	A-30	30 µg	34	33	32-34	33-33	≥19*
Clindamycin	CC-2	2 µg	29	24	28-30	24-25	≥21
Erythromycin	E-15	15 µg	31	27	30-32	27-28	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	13	13	13-14	13-14	≥20
Sulfathiazole	ST-300	300 µg	14	16	14-14	13-17	≥17
Tetracycline	TE-30	30 µg	34	32	32-36	32-33	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	33	28	33-33	27-28	≥13*
Lincomycin	L-2	2 µg	14	15	14-14	14-15	≥21*
Neomycin	N-5	5 µg	17	16	14-18	16-16	≥17*

* From Difco antibiotic insert, 1989

Table XIVa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 189***											Date: 7.10 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 16 hrs; pH 4.18 (broth pH = 6.71) OD @600 = 0.552 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	26	26	25	26	26	21	21	21	20	20
Penicillin G	P-10	10 µg	34	34	34	34	34	27	28	28	27	28
Streptomycin	S-10	10 µg	17	17	17	17	18	18	19	19	18	18
Tobramycin	TM-10	10 µg	24	23	23	23	23	23	23	23	22	23
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	28	28	28	28	28	27	24	27	27	28
Chloramphenicol	C-30	30 µg	30	30	30	34	30	29	27	27	29	28
Chlortetracycline	A-30	30 µg	36	36	36	25	36	29	32	33	32	32
Clindamycin	CC-2	2 µg	32	33	32	32	31	26	25	26	26	26
Erythromycin	E-15	15 µg	35	32	32	32	32	28	27	27	28	28
Nitrofurantoin	FD-300	300 µg	0	0	0	0	0	0	0	0	0	0
Rifampin	RA-5	5 µg	13	13	13	13	13	13	13	13	13	13
Sulfathiazole	ST-300	300 µg	14	14	14	14	14	14	14	15	14	14
Tetracycline	TE-30	30 µg	32	30	32	32	31	32	31	31	32	32
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	31	31	29	30	30	29	28	28	29	28
Lincomycin	L-2	2 µg	17	16	16	17	16	18	18	19	18	18
Neomycin	N-5	5 µg	17	17	17	17	17	16	17	16	16	16

Table XIVb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 189***						Date: 7.10.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 16 hrs; pH 4.18 (broth pH = 6.71) OD @600 = 0.552 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	26	21	25-26	20-21	≥17
Penicillin G	P-10	10 µg	34	28	34-34	27-28	≥28
Streptomycin	S-10	10 µg	17	18	17-18	18-19	≥15
Tobramycin	TM-10	10 µg	23	23	23-24	22-23	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	28	27	28-28	24-28	≥18
Chloramphenicol	C-30	30 µg	31	28	30-34	27-30	≥18
Chlortetracycline	A-30	30 µg	36	32	35-36	29-33	≥19*
Clindamycin	CC-2	2 µg	32	26	31-33	25-26	≥21
Erythromycin	E-15	15 µg	33	28	32-35	27-28	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	13	13	13-13	13-13	≥20
Sulfathiazole	ST-300	300 µg	14	14	14-14	14-15	≥17
Tetracycline	TE-30	30 µg	31	32	30-32	31-32	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	30	28	29-31	28-29	≥13*
Lincomycin	L-2	2 µg	16	18	16-17	18-19	≥21*
Neomycin	N-5	5 µg	17	16	17-17	16-17	≥17*

* From Difco antibiotic insert, 1989

Table XVa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 190***											Date: 7.12 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 17.5 hrs; pH 4.23 (broth pH = 6.73) OD @600 = 0.715 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	20	20	19	20	20	20	20	21	20	20
Penicillin G	P-10	10 µg	32	31	33	33	33	30	31	32	31	31
Streptomycin	S-10	10 µg	16	15	16	16	17	16	17	16	16	16
Tobramycin	TM-10	10 µg	22	21	22	21	23	22	22	22	22	22
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	34	33	33	34	33	31	31	31	32	31
Chloramphenicol	C-30	30 µg	34	34	34	35	34	30	31	31	31	31
Chlortetracycline	A-30	30 µg	34	34	34	33	34	33	33	33	32	33
Clindamycin	CC-2	2 µg	29	29	30	30	30	28	28	28	28	28
Erythromycin	E-15	15 µg	28	28	28	29	29	28	28	27	28	28
Nitrofurantoin	FD-300	300 µg	16	17	16	16	16	17	17	17	16	17
Rifampin	RA-5	5 µg	15	16	16	14	17	16	16	16	16	16
Sulfathiazole	ST-300	300 µg	17	18	17	17	16	16	16	16	17	17
Tetracycline	TE-30	30 µg	31	32	32	31	32	32	32	32	32	31
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	29	29	29	30	29	26	26	26	26	26
Lincomycin	L-2	2 µg	20	20	20	20	22	20	20	20	21	20
Neomycin	N-5	5 µg	16	16	16	17	16	16	16	16	16	16

Table XVb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 190***						Date: 7.12.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 17.5 hrs; pH 4.23 (broth pH = 6.73) OD @600=0.715 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	20	20	19-20	20-21	≥17
Penicillin G	P-10	10 µg	32	31	31-33	30-32	≥28
Streptomycin	S-10	10 µg	16	16	15-17	16-17	≥15
Tobramycin	TM-10	10 µg	22	22	21-23	22-22	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	33	31	33-34	31-32	≥18
Chloramphenicol	C-30	30 µg	34	31	34-35	30-31	≥18
Chlortetracycline	A-30	30 µg	34	33	33-34	32-33	≥19*
Clindamycin	CC-2	2 µg	30	28	29-30	28-28	≥21
Erythromycin	E-15	15 µg	28	28	28-29	27-28	≥23
Nitrofurantoin	FD-300	300 µg	16	17	16-17	16-17	≥17
Rifampin	RA-5	5 µg	16	16	14-17	16-16	≥20
Sulfathiazole	ST-300	300 µg	17	16	16-18	16-17	≥17
Tetracycline	TE-30	30 µg	32	32	31-32	31-32	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	29	26	29-30	26-26	≥13*
Lincomycin	L-2	2 µg	20	20	20-22	20-21	≥21*
Neomycin	N-5	5 µg	16	16	16-17	16-16	≥17*

* From Difco antibiotic insert, 1989

Table XVIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 196***										Date: 7.10 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 16 hrs; pH 4.27 (broth pH = 6.71) OD @600 = 0.597 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	20	20	19	22	22	20	21	20	20	20
Penicillin G	P-10	10 µg	25	25	25	25	25	26	27	25	26	26
Streptomycin	S-10	10 µg	17	17	17	17	17	17	17	17	17	17
Tobramycin	TM-10	10 µg	24	25	24	24	24	22	22	22	22	22
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	26	26	26	26	27	26	25	26	27	27
Chloramphenicol	C-30	30 µg	27	27	27	27	29	27	27	27	27	27
Chlortetracycline	A-30	30 µg	30	31	31	31	31	31	32	30	31	31
Clindamycin	CC-2	2 µg	21	22	22	23	23	22	21	21	21	22
Erythromycin	E-15	15 µg	25	26	26	25	27	26	25	26	26	25
Nitrofurantoin	FD-300	300 µg	20	21	21	20	21	21	21	21	21	21
Rifampin	RA-5	5 µg	15	16	15	15	15	16	16	16	16	15
Sulfathiazole	ST-300	300 µg	16	17	16	16	17	17	15	16	16	16
Tetracycline	TE-30	30 µg	31	31	31	30	30	31	31	31	31	30
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	27	27	27	27	27	26	26	26	26	26
Lincomycin	L-2	2 µg	13	13	13	13	13	14	14	14	13	13
Neomycin	N-5	5 µg	17	17	17	17	17	15	15	15	16	15

Table XVIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 196***						Date: 7.10.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 16 hrs; pH 4.27 (broth pH = 6.71) OD @600 = 0.597 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	21	20	19-22	20-21	≥17
Penicillin G	P-10	10 µg	25	26	25-25	25-27	≥28
Streptomycin	S-10	10 µg	17	17	17-17	17-17	≥15
Tobramycin	TM-10	10 µg	24	22	24-25	22-22	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	26	26	26-27	25-27	≥18
Chloramphenicol	C-30	30 µg	27	27	27-29	27-29	≥18
Chlortetracycline	A-30	30 µg	31	31	30-31	30-32	≥19*
Clindamycin	CC-2	2 µg	22	21	21-23	21-22	≥21
Erythromycin	E-15	15 µg	26	26	25-27	25-26	≥23
Nitrofurantoin	FD-300	300 µg	21	21	20-21	21-21	≥17
Rifampin	RA-5	5 µg	15	16	15-16	15-16	≥20
Sulfathiazole	ST-300	300 µg	16	16	16-17	15-17	≥17
Tetracycline	TE-30	30 µg	31	31	30-31	30-31	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	27	26	27-27	26-26	≥13*
Lincomycin	L-2	2 µg	13	14	13-13	13-14	≥21*
Neomycin	N-5	5 µg	17	15	17-17	15-16	≥17*

* From Difco antibiotic insert, 1989

Table XVIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 203***										Date: 7.12 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 17.5 hrs; pH 4.17 (broth pH = 6.73) OD @600 = 0.659 Plates (made on 6/6/89) incubated at 30°C for 19 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	17	17	17	18	16	14	14	15	14	14
Penicillin G	P-10	10 µg	25	26	25	25	26	24	24	23	22	24
Streptomycin	S-10	10 µg	13	13	13	12	13	11	11	10	11	11
Tobramycin	TM-10	10 µg	18	18	18	18	19	17	17	17	16	17
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	22	23	22	22	22	22	22	20	21	21
Chloramphenicol	C-30	30 µg	27	24	24	25	24	26	25	25	25	26
Chlortetracycline	A-30	30 µg	26	26	26	26	26	26	26	25	26	26
Clindamycin	CC-2	2 µg	23	23	24	25	25	27	25	23	22	23
Erythromycin	E-15	15 µg	27	28	28	26	26	25	25	25	25	24
Nitrofurantoin	FD-300	300 µg	15	15	15	15	14	15	14	15	14	15
Rifampin	RA-5	5 µg	16	17	16	16	16	15	14	14	13	13
Sulfathiazole	ST-300	300 µg	0	0	0	0	0	0	0	0	0	0
Tetracycline	TE-30	30 µg	26	27	27	28	26	28	28	28	26	26
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	24	23	24	24	24	22	22	22	23	22
Lincomycin	L-2	2 µg	18	19	17	18	18	18	18	18	18	17
Neomycin	N-5	5 µg	13	13	12	13	13	12	12	11	12	12

Table XVIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 203***					Date: 7.12.89	
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 17.5 hrs; pH 4.17 (broth pH = 6.73) OD @600=0.659 Plates (made on 6/6/89) incubated at 30°C for 19 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	17	14	16-18	14-15	≥17
Penicillin G	P-10	10 µg	25	23	25-26	23-24	≥28
Streptomycin	S-10	10 µg	13	11	12-13	10-11	≥15
Tobramycin	TM-10	10 µg	18	17	18-19	16-17	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	22	22	22-23	20-22	≥18
Chloramphenicol	C-30	30 µg	25	25	24-27	25-26	≥18
Chlortetracycline	A-30	30 µg	26	26	26-26	25-26	≥19*
Clindamycin	CC-2	2 µg	24	24	23-25	22-27	≥21
Erythromycin	E-15	15 µg	27	25	26-28	24-25	≥23
Nitrofurantoin	FD-300	300 µg	15	15	14-15	14-15	≥17
Rifampin	RA-5	5 µg	16	14	16-17	13-15	≥20
Sulfathiazole	ST-300	300 µg	0	0	0	0	≥17
Tetracycline	TE-30	30 µg	27	27	26-27	26-28	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	24	22	23-24	22-23	≥13*
Lincomycin	L-2	2 µg	18	18	17-19	17-18	≥21*
Neomycin	N-5	5 µg	13	12	12-13	11-12	≥17*

* From Difco antibiotic insert, 1989

Table XVIIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 205***											Date: 7.14 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 19.5 hrs; pH 4.09 (broth pH = 6.70) OD @600 = 0.642 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made for overlay only (5 ml culture + 5 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	22	20	22	21	22	17	17	17	16	16
Penicillin G	P-10	10 µg	29	29	28	27	27	24	24	24	24	24
Streptomycin	S-10	10 µg	19	18	19	19	18	16	16	16	16	16
Tobramycin	TM-10	10 µg	20	22	20	20	22	22	18	19	19	19
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	23	22	22	23	23	24	23	23	23	22
Chloramphenicol	C-30	30 µg	24	24	24	23	24	26	27	24	24	24
Chlortetracycline	A-30	30 µg	31	28	31	31	31	27	28	28	27	28
Clindamycin	CC-2	2 µg	22	22	22	22	22	21	22	21	22	22
Erythromycin	E-15	15 µg	24	25	25	25	25	25	24	24	24	24
Nitrofurantoin	FD-300	300 µg	0	0	0	0	0	0	0	0	0	0
Rifampin	RA-5	5 µg	14	12	12	12	12	14	13	13	14	13
Sulfathiazole	ST-300	300 µg	0	0	0	0	0	0	0	0	0	0
Tetracycline	TE-30	30 µg	30	30	30	30	29	28	29	29	28	29
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	24	24	25	25	24	23	24	23	24	24
Lincomycin	L-2	2 µg	16	15	16	16	16	17	16	16	16	17
Neomycin	N-5	5 µg	15	14	15	15	15	13	13	13	13	13

Table XVIIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 205***					Date: 7.14.89	
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 19.5 hrs; pH 4.09 (broth pH = 6.70) OD @600=0.642 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm				
			Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989
			Swab	Overlay	Swab	Overlay	Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	21	17	20-22	16-17	≥17
Penicillin G	P-10	10 µg	28	24	27-29	24-24	≥28
Streptomycin	S-10	10 µg	19	16	18-19	16-16	≥15
Tobramycin	TM-10	10 µg	21	19	20-22	18-22	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	23	23	22-23	22-24	≥18
Chloramphenicol	C-30	30 µg	24	24	23-24	24-27	≥18
Chlortetracycline	A-30	30 µg	31	28	28-31	27-28	≥19*
Clindamycin	CC-2	2 µg	22	22	22-22	21-22	≥21
Erythromycin	E-15	15 µg	25	24	24-25	24-25	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	12	13	12-14	13-14	≥20
Sulfathiazole	ST-300	300 µg	0	0	0	0	≥17
Tetracycline	TE-30	30 µg	30	29	29-30	28-29	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	24	24	24-25	23-24	≥13*
Lincomycin	L-2	2 µg	16	16	15-16	16-17	≥21*
Neomycin	N-5	5 µg	15	13	14-15	13-13	≥17*

* From Difco antibiotic insert, 1989

Table XIXa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 211***										Date: 7.31 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.05 (broth pH = 6.73) OD @600 = 0.608 Plates (made on 6/6/89) incubated at 30°C for 18.33 hrs. No dilution made (well, clear zones seen)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	22	21	23	21	21	20	20	20	20	21
Penicillin G	P-10	10 µg	31	32	31	32	33	30	30	30	29	29
Streptomycin	S-10	10 µg	18	19	17	18	18	17	17	17	17	17
Tobramycin	TM-10	10 µg	22	21	23	22	23	20	20	20	21	21
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	30	32	30	31	31	28	26	26	26	29
Chloramphenicol	C-30	30 µg	26	28	29	28	27	27	25	28	27	27
Chlortetracycline	A-30	30 µg	31	33	32	33	32	30	29	30	28	26
Clindamycin	CC-2	2 µg	24	23	24	24	25	23	23	22	23	23
Erythromycin	E-15	15 µg	25	26	26	26	25	24	25	25	25	25
Nitrofurantoin	FD-300	300 µg	0	0	0	0	0	0	0	0	0	0
Rifampin	RA-5	5 µg	12	12	12	12	12	13	14	14	14	14
Sulfathiazole	ST-300	300 µg	16	18	18	18	18	16	16	16	16	17
Tetracycline	TE-30	30 µg	34	33	34	35	33	30	29	30	30	29
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	27	26	27	27	26	27	26	26	26	26
Lincomycin	L-2	2 µg	13	13	13	14	13	18	18	17	18	18
Neomycin	N-5	5 µg	17	16	17	16	14	15	15	15	15	15

Table XIXb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 211***						Date: 7.31.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.05 (broth pH = 6.73) OD @600 = 0.608 Plates (made on 6/6/89) incubated at 30°C for 18.33 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	22	20	21-23	20-21	≥17
Penicillin G	P-10	10 µg	32	30	31-33	29-30	≥28
Streptomycin	S-10	10 µg	18	17	17-19	17-17	≥15
Tobramycin	TM-10	10 µg	22	20	21-23	20-21	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	31	27	30-32	26-29	≥18
Chloramphenicol	C-30	30 µg	28	27	26-29	25-27	≥18
Chlortetracycline	A-30	30 µg	32	29	31-33	26-30	≥19*
Clindamycin	CC-2	2 µg	24	23	23-25	22-23	≥21
Erythromycin	E-15	15 µg	26	25	25-26	24-25	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	12	14	12-12	13-14	≥20
Sulfathiazole	ST-300	300 µg	18	16	16-18	16-17	≥17
Tetracycline	TE-30	30 µg	34	30	33-35	29-30	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	27	26	26-27	26-27	≥13*
Lincomycin	L-2	2 µg	13	18	13-14	17-18	≥21*
Neomycin	N-5	5 µg	17	15	16-17	14-15	≥17*

* From Difco antibiotic insert, 1989

Table XXa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 217***											Date: 7.10 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 16 hrs; pH 4.69 (broth pH = 6.71) OD @600 = 0.642 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	18	18	18	19	15	14	15	15	15	15
Penicillin G	P-10	10 µg	31	32	31	30	31	27	27	28	27	27
Streptomycin	S-10	10 µg	16	18	18	16	16	11	11	11	11	11
Tobramycin	TM-10	10 µg	21	21	21	21	21	16	16	16	16	17
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	32	34	32	32	32	25	26	26	25	26
Chloramphenicol	C-30	30 µg	33	34	34	34	34	28	26	29	29	29
Chlortetracycline	A-30	30 µg	30	30	30	30	30	28	29	30	30	30
Clindamycin	CC-2	2 µg	24	24	24	24	23	23	22	22	23	22
Erythromycin	E-15	15 µg	28	28	28	27	27	25	25	26	25	25
Nitrofurantoin	FD-300	300 µg	11	11	11	10	11	9	8	9	8	8
Rifampin	RA-5	5 µg	18	18	18	18	12	16	16	16	16	15
Sulfathiazole	ST-300	300 µg	17	17	17	16	17	16	17	17	18	17
Tetracycline	TE-30	30 µg	32	32	31	31	32	29	30	29	29	27
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	30	30	31	27	29	26	26	26	26	26
Lincomycin	L-2	2 µg	17	17	18	18	17	18	18	18	17	18
Neomycin	N-5	5 µg	19	15	15	15	16	11	11	10	11	10

Table XXb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 217***					Date: 7.10.89	
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 16 hrs; pH 4.69 (broth pH = 6.71) OD @600 = 0.642 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	18	15	15-18	14-15	≥17
Penicillin G	P-10	10 µg	31	27	30-32	27-28	≥28
Streptomycin	S-10	10 µg	17	11	16-18	11-11	≥15
Tobramycin	TM-10	10 µg	21	16	21-21	16-17	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	22	26	32-34	25-26	≥18
Chloramphenicol	C-30	30 µg	34	28	33-34	26-29	≥18
Chlortetracycline	A-30	30 µg	30	29	30-30	28-30	≥19*
Clindamycin	CC-2	2 µg	24	22	24-24	22-23	≥21
Erythromycin	E-15	15 µg	28	25	27-28	25-26	≥23
Nitrofurantoin	FD-300	300 µg	11	8	10-11	8-9	≥17
Rifampin	RA-5	5 µg	17	16	12-18	15-16	≥20
Sulfathiazole	ST-300	300 µg	17	17	16-17	17-18	≥17
Tetracycline	TE-30	30 µg	32	29	31-32	27-30	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	30	26	27-30	26-26	≥13*
Lincomycin	L-2	2 µg	17	18	17-18	17-18	≥21*
Neomycin	N-5	5 µg	15	11	15-16	10-11	≥17*

* From Difco antibiotic insert, 1989

Table XXIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 220***										Date: 7.17 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 20 hrs; pH 4.04 (broth pH = 6.77) OD @600 = 0.588 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made for overlay only (5 ml culture + 5 ml Mueller broth)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	18	20	19	20	20	17	18	17	18	18
Penicillin G	P-10	10 µg	32	31	30	30	31	30	30	30	30	30
Streptomycin	S-10	10 µg	15	14	14	13	14	14	14	14	13	14
Tobramycin	TM-10	10 µg	17	19	18	17	17	19	20	20	20	20
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	31	30	31	30	30	30	29	29	31	29
Chloramphenicol	C-30	30 µg	29	30	29	28	29	27	26	26	27	27
Chlortetracycline	A-30	30 µg	34	33	35	34	34	32	33	32	33	33
Clindamycin	CC-2	2 µg	25	24	26	25	26	23	24	25	24	24
Erythromycin	E-15	15 µg	27	28	27	27	28	25	25	26	25	26
Nitrofurantoin	FD-300	300 µg	17	16	15	16	16	16	16	16	16	16
Rifampin	RA-5	5 µg	13	13	13	12	12	13	13	13	12	13
Sulfathiazole	ST-300	300 µg	0	0	0	0	0	0	0	0	0	0
Tetracycline	TE-30	30 µg	34	35	34	35	36	30	30	30	33	32
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	24	25	26	25	25	24	24	25	24	24
Lincomycin	L-2	2 µg	22	21	22	21	22	18	18	19	19	19
Neomycin	N-5	5 µg	15	14	15	15	13	15	15	15	14	15

Table XXIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 220***						Date: 7.17.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 20 hrs; pH 4.04 (broth pH = 6.77) OD @600 = 0.588 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made for overlay only (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	19	18	18-20	17-18	≥17
Penicillin G	P-10	10 µg	31	30	30-32	30-30	≥28
Streptomycin	S-10	10 µg	14	14	13-15	13-14	≥15
Tobramycin	TM-10	10 µg	18	20	17-19	19-20	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	30	30	30-31	29-31	≥18
Chloramphenicol	C-30	30 µg	29	27	28-30	26-27	≥18
Chlortetracycline	A-30	30 µg	34	33	33-35	32-33	≥19*
Clindamycin	CC-2	2 µg	25	24	24-26	23-25	≥21
Erythromycin	E-15	15 µg	27	25	27-28	25-26	≥23
Nitrofurantoin	FD-300	300 µg	16	16	15-17	16-16	≥17
Rifampin	RA-5	5 µg	13	13	12-13	12-13	≥20
Sulfathiazole	ST-300	300 µg	0	0	0	0	≥17
Tetracycline	TE-30	30 µg	35	31	34-36	30-33	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	25	24	24-26	24-25	≥13*
Lincomycin	L-2	2 µg	22	19	21-22	18-19	≥21*
Neomycin	N-5	5 µg	15	15	13-15	14-15	≥17*

* From Difco antibiotic insert, 1989

Table XXIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 222***										Date: 7.31 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.09 (broth pH = 6.73) OD @600 = 0.647 Plates (made on 6/6/89) incubated at 30°C for 18.58 hrs. No dilution made (nice, well defined zones seen)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm								
			Trials								
			Swab					Overlay			
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	23	23	23	21	23	23	23	23	23	23
Penicillin G	P-10	10 µg	31	32	31	31	31	28	30	29	30	30
Streptomycin	S-10	10 µg	17	17	17	18	18	18	18	17	18	18
Tobramycin	TM-10	10 µg	23	24	23	23	23	24	23	24	23	24
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	31	31	31	30	30	30	31	30	29	29
Chloramphenicol	C-30	30 µg	25	29	25	25	25	27	27	25	25	27
Chlortetracycline	A-30	30 µg	29	27	27	27	30	29	29	30	29	30
Clindamycin	CC-2	2 µg	29	26	26	27	27	25	24	25	24	24
Erythromycin	E-15	15 µg	28	29	27	29	29	28	25	27	27	27
Nitrofurantoin	FD-300	300 µg	14	16	16	17	16	17	17	16	17	15
Rifampin	RA-5	5 µg	14	14	13	13	15	13	14	14	13	13
Sulfathiazole	ST-300	300 µg	18	19	18	18	17	17	17	18	20	17
Tetracycline	TE-30	30 µg	30	31	31	32	31	31	31	31	30	31
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	27	29	31	30	31	28	27	26	26	26
Lincomycin	L-2	2 µg	17	17	17	16	17	18	17	16	17	17
Neomycin	N-5	5 µg	17	17	17	16	17	18	17	17	17	17

Table XXIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 222***						Date: 7.31.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.09 (broth pH = 6.73) OD @600 = 0.647 Plates (made on 6/6/89) incubated at 30°C for 18.58 hrs. No dilution made (nice, well defined zones seen)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989
			Swab	Overlay	Swab	Overlay	Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	23	23	21-23	23-23	≥17
Penicillin G	P-10	10 µg	31	30	31-32	28-30	≥28
Streptomycin	S-10	10 µg	17	18	17-18	17-18	≥15
Tobramycin	TM-10	10 µg	23	24	23-24	23-24	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	31	30	30-31	29-31	≥18
Chloramphenicol	C-30	30 µg	26	26	25-29	25-27	≥18
Chlortetracycline	A-30	30 µg	28	29	27-30	29-30	≥19*
Clindamycin	CC-2	2 µg	27	24	26-29	24-25	≥21
Erythromycin	E-15	15 µg	28	27	27-29	25-29	≥23
Nitrofurantoin	FD-300	300 µg	16	17	14-17	15-17	≥17
Rifampin	RA-5	5 µg	14	13	13-15	13-14	≥20
Sulfathiazole	ST-300	300 µg	18	18	17-19	17-20	≥17
Tetracycline	TE-30	30 µg	31	31	30-32	30-31	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	31	27	27-31	26-28	≥13*
Lincomycin	L-2	2 µg	17	17	16-17	16-18	≥21*
Neomycin	N-5	5 µg	17	17	16-17	17-18	≥17*

* From Difco antibiotic insert, 1989

Table XXIIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 223***											Date: 7.17 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 20 hrs; pH 4.05 (broth pH = 6.77) OD @600 = 0.578 Plates (made on 6/6/89) incubated at 30°C for 19.5 hrs. 50% dilution made for overlay only (5 ml culture + 5 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	22	23	24	23	23	22	20	22	22	22
Penicillin G	P-10	10 µg	34	34	36	36	36	30	29	30	30	30
Streptomycin	S-10	10 µg	18	19	18	19	19	18	18	18	20	20
Tobramycin	TM-10	10 µg	25	24	23	24	24	22	22	22	22	22
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	31	28	31	31	31	26	26	27	25	25
Chloramphenicol	C-30	30 µg	26	25	26	25	27	27	26	27	29	27
Chlortetracycline	A-30	30 µg	33	34	34	34	33	31	32	31	32	31
Clindamycin	CC-2	2 µg	30	32	30	30	29	24	24	25	24	24
Erythromycin	E-15	15 µg	30	33	32	30	30	26	26	26	26	26
Nitrofurantoin	FD-300	300 µg	0	0	0	0	0	0	0	0	0	0
Rifampin	RA-5	5 µg	13	13	12	13	13	13	13	13	13	13
Sulfathiazole	ST-300	300 µg	14	14	15	14	14	14	14	14	14	14
Tetracycline	TE-30	30 µg	34	34	34	34	34	32	31	32	31	32
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	26	26	26	26	25	24	24	25	24	24
Lincomycin	L-2	2 µg	22	20	20	30	22	18	20	17	17	17
Neomycin	N-5	5 µg	17	17	17	17	18	16	16	16	15	16

Table XXIIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 223***					Date: 7.17.89	
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 20 hrs; pH 4.05 (broth pH = 6.77) OD @600 = 0.578 Plates (made on 6/6/89) incubated at 30°C for 19.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989 Susceptible
			Swab	Overlay	Swab	Overlay	
Primary Grouping:							
Amikacin	AN-30	30 µg	23	22	22-24	20-22	≥17
Penicillin G	P-10	10 µg	35	30	34-36	29-30	≥28
Streptomycin	S-10	10 µg	19	19	18-19	18-20	≥15
Tobramycin	TM-10	10 µg	24	22	23-25	22-22	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	30	26	28-31	25-27	≥18
Chloramphenicol	C-30	30 µg	26	27	25-27	26-29	≥18
Chlortetracycline	A-30	30 µg	34	31	33-34	31-32	≥19*
Clindamycin	CC-2	2 µg	30	24	29-32	24-25	≥21
Erythromycin	E-15	15 µg	31	26	30-32	26-26	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	13	13	12-13	13-13	≥20
Sulfathiazole	ST-300	300 µg	14	14	14-15	14-14	≥17
Tetracycline	TE-30	30 µg	34	31	34-34	31-32	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	26	24	25-26	24-25	≥13*
Lincomycin	L-2	2 µg	21	18	20-22	17-20	≥21*
Neomycin	N-5	5 µg	17	16	17-18	15-16	≥17*

* From Difco antibiotic insert, 1989

Table XXIVa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 459***											Date: 7.14 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 19.5 hrs; pH 4.13 (broth pH = 6.70) OD @600 = 0.603 Plates (made on 6/6/89) incubated at 30°C for 19 hrs. 50% dilution made for overlay only (5 ml culture + 5 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	23	22	23	23	24	22	20	22	22	22
Penicillin G	P-10	10 µg	34	35	36	36	35	31	30	29	30	30
Streptomycin	S-10	10 µg	20	21	21	22	22	19	18	18	18	18
Tobramycin	TM-10	10 µg	26	25	23	25	25	23	23	23	23	24
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	36	35	35	36	34	29	30	30	29	28
Chloramphenicol	C-30	30 µg	36	34	36	35	36	30	30	28	27	28
Chlortetracycline	A-30	30 µg	32	30	32	32	32	32	33	33	32	33
Clindamycin	CC-2	2 µg	31	29	30	32	32	25	25	24	25	25
Erythromycin	E-15	15 µg	32	32	30	32	32	27	27	27	26	27
Nitrofurantoin	FD-300	300 µg	16	16	15	16	16	16	17	17	16	16
Rifampin	RA-5	5 µg	12	12	12	11	12	13	12	13	12	12
Sulfathiazole	ST-300	300 µg	16	16	17	16	17	16	16	17	16	16
Tetracycline	TE-30	30 µg	34	34	35	34	34	35	34	35	34	34
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	30	29	30	30	30	22	22	22	22	22
Lincomycin	L-2	2 µg	16	17	16	16	17	15	15	16	16	16
Neomycin	N-5	5 µg	19	19	20	19	19	18	18	18	18	18

Table XXIVb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 459***						Date: 7.14.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 19.5 hrs; pH 4.13 (broth pH = 6.70) OD @600=0.603 Plates (made on 6/6/89) incubated at 30°C for 19 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	23	22	22-24	20-22	≥17
Penicillin G	P-10	10 µg	35	30	34-36	29-31	≥28
Streptomycin	S-10	10 µg	21	18	20-22	18-19	≥15
Tobramycin	TM-10	10 µg	25	23	23-26	23-24	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	35	29	34-36	28-30	≥18
Chloramphenicol	C-30	30 µg	35	29	34-36	27-30	≥18
Chlortetracycline	A-30	30 µg	32	33	30-32	32-33	≥19*
Clindamycin	CC-2	2 µg	31	25	29-32	24-25	≥21
Erythromycin	E-15	15 µg	32	27	30-32	26-27	≥23
Nitrofurantoin	FD-300	300 µg	16	16	15-16	16-17	≥17
Rifampin	RA-5	5 µg	12	12	11-12	12-13	≥20
Sulfathiazole	ST-300	300 µg	16	16	16-17	16-17	≥17
Tetracycline	TE-30	30 µg	34	34	34-35	34-35	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	30	22	29-30	22-22	≥13*
Lincomycin	L-2	2 µg	16	16	16-17	15-16	≥21*
Neomycin	N-5	5 µg	19	18	19-20	18-18	≥17*

* From Difco antibiotic insert, 1989

Table XXVa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 799***										Date: 7.31 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.07 (broth pH = 6.73) OD @600 = 0.715 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. No dilution made (nice, well defined zones seen)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm								
			Trials								
			Swab					Overlay			
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	17	17	17	17	17	18	17	17	17	17
Penicillin G	P-10	10 µg	30	29	29	29	29	28	29	28	29	29
Streptomycin	S-10	10 µg	14	14	13	14	14	15	15	14	14	14
Tobramycin	TM-10	10 µg	20	20	19	19	20	21	19	19	19	20
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	26	27	28	28	28	27	28	27	27	27
Chloramphenicol	C-30	30 µg	27	26	29	29	28	25	28	27	25	25
Chlortetracycline	A-30	30 µg	29	29	29	29	30	28	28	29	30	28
Clindamycin	CC-2	2 µg	27	26	26	27	26	25	24	24	24	25
Erythromycin	E-15	15 µg	26	27	27	27	27	26	25	24	25	25
Nitrofurantoin	FD-300	300 µg	17	17	16	15	15	16	17	16	16	17
Rifampin	RA-5	5 µg	12	13	12	12	12	15	16	15	15	16
Sulfathiazole	ST-300	300 µg	0	0	0	0	0	0	0	0	0	0
Tetracycline	TE-30	30 µg	32	32	31	31	31	29	30	31	31	31
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	27	28	29	28	28	23	26	26	25	26
Lincomycin	L-2	2 µg	19	17	17	18	18	19	20	19	19	19
Neomycin	N-5	5 µg	14	15	14	16	15	14	14	14	14	14

Table XXVb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 799***						Date: 7.31.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.07 (broth pH = 6.73) OD @600 = 0.715 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989
			Swab	Overlay	Swab	Overlay	Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	17	17	17-17	17-18	≥17
Penicillin G	P-10	10 µg	29	29	29-30	28-29	≥28
Streptomycin	S-10	10 µg	14	14	13-14	14-15	≥15
Tobramycin	TM-10	10 µg	20	19	19-20	19-20	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	27	27	26-28	27-28	≥18
Chloramphenicol	C-30	30 µg	28	26	26-29	25-28	≥18
Chlortetracycline	A-30	30 µg	29	29	29-30	28-30	≥19*
Clindamycin	CC-2	2 µg	26	24	26-27	24-25	≥21
Erythromycin	E-15	15 µg	27	25	26-27	24-26	≥23
Nitrofurantoin	FD-300	300 µg	16	16	15-17	16-17	≥17
Rifampin	RA-5	5 µg	12	15	12-13	15-16	≥20
Sulfathiazole	ST-300	300 µg	0	0	0	0	≥17
Tetracycline	TE-30	30 µg	31	30	31-32	29-31	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	28	25	27-29	23-26	≥13*
Lincomycin	L-2	2 µg	18	19	17-19	19-20	≥21*
Neomycin	N-5	5 µg	15	14	14-16	14-14	≥17*

* From Difco antibiotic insert, 1989

Table XXVIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 819***											Date: 8.04 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 19 hrs; pH 4.17 (broth pH = 6.64) OD @600 = 0.648 Plates (made on 8/1/89) incubated at 30°C for 18.67 hrs. 50% dilution made (2 ml culture + 2 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	23	25	24	23	23	20	20	20	20	20
Penicillin G	P-10	10 µg	31	31	31	30	31	32	31	31	31	31
Streptomycin	S-10	10 µg	18	17	18	17	17	18	17	17	17	17
Tobramycin	TM-10	10 µg	24	23	22	22	22	21	21	21	21	22
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	28	31	32	31	31	28	27	30	30	30
Chloramphenicol	C-30	30 µg	25	26	26	26	26	26	27	27	26	26
Chlortetracycline	A-30	30 µg	31	31	31	31	30	30	31	30	30	31
Clindamycin	CC-2	2 µg	21	23	24	24	24	22	23	22	22	23
Erythromycin	E-15	15 µg	27	28	28	28	27	27	26	27	28	27
Nitrofurantoin	FD-300	300 µg	0	0	0	0	0	0	0	0	0	0
Rifampin	RA-5	5 µg	15	14	14	14	14	14	14	13	14	14
Sulfathiazole	ST-300	300 µg	18	20	18	18	18	17	17	16	17	16
Tetracycline	TE-30	30 µg	32	32	32	32	31	31	31	31	28	31
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	27	28	28	27	28	27	27	26	26	27
Lincomycin	L-2	2 µg	15	16	16	16	16	15	15	15	15	15
Neomycin	N-5	5 µg	16	17	16	16	16	14	15	15	15	15

Table XXVIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 819***						Date: 8.04.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 19 hrs; pH 4.17 (broth pH = 6.64) OD @600 = 0.648 Plates (made on 8/1/89) incubated at 30°C for 18.67 hrs. 50% dilution made (2 ml culture + 2 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	24	20	23-25	20-20	≥17
Penicillin G	P-10	10 µg	31	31	30-31	31-32	≥28
Streptomycin	S-10	10 µg	17	17	17-18	17-18	≥15
Tobramycin	TM-10	10 µg	23	21	22-24	21-22	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	31	29	28-32	27-30	≥18
Chloramphenicol	C-30	30 µg	26	26	25-26	26-27	≥18
Chlortetracycline	A-30	30 µg	31	30	30-31	30-31	≥19*
Clindamycin	CC-2	2 µg	23	22	21-24	22-23	≥21
Erythromycin	E-15	15 µg	28	27	27-28	26-28	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	14	14	14-15	13-14	≥20
Sulfathiazole	ST-300	300 µg	18	17	18-20	16-17	≥17
Tetracycline	TE-30	30 µg	32	31	31-32	28-31	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	28	27	27-28	26-27	≥13*
Lincomycin	L-2	2 µg	16	15	15-16	15-15	≥21*
Neomycin	N-5	5 µg	16	15	16-17	14-15	≥17*

* From Difco antibiotic insert, 1989

Table XXVIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 852***										Date: 7.31 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.07 (broth pH = 6.73) OD @600 = 0.730 Plates (made on 6/6/89) incubated at 30°C for 18.25 hrs. No dilution made (well, clear zones seen)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm								
			Trials								
			Swab					Overlay			
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	24	23	25	25	25	22	22	23	22	22
Penicillin G	P-10	10 µg	33	33	34	33	33	32	32	32	32	32
Streptomycin	S-10	10 µg	18	19	19	18	19	19	20	20	19	19
Tobramycin	TM-10	10 µg	25	25	23	23	23	24	23	23	23	23
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	29	31	32	31	32	32	31	32	32	31
Chloramphenicol	C-30	30 µg	23	23	23	24	24	25	26	25	26	25
Chlortetracycline	A-30	30 µg	32	31	33	32	33	31	32	31	31	31
Clindamycin	CC-2	2 µg	25	27	26	26	25	23	23	24	23	23
Erythromycin	E-15	15 µg	31	28	28	29	28	26	26	26	26	26
Nitrofurantoin	FD-300	300 µg	0	0	0	0	0	0	0	0	0	0
Rifampin	RA-5	5 µg	14	15	14	14	13	14	14	15	14	14
Sulfathiazole	ST-300	300 µg	18	19	19	18	19	19	18	19	19	19
Tetracycline	TE-30	30 µg	32	32	34	33	33	31	32	32	32	32
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	31	33	32	27	31	28	29	28	29	28
Lincomycin	L-2	2 µg	14	15	15	15	15	15	16	16	15	15
Neomycin	N-5	5 µg	18	17	17	17	17	17	16	17	17	17

Table XXVIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 852***						Date: 7.31.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.07 (broth pH = 6.73) OD @600 = 0.730 Plates (made on 6/6/89) incubated at 30°C for 18.25 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm				
			Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989
			Swab	Overlay	Swab	Overlay	Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	24	22	23-25	22-23	≥17
Penicillin G	P-10	10 µg	33	32	33-34	32-32	≥28
Streptomycin	S-10	10 µg	19	19	18-19	19-20	≥15
Tobramycin	TM-10	10 µg	24	23	23-25	23-24	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	31	32	29-32	31-32	≥18
Chloramphenicol	C-30	30 µg	23	25	23-24	25-26	≥18
Chlortetracycline	A-30	30 µg	32	31	31-33	31-32	≥19*
Clindamycin	CC-2	2 µg	26	23	25-27	23-24	≥21
Erythromycin	E-15	15 µg	29	26	28-31	26-26	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	14	14	13-15	14-15	≥20
Sulfathiazole	ST-300	300 µg	19	19	18-19	18-19	≥17
Tetracycline	TE-30	30 µg	33	32	32-34	31-32	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	31	28	27-32	28-29	≥13*
Lincomycin	L-2	2 µg	15	15	14-15	15-16	≥21*
Neomycin	N-5	5 µg	17	17	17-18	16-17	≥17*

* From Difco antibiotic insert, 1989

Table XXVIIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 865***										Date: 7.17 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 20 hrs; pH 4.62 (broth pH = 6.77) OD @600 = 0.530 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made for overlay only (5 ml culture + 5 ml Mueller broth)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	22	22	22	22	22	17	17	17	18	17
Penicillin G	P-10	10 µg	29	31	29	29	29	28	28	28	28	27
Streptomycin	S-10	10 µg	16	16	17	17	17	15	15	15	16	15
Tobramycin	TM-10	10 µg	22	20	22	22	22	19	19	20	19	18
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	31	31	32	31	31	23	23	23	25	24
Chloramphenicol	C-30	30 µg	27	26	28	27	26	24	23	23	23	23
Chlortetracycline	A-30	30 µg	34	32	34	34	34	29	29	28	29	29
Clindamycin	CC-2	2 µg	28	25	28	28	28	22	22	22	22	22
Erythromycin	E-15	15 µg	28	28	28	27	28	23	23	24	24	24
Nitrofurantoin	FD-300	300 µg	0	0	0	0	0	0	0	0	0	0
Rifampin	RA-5	5 µg	17	17	17	15	16	13	13	13	12	13
Sulfathiazole	ST-300	300 µg	17	17	17	17	17	15	16	15	15	16
Tetracycline	TE-30	30 µg	34	32	32	34	34	29	28	28	27	28
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	26	27	26	26	26	26	26	26	26	26
Lincomycin	L-2	2 µg	21	18	20	18	18	17	17	17	17	17
Neomycin	N-5	5 µg	16	16	16	15	16	14	14	14	14	13

Table XXVIIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 865***					Date: 7.17.89	
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 20 hrs; pH 4.62 (broth pH = 6.77) OD @600 = 0.530 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989 Susceptible
			Swab	Overlay	Swab	Overlay	
Primary Grouping:							
Amikacin	AN-30	30 µg	22	17	22-22	17-18	≥17
Penicillin G	P-10	10 µg	29	28	29-31	27-28	≥28
Streptomycin	S-10	10 µg	17	15	16-17	15-16	≥15
Tobramycin	TM-10	10 µg	22	19	20-22	18-20	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	31	24	31-32	23-25	≥18
Chloramphenicol	C-30	30 µg	27	23	26-28	23-24	≥18
Chlortetracycline	A-30	30 µg	34	29	32-34	28-29	≥19*
Clindamycin	CC-2	2 µg	27	22	25-28	22-22	≥21
Erythromycin	E-15	15 µg	28	24	27-28	23-24	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	16	13	15-17	12-13	≥20
Sulfathiazole	ST-300	300 µg	17	15	17-17	15-16	≥17
Tetracycline	TE-30	30 µg	33	28	32-34	27-29	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	26	26	26-27	26-26	≥13*
Lincomycin	L-2	2 µg	19	17	18-21	17-17	≥21*
Neomycin	N-5	5 µg	16	14	15-16	13-14	≥17*

* From Difco antibiotic insert, 1989

Table XXIXa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 990***											Date: 7.14 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.22 (broth pH = 6.73) OD @600 = 0.693 Plates (made on 6/6/89) incubated at 30°C for 17.5 hrs. No dilution made (nice, well defined zones seen)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm									
			Trials									
			Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	17	19	18	18	17	18	20	19	19	19
Penicillin G	P-10	10 µg	31	31	32	32	31	31	32	32	31	30
Streptomycin	S-10	10 µg	14	15	14	13	14	13	15	14	15	15
Tobramycin	TM-10	10 µg	19	19	19	19	19	20	20	19	20	19
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	32	31	32	31	31	31	32	32	31	31
Chloramphenicol	C-30	30 µg	27	25	25	27	27	27	28	27	27	28
Chlortetracycline	A-30	30 µg	31	31	30	31	31	32	31	29	30	32
Clindamycin	CC-2	2 µg	27	27	26	26	26	26	26	24	27	26
Erythromycin	E-15	15 µg	27	26	27	26	26	28	27	28	29	28
Nitrofurantoin	FD-300	300 µg	17	17	17	17	17	13	14	14	15	14
Rifampin	RA-5	5 µg	17	17	17	17	17	18	19	18	18	19
Sulfathiazole	ST-300	300 µg	21	21	21	20	21	24	25	24	24	25
Tetracycline	TE-30	30 µg	31	31	29	31	29	32	33	33	32	30
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	27	27	26	28	27	27	28	28	28	28
Lincomycin	L-2	2 µg	12	12	12	12	12	13	12	14	13	13
Neomycin	N-5	5 µg	13	14	13	13	13	15	12	15	15	15

Table XXIXb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 990***						Date: 7.31.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.22 (broth pH = 6.73) OD @600 = 0.693 Plates (made on 6/6/89) incubated at 30°C for 17.5 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm				
			Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989
			Swab	Overlay	Swab	Overlay	Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	18	19	17-19	18-20	≥17
Penicillin G	P-10	10 µg	31	31	31-32	30-32	≥28
Streptomycin	S-10	10 µg	14	14	13-15	13-15	≥15
Tobramycin	TM-10	10 µg	19	20	19-19	19-20	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	31	31	31-32	31-32	≥18
Chloramphenicol	C-30	30 µg	26	27	25-27	27-28	≥18
Chlortetracycline	A-30	30 µg	31	31	30-31	29-32	≥19*
Clindamycin	CC-2	2 µg	26	26	26-27	24-27	≥21
Erythromycin	E-15	15 µg	26	28	26-27	27-29	≥23
Nitrofurantoin	FD-300	300 µg	17	14	17-17	13-15	≥17
Rifampin	RA-5	5 µg	17	18	17-17	18-19	≥20
Sulfathiazole	ST-300	300 µg	21	24	20-21	24-25	≥17
Tetracycline	TE-30	30 µg	30	32	29-31	30-33	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	27	28	26-28	27-28	≥13*
Lincomycin	L-2	2 µg	12	13	12-12	12-14	≥21*
Neomycin	N-5	5 µg	13	14	13-14	12-15	≥17*

* From Difco antibiotic insert, 1989

Table XXXa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris BK5***											Date: 7.31 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.04 (broth pH = 6.73) OD @600 = 0.757 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. No dilution made (nice, well defined zones seen)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	18	18	18	17	17	15	15	15	15	16
Penicillin G	P-10	10 µg	27	27	28	27	28	25	24	25	25	25
Streptomycin	S-10	10 µg	14	14	13	13	13	12	12	11	11	11
Tobramycin	TM-10	10 µg	20	19	19	20	19	18	17	18	18	18
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	24	24	24	24	25	25	24	24	24	24
Chloramphenicol	C-30	30 µg	26	26	26	25	26	26	26	26	26	26
Chlortetracycline	A-30	30 µg	29	29	29	29	30	30	30	30	30	30
Clindamycin	CC-2	2 µg	20	19	19	19	19	20	20	20	20	20
Erythromycin	E-15	15 µg	25	24	24	24	24	24	24	24	24	24
Nitrofurantoin	FD-300	300 µg	17	18	17	17	17	21	21	21	21	21
Rifampin	RA-5	5 µg	14	14	13	14	14	16	16	16	16	16
Sulfathiazole	ST-300	300 µg	0	0	0	0	0	0	0	0	0	0
Tetracycline	TE-30	30 µg	29	29	29	30	29	28	28	28	28	30
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	23	23	23	22	24	22	23	23	22	23
Lincomycin	L-2	2 µg	13	13	13	13	13	13	13	13	13	13
Neomycin	N-5	5 µg	14	14	14	14	14	13	13	13	13	13

Table XXXb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris BK5***					Date: 7.31.89	
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.04 (broth pH = 6.73) OD @600 = 0.757 Plates (made on 6/6/89) incubated at 30°C for 18 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm				
			Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989
			Swab	Overlay	Swab	Overlay	Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	18	15	18-17	15-16	≥17
Penicillin G	P-10	10 µg	27	25	27-28	24-25	≥28
Streptomycin	S-10	10 µg	13	11	13-14	11-12	≥15
Tobramycin	TM-10	10 µg	19	18	19-20	17-18	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	24	24	24-25	24-25	≥18
Chloramphenicol	C-30	30 µg	26	26	25-26	26-26	≥18
Chlortetracycline	A-30	30 µg	29	30	29-30	29-30	≥19*
Clindamycin	CC-2	2 µg	19	20	19-20	20-20	≥21
Erythromycin	E-15	15 µg	24	24	24-25	24-24	≥23
Nitrofurantoin	FD-300	300 µg	17	21	17-18	21-21	≥17
Rifampin	RA-5	5 µg	14	16	13-14	16-16	≥20
Sulfathiazole	ST-300	300 µg	0	0	0	0	≥17
Tetracycline	TE-30	30 µg	29	28	29-30	28-30	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	23	22	22-24	22-23	≥13*
Lincomycin	L-2	2 µg	13	13-13	13	13-13	≥21*
Neomycin	N-5	5 µg	14	13	14-14	13-13	≥17*

* From Difco antibiotic insert, 1989

Table XXXIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris Cl***											Date: 8.02 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.27 (broth pH = 6.72) OD @600 = 0.612 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. No dilution made (nice, well defined zones seen)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	24	24	23	24	24	20	18	20	19	19
Penicillin G	P-10	10 µg	32	31	32	34	32	27	27	29	28	28
Streptomycin	S-10	10 µg	17	21	17	17	17	17	15	17	17	15
Tobramycin	TM-10	10 µg	22	22	22	23	21	26	20	20	20	20
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	33	32	34	32	32	26	25	26	26	26
Chloramphenicol	C-30	30 µg	24	24	23	22	22	25	25	25	25	25
Chlortetracycline	A-30	30 µg	30	30	29	29	29	26	27	26	28	26
Clindamycin	CC-2	2 µg	21	22	21	21	21	21	23	21	22	21
Erythromycin	E-15	15 µg	25	25	25	26	27	25	25	23	25	24
Nitrofurantoin	FD-300	300 µg	0	0	0	0	0	0	0	0	0	0
Rifampin	RA-5	5 µg	14	14	14	14	14	13	14	13	14	14
Sulfathiazole	ST-300	300 µg	16	16	17	19	16	16	14	14	16	16
Tetracycline	TE-30	30 µg	34	34	34	34	34	29	29	30	29	30
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	29	27	29	30	29	26	25	26	25	25
Lincomycin	L-2	2 µg	18	17	18	17	17	17	18	18	18	18
Neomycin	N-5	5 µg	16	15	15	16	16	15	15	15	15	15

Table XXXIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris Cl***					Date: 8.02.89	
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.27 (broth pH = 6.72) OD @600 = 0.612 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989
			Swab	Overlay	Swab	Overlay	Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	24	19	23-24	18-20	≥17
Penicillin G	P-10	10 µg	32	28	31-34	27-29	≥28
Streptomycin	S-10	10 µg	18	16	17-21	15-17	≥15
Tobramycin	TM-10	10 µg	22	21	21-23	20-26	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	33	26	32-34	25-26	≥18
Chloramphenicol	C-30	30 µg	23	25	22-24	25-25	≥18
Chlortetracycline	A-30	30 µg	29	27	29-30	26-28	≥19*
Clindamycin	CC-2	2 µg	21	22	21-22	21-23	≥21
Erythromycin	E-15	15 µg	26	24	25-27	23-25	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	14	14	14-14	13-14	≥20
Sulfathiazole	ST-300	300 µg	17	15	16-19	14-16	≥17
Tetracycline	TE-30	30 µg	34	29	34-34	29-30	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	29	25	27-30	25-26	≥13*
Lincomycin	L-2	2 µg	17	18	17-18	17-18	≥21*
Neomycin	N-5	5 µg	16	15	15-16	15-15	≥17*

* From Difco antibiotic insert, 1989

Table XXXIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris C3***											Date: 6.30 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 21 hrs; pH 7.17 (broth pH = 7.37) OD @600 = 0.037 Plates (made on 6/6/89) incubated at 30°C for 17 hrs. No dilution made (not good lawn)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	30	30	30	32	30	28	26	28	27	28
Penicillin G	P-10	10 µg	36	38	40	40	40	36	36	36	35	36
Streptomycin	S-10	10 µg	24	25	24	23	24	22	23	22	22	23
Tobramycin	TM-10	10 µg	28	27	28	28	28	29	29	27	28	29
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	41	42	42	41	40	40	40	42	40	40
Chloramphenicol	C-30	30 µg	34	34	34	34	34	32	36	36	36	34
Chlortetracycline	A-30	30 µg	30	32	34	30	30	38	39	38	39	39
Clindamycin	CC-2	2 µg	30	29	29	30	30	30	30	29	29	29
Erythromycin	E-15	15 µg	34	34	34	34	34	34	34	34	32	32
Nitrofurantoin	FD-300	300 µg	13	13	12	12	12	13	13	13	13	13
Rifampin	RA-5	5 µg	13	12	12	12	13	13	14	13	13	13
Sulfathiazole	ST-300	300 µg	23	23	22	24	23	25	25	25	24	25
Tetracycline	TE-30	30 µg	41	42	41	42	39	40	39	39	39	39
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	34	34	34	34	34	42	41	42	42	42
Lincomycin	L-2	2 µg	19	19	20	19	21	18	17	19	18	18
Neomycin	N-5	5 µg	19	18	18	18	18	19	18	19	19	19

Table XXXIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris C3***						Date: 6.30.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 21 hrs; pH 7.17 (broth pH = 7.37) OD @600 = 0.037 Plates (made on 6/6/89) incubated at 30°C for 17 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm				
			Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989
			Swab	Overlay	Swab	Overlay	Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	30	28	30-32	26-28	≥17
Penicillin G	P-10	10 µg	39	36	36-40	35-36	≥28
Streptomycin	S-10	10 µg	24	22	23-25	22-23	≥15
Tobramycin	TM-10	10 µg	28	28	27-28	27-29	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	41	40	40-42	40-42	≥18
Chloramphenicol	C-30	30 µg	34	35	34-34	32-36	≥18
Chlortetracycline	A-30	30 µg	31	39	30-34	38-39	≥19*
Clindamycin	CC-2	2 µg	30	29	29-30	29-30	≥21
Erythromycin	E-15	15 µg	34	33	34-34	32-34	≥23
Nitrofurantoin	FD-300	300 µg	12	13	12-13	13-13	≥17
Rifampin	RA-5	5 µg	12	13	12-13	13-14	≥20
Sulfathiazole	ST-300	300 µg	23	25	22-24	24-25	≥17
Tetracycline	TE-30	30 µg	41	39	39-42	39-40	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	34	42	34-34	41-42	≥13*
Lincomycin	L-2	2 µg	20	18	19-21	17-19	≥21*
Neomycin	N-5	5 µg	18	19	18-19	18-19	≥17*

* From Difco antibiotic insert, 1989

Table XXXIIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris C11***										Date: 7.17 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 20 hrs; pH 4.29 (broth pH = 6.77) OD @600 = 0.625 Plates (made on 6/6/89) incubated at 30°C for 20 hrs. 50% dilution made for overlay only (5 ml culture + 5 ml Mueller broth)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm								
			Trials								
			Swab					Overlay			
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	24	24	25	24	24	22	20	20	20	20
Penicillin G	P-10	10 µg	34	36	34	36	36	30	30	30	31	32
Streptomycin	S-10	10 µg	19	20	20	19	19	19	18	18	18	18
Tobramycin	TM-10	10 µg	24	24	24	24	25	22	22	22	23	22
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	28	29	32	32	33	28	28	29	28	28
Chloramphenicol	C-30	30 µg	30	30	30	28	30	32	33	33	33	33
Chlortetracycline	A-30	30 µg	36	36	36	36	36	34	34	35	34	34
Clindamycin	CC-2	2 µg	36	32	32	36	34	28	28	27	26	28
Erythromycin	E-15	15 µg	31	31	31	31	31	30	30	31	30	30
Nitrofurantoin	FD-300	300 µg	0	0	0	0	0	0	0	0	0	0
Rifampin	RA-5	5 µg	12	12	12	12	12	12	13	12	12	13
Sulfathiazole	ST-300	300 µg	0	0	0	0	0	0	0	0	0	0
Tetracycline	TE-30	30 µg	35	35	35	37	35	35	35	35	35	35
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	22	22	22	22	27	27	27	27	27	27
Lincomycin	L-2	2 µg	19	20	19	19	20	20	20	20	19	20
Neomycin	N-5	5 µg	15	16	15	15	15	15	15	15	15	15

Table XXXIIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris C11***						Date: 7.17.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 20 hrs; pH 4.29 (broth pH = 6.77) OD @600 = 0.625 Plates (made on 6/6/89) incubated at 30°C for 20 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	24	20	24-25	20-22	≥17
Penicillin G	P-10	10 µg	35	30	34-36	30-32	≥28
Streptomycin	S-10	10 µg	19	18	19-20	18-19	≥15
Tobramycin	TM-10	10 µg	24	22	24-25	22-23	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	31	28	28-33	28-29	≥18
Chloramphenicol	C-30	30 µg	30	33	28-30	32-33	≥18
Chlortetracycline	A-30	30 µg	36	34	36-36	34-35	≥19*
Clindamycin	CC-2	2 µg	34	27	32-36	26-28	≥21
Erythromycin	E-15	15 µg	31	30	31-31	30-31	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	12	12	12-12	12-13	≥20
Sulfathiazole	ST-300	300 µg	0	0	0	0	≥17
Tetracycline	TE-30	30 µg	35	35	35-37	35-35	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	22	27	22-22	27-27	≥13*
Lincomycin	L-2	2 µg	19	20	19-20	19-20	≥21*
Neomycin	N-5	5 µg	15	15	15-16	15-15	≥17*

* From Difco antibiotic insert, 1989

Table XXXIVa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris C13***										Date: 7.17 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 20 hrs; pH 4.36 (broth pH = 6.77) OD @600 = 0.546 Plates (made on 6/6/89) incubated at 30°C for 19.5 hrs. 50% dilution made for overlay only (5 ml culture + 5 ml Mueller broth)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	23	22	21	22	23	22	20	20	20	22
Penicillin G	P-10	10 µg	31	32	30	31	31	30	30	30	32	31
Streptomycin	S-10	10 µg	18	17	17	17	18	17	17	17	17	17
Tobramycin	TM-10	10 µg	22	22	22	22	22	22	22	22	22	22
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	29	28	28	27	28	28	27	28	27	28
Chloramphenicol	C-30	30 µg	29	27	27	27	27	27	27	28	27	27
Chlortetracycline	A-30	30 µg	35	36	35	34	35	34	34	33	33	33
Clindamycin	CC-2	2 µg	32	31	31	30	30	28	28	27	28	27
Erythromycin	E-15	15 µg	31	30	31	30	29	28	29	28	28	28
Nitrofurantoin	FD-300	300 µg	0	0	0	0	0	0	0	0	0	0
Rifampin	RA-5	5 µg	14	15	15	15	14	14	16	15	15	15
Sulfathiazole	ST-300	300 µg	14	14	15	14	14	14	14	14	14	15
Tetracycline	TE-30	30 µg	32	32	33	32	32	32	34	32	32	32
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	28	28	28	28	28	26	26	26	26	26
Lincomycin	L-2	2 µg	20	19	20	19	19	20	20	20	20	20
Neomycin	N-5	5 µg	17	17	17	17	17	18	17	17	17	17

Table XXXIVb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris C13***						Date: 7.17.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 20 hrs; pH 4.36 (broth pH = 6.77) OD @600 = 0.546 Plates (made on 6/6/89) incubated at 30°C for 19.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	22	21	21-23	20-22	≥17
Penicillin G	P-10	10 µg	31	31	30-32	30-32	≥28
Streptomycin	S-10	10 µg	17	17	17-18	17-17	≥15
Tobramycin	TM-10	10 µg	22	22	22-22	22-22	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	28	28	27-29	27-28	≥18
Chloramphenicol	C-30	30 µg	27	27	27-29	27-29	≥18
Chlortetracycline	A-30	30 µg	35	33	34-36	33-34	≥19*
Clindamycin	CC-2	2 µg	31	28	30-32	27-28	≥21
Erythromycin	E-15	15 µg	30	28	29-31	28-29	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	15	15	14-15	14-16	≥20
Sulfathiazole	ST-300	300 µg	14	14	14-15	14-15	≥17
Tetracycline	TE-30	30 µg	32	32	32-33	32-34	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	28	26	28-28	26-26	≥13*
Lincomycin	L-2	2 µg	19	20	19-20	20-20	≥21*
Neomycin	N-5	5 µg	17	17	17-17	17-18	≥17*

* From Difco antibiotic insert, 1989

Table XXXVa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris E8***											Date: 7.17 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 20 hrs; pH 4.26 (broth pH = 6.77) OD @600 = 0.673 Plates (made on 6/6/89) incubated at 30°C for 19.5 hrs. 50% dilution made for overlay only (5 ml culture + 5 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	18	18	19	18	16	16	16	15	16	16
Penicillin G	P-10	10 µg	29	29	29	29	30	25	25	26	25	25
Streptomycin	S-10	10 µg	13	14	13	13	13	13	13	13	13	13
Tobramycin	TM-10	10 µg	20	19	19	19	20	17	17	17	18	17
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	25	27	26	25	25	25	25	25	26	25
Chloramphenicol	C-30	30 µg	24	23	24	25	24	26	25	25	25	24
Chlortetracycline	A-30	30 µg	28	28	27	28	28	30	30	28	29	30
Clindamycin	CC-2	2 µg	25	24	25	24	25	22	22	22	22	26
Erythromycin	E-15	15 µg	25	25	25	25	25	24	23	24	24	24
Nitrofurantoin	FD-300	300 µg	13	13	13	13	13	15	14	15	14	14
Rifampin	RA-5	5 µg	13	13	13	12	12	15	15	14	15	14
Sulfathiazole	ST-300	300 µg	0	0	0	0	0	0	0	0	0	0
Tetracycline	TE-30	30 µg	29	29	29	31	29	27	27	28	27	28
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	27	26	26	26	26	24	24	24	24	24
Lincomycin	L-2	2 µg	19	17	18	17	17	17	17	17	17	17
Neomycin	N-5	5 µg	14	15	14	15	16	13	13	13	13	15

Table XXXVb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris E8***					Date: 7.17.89	
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 20 hrs; pH 4.26 (broth pH = 6.77) OD @600 = 0.673 Plates (made on 6/6/89) incubated at 30°C for 19.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	18	16	16-19	15-16	≥17
Penicillin G	P-10	10 µg	29	25	29-30	25-26	≥28
Streptomycin	S-10	10 µg	13	13	13-14	13-13	≥15
Tobramycin	TM-10	10 µg	19	17	19-20	17-18	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	26	25	25-27	25-26	≥18
Chloramphenicol	C-30	30 µg	24	25	23-25	24-26	≥18
Chlortetracycline	A-30	30 µg	28	29	27-28	28-30	≥19*
Clindamycin	CC-2	2 µg	25	23	24-25	22-26	≥21
Erythromycin	E-15	15 µg	25	24	25-25	23-24	≥23
Nitrofurantoin	FD-300	300 µg	13	14	13-13	14-15	≥17
Rifampin	RA-5	5 µg	13	15	12-13	14-15	≥20
Sulfathiazole	ST-300	300 µg	0	0	0	0	≥17
Tetracycline	TE-30	30 µg	29	27	29-31	27-28	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	26	24	26-27	24-24	≥13*
Lincomycin	L-2	2 µg	18	17	17-19	17-17	≥21*
Neomycin	N-5	5 µg	15	13	14-16	13-15	≥17*

* From Difco antibiotic insert, 1989

Table XXXVIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris EB2***											Date: 7.14 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 19.5 hrs; pH 4.16 (broth pH = 6.70) OD @600 = 0.661 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made for overlay only (5 ml culture + 5 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	30	28	28	27	27	22	21	22	21	21
Penicillin G	P-10	10 µg	39	40	40	40	40	31	30	30	32	30
Streptomycin	S-10	10 µg	21	20	21	23	21	21	19	18	17	17
Tobramycin	TM-10	10 µg	25	25	25	25	26	24	23	23	23	23
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	38	39	38	38	39	31	32	32	31	32
Chloramphenicol	C-30	30 µg	30	29	28	28	29	24	26	26	26	28
Chlortetracycline	A-30	30 µg	41	41	40	41	41	33	34	34	34	33
Clindamycin	CC-2	2 µg	35	34	34	34	36	29	28	29	28	28
Erythromycin	E-15	15 µg	36	36	34	34	36	29	28	28	28	29
Nitrofurantoin	FD-300	300 µg	26	26	26	26	26	23	24	23	23	23
Rifampin	RA-5	5 µg	16	16	16	16	16	17	16	17	17	16
Sulfathiazole	ST-300	300 µg	25	25	27	26	24	18	17	18	19	19
Tetracycline	TE-30	30 µg	40	40	40	40	39	34	34	34	33	33
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	27	26	26	26	27	26	26	27	26	26
Lincomycin	L-2	2 µg	18	20	19	20	20	19	20	19	19	19
Neomycin	N-5	5 µg	18	18	19	18	19	16	16	16	15	16

Table XXXVIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris EB2***						Date: 7.14.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 19.5 hrs; pH 4.16 (broth pH = 6.70) OD @600=0.661 Plates (made on 6/6/89) incubated at 30°C for 18.5 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	28	21	27-30	21-22	≥17
Penicillin G	P-10	10 µg	40	31	39-40	30-32	≥28
Streptomycin	S-10	10 µg	21	18	20-23	17-21	≥15
Tobramycin	TM-10	10 µg	25	23	25-26	23-24	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	38	32	38-39	31-32	≥18
Chloramphenicol	C-30	30 µg	29	26	28-30	24-28	≥18
Chlortetracycline	A-30	30 µg	41	34	40-41	33-34	≥19*
Clindamycin	CC-2	2 µg	35	28	34-36	28-29	≥21
Erythromycin	E-15	15 µg	35	28	34-36	28-29	≥23
Nitrofurantoin	FD-300	300 µg	26	23	26-26	23-24	≥17
Rifampin	RA-5	5 µg	16	17	16-16	16-17	≥20
Sulfathiazole	ST-300	300 µg	25	18	24-27	17-19	≥17
Tetracycline	TE-30	30 µg	40	34	39-40	33-34	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	26	26	26-27	26-27	≥13*
Lincomycin	L-2	2 µg	19	19	18-20	19-20	≥21*
Neomycin	N-5	5 µg	18	16	18-19	15-16	≥17*

* From Difco antibiotic insert, 1989

Table XXXVIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris EB4***											Date: 8.04 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 19 hrs; pH 4.14 (broth pH = 6.64) OD @600 = 0.583 Plates (made on 8/1/89) incubated at 30°C for 18.25 hrs. 50% dilution made (2 ml culture + 2 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	20	21	22	20	20	19	20	21	20	20
Penicillin G	P-10	10 µg	31	31	32	31	32	31	31	31	31	31
Streptomycin	S-10	10 µg	16	16	16	16	16	17	17	18	17	17
Tobramycin	TM-10	10 µg	22	22	22	22	22	20	22	21	20	20
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	31	31	31	29	30	28	29	28	28	28
Chloramphenicol	C-30	30 µg	24	25	24	26	24	26	26	27	28	27
Chlortetracycline	A-30	30 µg	31	31	29	31	30	30	31	29	30	30
Clindamycin	CC-2	2 µg	22	23	22	22	22	24	23	23	23	24
Erythromycin	E-15	15 µg	27	27	28	27	27	26	27	27	27	26
Nitrofurantoin	FD-300	300 µg	16	15	16	15	16	18	18	18	17	17
Rifampin	RA-5	5 µg	14	14	15	14	14	14	15	14	14	14
Sulfathiazole	ST-300	300 µg	0	0	0	0	0	0	0	0	0	0
Tetracycline	TE-30	30 µg	31	30	30	29	30	31	31	32	31	31
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	25	26	26	27	26	27	28	27	27	27
Lincomycin	L-2	2 µg	14	14	14	14	13	14	15	13	14	14
Neomycin	N-5	5 µg	14	14	15	14	14	14	14	15	14	14

Table XXXVIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris EB4***					Date: 8.04.89	
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 19 hrs; pH 4.14 (broth pH = 6.64) OD @600 = 0.583 Plates (made on 8/1/89) incubated at 30°C for 18.25 hrs. 50% dilution made (2 ml culture + 2 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989 Susceptible
			Swab	Overlay	Swab	Overlay	
Primary Grouping:							
Amikacin	AN-30	30 µg	21	20	20-22	19-21	≥17
Penicillin G	P-10	10 µg	31	31	31-32	31-31	≥28
Streptomycin	S-10	10 µg	16	17	16-16	17-18	≥15
Tobramycin	TM-10	10 µg	22	21	22-22	20-22	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	30	28	29-31	28-29	≥18
Chloramphenicol	C-30	30 µg	25	27	24-26	26-28	≥18
Chlortetracycline	A-30	30 µg	30	30	29-31	29-31	≥19*
Clindamycin	CC-2	2 µg	22	23	22-23	23-24	≥21
Erythromycin	E-15	15 µg	27	27	27-28	26-27	≥23
Nitrofurantoin	FD-300	300 µg	16	18	15-16	17-18	≥17
Rifampin	RA-5	5 µg	14	14	14-15	14-15	≥20
Sulfathiazole	ST-300	300 µg	0	0	0	0	≥17
Tetracycline	TE-30	30 µg	30	31	29-31	31-32	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	26	27	25-27	27-28	≥13*
Lincomycin	L-2	2 µg	14	14	13-14	13-15	≥21*
Neomycin	N-5	5 µg	14	14	14-15	14-15	≥17*

* From Difco antibiotic insert, 1989

Table XXXVIIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris EB9***										Date: 8.04 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 19 hrs; pH 4.31 (broth pH = 6.64) OD @600 = 0.511 Plates (made on 8/1/89) incubated at 30°C for 18.5 hrs. No dilution made										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm								
			Trials								
			Swab					Overlay			
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	20	20	20	20	20	20	20	19	20	20
Penicillin G	P-10	10 µg	30	30	30	31	30	30	30	30	30	30
Streptomycin	S-10	10 µg	16	16	16	16	17	16	16	16	16	16
Tobramycin	TM-10	10 µg	22	22	22	22	22	21	21	22	22	22
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	28	27	27	27	29	28	28	28	28	27
Chloramphenicol	C-30	30 µg	27	27	26	27	26	27	27	27	28	27
Chlortetracycline	A-30	30 µg	29	28	29	29	29	27	27	28	28	28
Clindamycin	CC-2	2 µg	24	23	23	24	24	24	25	25	25	25
Erythromycin	E-15	15 µg	27	26	27	27	27	27	26	27	27	27
Nitrofurantoin	FD-300	300 µg	0	0	0	0	0	0	0	0	0	0
Rifampin	RA-5	5 µg	17	16	16	17	17	18	18	19	18	18
Sulfathiazole	ST-300	300 µg	20	21	20	21	21	19	19	20	19	20
Tetracycline	TE-30	30 µg	32	32	32	32	32	31	31	31	32	31
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	27	27	27	27	27	27	28	27	27	27
Lincomycin	L-2	2 µg	17	17	17	17	16	16	17	16	16	16
Neomycin	N-5	5 µg	15	15	15	15	15	15	15	15	15	15

Table XXXVIIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris EB9***						Date: 8.04.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 19 hrs; pH 4.31 (broth pH = 6.64) OD @600 = 0.511 Plates (made on 8/1/89) incubated at 30°C for 18.5 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	20	20	20-20	19-20	≥17
Penicillin G	P-10	10 µg	30	30	30-31	30-30	≥28
Streptomycin	S-10	10 µg	16	16	16-17	16-16	≥15
Tobramycin	TM-10	10 µg	22	22	22-22	21-22	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	28	28	27-29	27-28	≥18
Chloramphenicol	C-30	30 µg	27	27	26-27	27-28	≥18
Chlortetracycline	A-30	30 µg	29	28	28-29	27-28	≥19*
Clindamycin	CC-2	2 µg	24	25	23-24	24-25	≥21
Erythromycin	E-15	15 µg	27	27	26-27	26-27	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	17	18	16-17	18-19	≥20
Sulfathiazole	ST-300	300 µg	21	19	20-21	19-20	≥17
Tetracycline	TE-30	30 µg	32	31	32-32	31-32	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	27	27	27-27	27-28	≥13*
Lincomycin	L-2	2 µg	17	16	16-17	16-17	≥21*
Neomycin	N-5	5 µg	15	15	15-15	15-15	≥17*

* From Difco antibiotic insert, 1989

Table XXXIXa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris HP***											Date: 8.02 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.21 (broth pH = 6.72) OD @600 = 0.659 Plates (made on 8/1/89) incubated at 30°C for 19 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	16	15	15	15	15	18	20	20	20	20
Penicillin G	P-10	10 µg	26	27	27	27	27	27	27	27	27	27
Streptomycin	S-10	10 µg	10	10	10	10	10	10	11	10	10	10
Tobramycin	TM-10	10 µg	18	18	18	18	18	21	18	20	20	20
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	27	26	26	26	27	26	26	24	23	25
Chloramphenicol	C-30	30 µg	23	25	24	24	24	26	26	26	25	26
Chlortetracycline	A-30	30 µg	30	29	30	30	26	30	29	31	29	29
Clindamycin	CC-2	2 µg	20	21	21	20	22	22	22	22	23	22
Erythromycin	E-15	15 µg	24	24	24	23	23	25	25	25	26	25
Nitrofurantoin	FD-300	300 µg	13	14	13	13	13	14	13	14	13	14
Rifampin	RA-5	5 µg	14	14	14	14	14	16	15	16	16	16
Sulfathiazole	ST-300	300 µg	18	19	18	19	19	17	17	15	17	16
Tetracycline	TE-30	30 µg	26	27	26	28	26	30	29	30	30	30
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	23	24	24	24	25	23	22	22	23	22
Lincomycin	L-2	2 µg	13	14	13	14	15	15	15	15	15	16
Neomycin	N-5	5 µg	14	14	14	14	14	13	13	12	13	13

Table XXXIXb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris HP***						Date: 8.02.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.21 (broth pH = 6.72) OD @600 = 0.659 Plates (made on 8/1/89) incubated at 30°C for 19 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	15	20	15-16	18-20	≥17
Penicillin G	P-10	10 µg	27	27	26-27	27-27	≥28
Streptomycin	S-10	10 µg	10	10	10-10	10-11	≥15
Tobramycin	TM-10	10 µg	18	20	18-18	18-21	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	26	25	26-27	23-26	≥18
Chloramphenicol	C-30	30 µg	24	26	23-25	25-26	≥18
Chlortetracycline	A-30	30 µg	29	30	26-30	29-31	≥19*
Clindamycin	CC-2	2 µg	21	22	20-22	22-23	≥21
Erythromycin	E-15	15 µg	24	25	23-24	25-26	≥23
Nitrofurantoin	FD-300	300 µg	13	14	13-14	13-14	≥17
Rifampin	RA-5	5 µg	14	16	14-14	15-16	≥20
Sulfathiazole	ST-300	300 µg	19	16	18-19	16-17	≥17
Tetracycline	TE-30	30 µg	26	30	26-28	29-30	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	24	22	23-25	22-23	≥13*
Lincomycin	L-2	2 µg	14	15	13-15	15-15	≥21*
Neomycin	N-5	5 µg	14	13	14-14	12-13	≥17*

* From Difco antibiotic insert, 1989

Table XLa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris ML1***											Date: 8.02 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.33 (broth pH = 6.72) OD @600 = 0.487 Plates (made on 8/1/89) incubated at 30°C for 18.25 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	20	20	19	20	20	20	20	20	20	20
Penicillin G	P-10	10 µg	33	34	32	34	34	31	31	31	31	31
Streptomycin	S-10	10 µg	17	16	17	17	17	17	17	17	17	17
Tobramycin	TM-10	10 µg	22	22	22	22	22	20	21	21	21	22
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	32	31	32	30	31	30	30	31	29	30
Chloramphenicol	C-30	30 µg	25	25	23	24	25	27	26	27	26	27
Chlortetracycline	A-30	30 µg	29	28	29	27	28	23	23	24	24	23
Clindamycin	CC-2	2 µg	25	22	22	22	23	23	23	22	22	22
Erythromycin	E-15	15 µg	26	26	26	27	26	26	28	28	28	27
Nitrofurantoin	FD-300	300 µg	21	20	22	23	22	21	22	22	22	22
Rifampin	RA-5	5 µg	12	13	13	13	13	16	17	16	16	16
Sulfathiazole	ST-300	300 µg	24	23	22	23	23	22	23	22	22	21
Tetracycline	TE-30	30 µg	33	32	33	31	32	31	30	31	31	32
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	26	28	27	27	26	27	27	27	27	27
Lincomycin	L-2	2 µg	11	11	11	11	11	13	13	13	13	14
Neomycin	N-5	5 µg	15	14	15	15	15	14	14	14	15	14

Table XLb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris ML1***					Date: 8.02.89	
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.33 (broth pH = 6.72) OD @600 = 0.487 Plates (made on 8/1/89) incubated at 30°C for 18.25 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	20	20	19-20	20-20	≥17
Penicillin G	P-10	10 µg	33	31	32-34	31-31	≥28
Streptomycin	S-10	10 µg	17	17	16-17	17-17	≥15
Tobramycin	TM-10	10 µg	22	21	22-22	20-22	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	31	30	30-32	29-31	≥18
Chloramphenicol	C-30	30 µg	24	27	23-25	26-27	≥18
Chlortetracycline	A-30	30 µg	28	23	27-29	23-24	≥19*
Clindamycin	CC-2	2 µg	23	22	22-25	22-23	≥21
Erythromycin	E-15	15 µg	26	27	26-27	26-28	≥23
Nitrofurantoin	FD-300	300 µg	22	22	20-23	21-22	≥17
Rifampin	RA-5	5 µg	13	16	12-13	16-17	≥20
Sulfathiazole	ST-300	300 µg	23	22	22-24	21-23	≥17
Tetracycline	TE-30	30 µg	32	31	31-33	30-32	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	27	27	26-28	27-27	≥13*
Lincomycin	L-2	2 µg	11	13	11-11	13-14	≥21*
Neomycin	N-5	5 µg	15	14	14-15	14-15	≥17*

* From Difco antibiotic insert, 1989

Table XLIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris PR-108***											Date: 7.31 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.10 (broth pH = 6.73) OD @600 = 0.819 Plates (made on 6/6/89) incubated at 30°C for 16.17 hrs. No dilution made (nice, well defined zones seen)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	21	21	21	21	20	18	19	18	18	18
Penicillin G	P-10	10 µg	18	18	18	18	18	18	18	19	18	18
Streptomycin	S-10	10 µg	14	14	15	15	14	15	15	15	14	15
Tobramycin	TM-10	10 µg	24	25	24	23	23	20	20	20	20	20
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	30	30	30	30	30	22	23	22	24	22
Chloramphenicol	C-30	30 µg	23	24	24	23	24	27	26	27	26	26
Chlortetracycline	A-30	30 µg	28	28	28	28	26	29	20	28	27	27
Clindamycin	CC-2	2 µg	23	23	23	22	23	23	22	22	23	23
Erythromycin	E-15	15 µg	24	24	26	26	26	26	26	27	26	26
Nitrofurantoin	FD-300	300 µg	19	20	19	18	19	18	18	19	19	19
Rifampin	RA-5	5 µg	13	13	13	13	13	13	13	13	13	13
Sulfathiazole	ST-300	300 µg	20	20	19	19	18	18	19	19	18	18
Tetracycline	TE-30	30 µg	36	34	36	36	35	31	31	30	31	31
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	28	28	29	28	28	26	27	26	26	26
Lincomycin	L-2	2 µg	14	14	14	14	14	14	15	14	14	14
Neomycin	N-5	5 µg	16	16	16	16	16	15	15	15	15	15

Table XLIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris PR-108***						Date: 7.31.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.10 (broth pH = 6.73) OD @600 = 0.819 Plates (made on 6/6/89) incubated at 30°C for 16.17 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm				
			Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989
			Swab	Overlay	Swab	Overlay	Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	21	18	20-21	18-19	≥17
Penicillin G	P-10	10 µg	18	18	18-18	18-19	≥28
Streptomycin	S-10	10 µg	14	15	14-15	14-15	≥15
Tobramycin	TM-10	10 µg	24	20	23-25	20-20	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	30	23	30-30	22-24	≥18
Chloramphenicol	C-30	30 µg	24	26	23-24	26-27	≥18
Chlortetracycline	A-30	30 µg	28	28	26-28	27-30	≥19*
Clindamycin	CC-2	2 µg	23	23	22-23	22-23	≥21
Erythromycin	E-15	15 µg	25	26	24-26	26-27	≥23
Nitrofurantoin	FD-300	300 µg	19	19	18-20	198-19	≥17
Rifampin	RA-5	5 µg	13	13	13-13	13-13	≥20
Sulfathiazole	ST-300	300 µg	19	18	18-20	18-19	≥17
Tetracycline	TE-30	30 µg	35	31	34-36	30-31	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	28	26	28-29	26-27	≥13*
Lincomycin	L-2	2 µg	14	14	14-14	14-15	≥21*
Neomycin	N-5	5 µg	16	15	16-16	15-15	≥17*

* From Difco antibiotic insert, 1989

Table XLIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris 108***											Date: 7.31 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.26 (broth pH = 6.73) OD @600 = 0.658 Plates (made on 6/6/89) incubated at 30°C for 17 hrs. No dilution made (nice, well defined zones seen)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	21	21	20	21	22	20	20	20	20	20
Penicillin G	P-10	10 µg	29	29	30	30	29	29	28	28	29	29
Streptomycin	S-10	10 µg	17	17	16	16	16	17	16	17	16	17
Tobramycin	TM-10	10 µg	21	22	21	22	22	21	21	21	21	21
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	29	27	29	28	29	27	28	27	28	28
Chloramphenicol	C-30	30 µg	25	25	23	26	25	25	26	26	25	26
Chlortetracycline	A-30	30 µg	29	29	27	26	29	29	29	29	28	29
Clindamycin	CC-2	2 µg	21	22	21	20	20	22	22	22	22	21
Erythromycin	E-15	15 µg	26	26	26	26	26	25	25	25	25	25
Nitrofurantoin	FD-300	300 µg	0	0	0	0	0	0	0	0	0	0
Rifampin	RA-5	5 µg	14	13	14	14	14	14	14	14	13	14
Sulfathiazole	ST-300	300 µg	19	18	20	19	18	18	18	18	18	19
Tetracycline	TE-30	30 µg	30	28	29	28	28	29	28	30	29	29
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	26	25	26	27	26	26	26	26	26	26
Lincomycin	L-2	2 µg	11	11	11	11	11	12	11	11	12	12
Neomycin	N-5	5 µg	15	15	16	16	15	15	15	16	15	15

Table XLIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris 108***						Date: 7.31.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.26 (broth pH = 6.73) OD @600 = 0.658 Plates (made on 6/6/89) incubated at 30°C for 17 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	21	20	20-22	20-20	≥17
Penicillin G	P-10	10 µg	29	29	29-30	28-29	≥28
Streptomycin	S-10	10 µg	16	17	16-17	16-17	≥15
Tobramycin	TM-10	10 µg	22	21	21-22	21-21	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	28	28	27-29	27-28	≥18
Chloramphenicol	C-30	30 µg	25	26	23-26	25-26	≥18
Chlortetracycline	A-30	30 µg	28	29	26-29	28-29	≥19*
Clindamycin	CC-2	2 µg	21	22	20-22	21-22	≥21
Erythromycin	E-15	15 µg	26	25	26-26	25-25	≥23
Nitrofurantoin	FD-300	300 µg	0	0	0	0	≥17
Rifampin	RA-5	5 µg	14	14	13-14	13-14	≥20
Sulfathiazole	ST-300	300 µg	19	18	18-20	18-19	≥17
Tetracycline	TE-30	30 µg	29	29	28-30	28-30	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	26	26	25-27	26-26	≥13*
Lincomycin	L-2	2 µg	11	12	11-11	11-12	≥21*
Neomycin	N-5	5 µg	15	15	15-16	15-16	≥17*

* From Difco antibiotic insert, 1989

Table XLIIIa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris from Hansen 101***											Date: 2.11 .90
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 16 hrs; pH 4.16 (broth pH = 7.10) OD @600 = 0.438 Plates (made on 2/6/90) incubated at 30°C for 17 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	20	22	22	21	22	17	19	20	20	19
Penicillin G	P-10	10 µg	30	33	30	31	32	29	30	30	29	30
Streptomycin	S-10	10 µg	14	16	15	15	19	11	12	11	12	11
Tobramycin	TM-10	10 µg	19	20	19	18	19	18	18	18	18	18
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	32	32	30	32	32	29	30	31	30	31
Chloramphenicol	C-30	30 µg	27	28	29	29	29	29	30	30	30	29
Chlortetracycline	A-30	30 µg	33	35	32	32	32	35	37	35	35	34
Clindamycin	CC-2	2 µg	23	22	22	22	23	23	22	23	24	23
Erythromycin	E-15	15 µg	28	27	27	27	27	27	29	29	28	29
Nitrofurantoin	FD-300	300 µg	22	25	25	24	24	19	21	21	22	19
Rifampin	RA-5	5 µg	18	17	17	18	18	18	20	20	20	19
Sulfathiazole	ST-300	300 µg	15	16	14	15	17	17	18	17	17	19
Tetracycline	TE-30	30 µg	32	32	33	31	32	34	34	33	34	34
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	27	28	27	27	27	28	30	29	30	30
Lincomycin	L-2	2 µg	15	16	17	16	16	17	16	15	15	15
Neomycin	N-5	5 µg	13	14	12	12	13	13	14	13	13	14

Table XLIIIb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris from Hansen 101***						Date: 2.11.90
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 16 hrs; pH 4.16 (broth pH = 7.10) OD @600 = 0.438 Plates (made on 2/6/90) incubated at 30°C for 17 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	21	19	20-22	17-20	≥17
Penicillin G	P-10	10 µg	31	30	30-33	29-30	≥28
Streptomycin	S-10	10 µg	15	11	14-19	11-12	15
Tobramycin	TM-10	10 µg	19	18	18-20	18-18	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	32	30	30-32	29-31	≥18
Chloramphenicol	C-30	30 µg	28	30	27-29	29-30	≥18
Chlortetracycline	A-30	30 µg	33	35	32-35	34-37	≥19*
Clindamycin	CC-2	2 µg	22	23	22-23	22-24	21
Erythromycin	E-15	15 µg	27	28	27-28	27-29	23
Nitrofurantoin	FD-300	300 µg	24	20	22-25	19-22	≥17
Rifampin	RA-5	5 µg	18	19	17-18	18-20	≥20
Sulfathiazole	ST-300	300 µg	15	18	14-17	17-19	≥17
Tetracycline	TE-30	30 µg	32	34	31-33	33-34	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	27	29	27-28	28-30	≥13*
Lincomycin	L-2	2 µg	16	16	15-17	15-17	≥21*
Neomycin	N-5	5 µg	13	13	12-14	13-14	≥17*

* From Difco antibiotic insert, 1989

Table XLIVa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Lactococcus cremoris from Microlife Technics***											Date: 2.11.90
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 16 hrs; pH 4.08 (broth pH = 7.10) OD @600 = 0.472 Plates (made on 2/6/90) incubated at 30°C for 17 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)											
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg	24	24	25	24	24	22	24	21	22	22
Penicillin G	P-10	10 µg	35	36	34	34	35	32	31	32	32	31
Streptomycin	S-10	10 µg	22	22	21	19	21	21	22	22	21	22
Tobramycin	TM-10	10 µg	20	20	21	21	21	20	20	20	19	20
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg	35	34	36	33	35	34	33	32	33	32
Chloramphenicol	C-30	30 µg	26	26	27	29	28	30	30	31	31	31
Chlortetracycline	A-30	30 µg	35	36	37	35	36	39	37	38	37	38
Clindamycin	CC-2	2 µg	29	25	27	27	27	28	28	27	27	28
Erythromycin	E-15	15 µg	32	31	32	33	32	33	33	32	33	33
Nitrofurantoin	FD-300	300 µg	23	22	22	23	23	23	22	22	24	24
Rifampin	RA-5	5 µg	16	14	16	16	16	18	17	18	18	17
Sulfathiazole	ST-300	300 µg	0	0	0	0	0	0	0	0	0	0
Tetracycline	TE-30	30 µg	37	36	37	38	38	37	37	37	36	37
Trimethoprim	TMP-5	5 µg	0	0	0	0	0	0	0	0	0	0
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg	31	31	31	31	30	30	32	31	31	32
Lincomycin	L-2	2 µg	14	15	14	14	13	14	13	14	14	15
Neomycin	N-5	5 µg	14	14	14	13	14	14	14	14	13	14

Table XLIVb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus cremoris from Microlife Technics***						Date: 2.11.90
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 16 hrs; pH 4.08 (broth pH = 7.10) OD @600 = 0.472 Plates (made on 2/6/90) incubated at 30°C for 17 hrs. 50% dilution made (5 ml culture + 5 ml Mueller broth)						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg	24	22	24-25	21-24	≥17
Penicillin G	P-10	10 µg	35	32	34-36	31-32	≥28
Streptomycin	S-10	10 µg	21	22	19-22	21-22	≥15
Tobramycin	TM-10	10 µg	21	20	20-21	19-20	≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg	34	33	33-36	32-34	≥18
Chloramphenicol	C-30	30 µg	27	31	26-29	30-31	≥18
Chlortetracycline	A-30	30 µg	36	38	35-37	37-39	≥19*
Clindamycin	CC-2	2 µg	27	28	25-29	27-28	21
Erythromycin	E-15	15 µg	32	33	31-33	32-33	23
Nitrofurantoin	FD-300	300 µg	23	23	22-23	22-24	≥17
Rifampin	RA-5	5 µg	16	18	14-16	17-18	≥20
Sulfathiazole	ST-300	300 µg	0	0	-	-	≥17
Tetracycline	TE-30	30 µg	37	37	36-38	36-37	≥19
Trimethoprim	TMP-5	5 µg	0	0	0	0	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg	31	31	30-31	30-32	≥13*
Lincomycin	L-2	2 µg	14	14	13-15	13-15	≥21*
Neomycin	N-5	5 µg	14	14	13-14	13-14	≥17*

* From Difco antibiotic insert, 1989

Table XLVa: Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:	***Streptococcus faecalis 29212 (ATCC)***										Date: 7.31 .89
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.17 (broth pH = 6.73) OD @600 = 0.827 Plates (made on 6/6/89) incubated at 30°C for 19.75 hrs. No dilution made (nice, well defined zones seen)										
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab					Overlay				
			1	2	3	4	5	1	2	3	4	5
Primary Grouping (NCCLS):												
Amikacin	AN-30	30 µg										
Penicillin G	P-10	10 µg										
Streptomycin	S-10	10 µg										
Tobramycin	TM-10	10 µg										
Secondary Grouping (NCCLS):												
Cephalothin	CR-30	30 µg										
Chloramphenicol	C-30	30 µg										
Chlortetracycline	A-30	30 µg										
Clindamycin	CC-2	2 µg										
Erythromycin	E-15	15 µg										
Nitrofurantoin	FD-300	300 µg										
Rifampin	RA-5	5 µg										
Sulfathiazole	ST-300	300 µg	24	25	24	25	24	23	24	23	23	24
Tetracycline	TE-30	30 µg										
Trimethoprim	TMP-5	5 µg	23	21	21	20	21	22	22	19	22	22
From Difco antibiotic insert, 1989												
Bacitracin	B-10	10 µg										
Lincomycin	L-2	2 µg										
Neomycin	N-5	5 µg										

Table XLVb. Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:	***Lactococcus faecalis 29212 (ATCC)***						Date: 7.31.89
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH 4.17 (broth pH = 6.73) OD @600 = 0.827 Plates (made on 6/6/89) incubated at 30°C for 19.75 hrs. No dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Mean 5 experiments Swab	Overlay	Range 5 experiments Swab	Overlay	Interpretive Standards (mm) NCCLS, 1989 Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg					≥17
Penicillin G	P-10	10 µg					≥28
Streptomycin	S-10	10 µg					≥15
Tobramycin	TM-10	10 µg					≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg					≥18
Chloramphenicol	C-30	30 µg					≥18
Chlortetracycline	A-30	30 µg					≥19*
Clindamycin	CC-2	2 µg					≥21
Erythromycin	E-15	15 µg					≥23
Nitrofurantoin	FD-300	300 µg					≥17
Rifampin	RA-5	5 µg					≥20
Sulfathiazole	ST-300	300 µg	24	23	24-25	23-24	≥17
Tetracycline	TE-30	30 µg					≥19
Trimethoprim	TMP-5	5 µg	21	21	20-23	19-22	≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg					≥13*
Lincomycin	L-2	2 µg					≥21*
Neomycin	N-5	5 µg					≥17*

* From Difco antibiotic insert, 1989

Inhibition zone (diameter measured to the nearest whole mm) by the action of different antibiotics. The antibiotic disks were placed on the surface of Mueller–Hinton agar plates, spread with a culture of bacterial strain indicated below. These antibiotics were also placed on the surface of seeded Mueller-Hinton agar plates. For further details see "growth conditions" indicated below.

Bacterial Strain:													Date:
Growth Conditions:	1% inoculum in Mueller Hinton II broth + 5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH (broth pH =) OD @600 = Plates (made on) incubated at 30°C for hrs. Dilution made												
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm Trials Swab 1	2	3	4	5	Overlay 1	2	3	4	5
Primary Grouping (NCCLS):													
Amikacin	AN-30	30 µg											
Penicillin G	P-10	10 µg											
Streptomycin	S-10	10 µg											
Tobramycin	TM-10	10 µg											
Secondary Grouping (NCCLS):													
Cephalothin	CR-30	30 µg											
Chloramphenicol	C-30	30 µg											
Chlortetracycline	A-30	30 µg											
Clindamycin	CC-2	2 µg											
Erythromycin	E-15	15 µg											
Nitrofurantoin	FD-300	300 µg											
Rifampin	RA-5	5 µg											
Sulfathiazole	ST-300	300 µg											
Tetracycline	TE-30	30 µg											
Trimethoprim	TMP-5	5 µg											
From Difco antibiotic insert, 1989													
Bacitracin	B-10	10 µg											
Lincomycin	L-2	2 µg											
Neomycin	N-5	5 µg											

Average of diameter zones for the 5 experiments from the preceeding table Ia along with the zone diameters for standards to indicate susceptibility as interpreted by NCCLS and by Difco in 1989.

Bacterial Strain:							Date:
Growth Conditions	1% inoculum in Mueller Hinton II broth +5 g/L yeast extract + 5 g/L glucose; incubated at 30°C for 18 hrs; pH (broth pH =) OD @600 = Plates (made on) incubated at 30°C for hrs. Dilution made						
Antimicrobial Agent (Difco)	Symbol	Disk Potency 6.5 mm diam	Zone Diameter, nearest whole mm				
			Mean 5 experiments		Range 5 experiments		Interpretive Standards (mm) NCCLS, 1989
			Swab	Overlay	Swab	Overlay	Susceptible
Primary Grouping:							
Amikacin	AN-30	30 µg					≥17
Penicillin G	P-10	10 µg					≥28
Streptomycin	S-10	10 µg					≥15
Tobramycin	TM-10	10 µg					≥15
Secondary Grouping:							
Cephalothin	CR-30	30 µg					≥18
Chloramphenicol	C-30	30 µg					≥18
Chlortetracycline	A-30	30 µg					≥19*
Clindamycin	CC-2	2 µg					≥21
Erythromycin	E-15	15 µg					≥23
Nitrofurantoin	FD-300	300 µg					≥17
Rifampin	RA-5	5 µg					≥20
Sulfathiazole	ST-300	300 µg					≥17
Tetracycline	TE-30	30 µg					≥19
Trimethoprim	TMP-5	5 µg					≥16
From Difco antibiotic insert, 1989							
Bacitracin	B-10	10 µg					≥13*
Lincomycin	L-2	2 µg					≥21*
Neomycin	N-5	5 µg					≥17*

* From Difco antibiotic insert, 1989