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Alfalfa and bean sprouts have become popular food items in the United States. Alfalfa sprouts are consumed raw and bean sprouts are often cooked briefly. Therefore, the presence of pathogenic bacteria would be a health hazard. Two outbreaks of Bacillus cereus food poisoning have resulted from sprouts. Products from eight producers were sampled from retail outlets and tested to identify the microbial flora, especially those bacteria which may cause illness in humans. Samples were analyzed for total numbers of aerobic bacteria; psychrotrophic bacteria; acid-producing bacteria; the indicator groups of fecal streptococci, coliforms and fecal coliforms; and specific bacteria, including B. cereus, Klebsiella, Pseudomonas aeruginosa, Yersinia enterocolitica, Salmonella and Shigella. Fecal coliform levels ($\geq$ 240,000 per g sprouts) and confirmed Klebsiella counts (0.1-72 x 10^6 cells per g) were consistently high for alfalfa and bean sprouts regardless of producer, sample date, retail outlet,

and whether sprouts were sold bulk or packaged. K. pneumoniae was the species responsible in large part for the fecal coliform positive response and was also the predominant species isolated from MacConkey-Inositol-Carbenicillin (MCIC) plates with about one-fourth being K. oxytoca. The alfalfa and bean sprouts sampled had similar total numbers of bacteria with a combined range of $1.2\text{-}13 \times 10^8$ cells per g. Psychrotrophs represented a large part of the population of both sprouts with a combined range of $0.66\text{-}62 \times 10^7$ cells per g. Coliform counts were similar for alfalfa and bean sprouts with a combined range of $1\text{-}1400 \times 10^5$ cells per g. Both alfalfa and bean sprouts had low total lactic and fecal streptococci counts. No B. cereus, P. aeruginosa, Y. enterocolitica, Salmonella or Shigella were isolated. The temperature range for sprouts in display cases was 7-22°C and no effects attributed to temperature differences were observed. Since sprouts are usually consumed raw or partially cooked, the presence of fecal coliform positive K. pneumoniae in large numbers is a potential public health problem especially for people with decreased resistance. It is suggested that agencies that find high levels of fecal coliforms with IMViC reactions typical of Enterobacter test to distinguish Klebsiella species.

Bacteria of Public Health Significance on Alfalfa and Bean Sprouts at the Retail Level

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BACTERIA OF PUBLIC HEALTH SIGNIFICANCE ON ALFALFA AND BEAN SPROUTS AT THE RETAIL LEVEL

INTRODUCTION

In the past few years alfalfa and bean sprouts have become increasingly popular food items in the United States. They are both available to consumers in restaurants and grocery stores. Raw alfalfa sprouts are most often included in sandwiches and salads. Bean sprouts are primarily eaten cooked as in stir fry vegetable dishes or egg rolls and are less frequently added raw to salads. Directions and sprouting kits have also been made available so that consumers can grow their own sprouts.

To date, there has not been a reported study of the microbial population on sprouts. This kind of information would be helpful for two reasons. One is a matter of public health. Since alfalfa sprouts, and bean sprouts to a lesser extent, are usually consumed raw, many of the microorganisms present would not be destroyed as in cooked foods. Thus, the presence of pathogenic bacteria would be a health hazard. Vegetable sprouts (raw and cooked) were the source of two outbreaks of Bacillus cereus food poisoning (Center for Disease Control, 1974, p. 10, 16; Gilbert and Parry, 1977). This particular type of foodborne illness is rarely reported in the United States, but may be more common than the identified cases indicate (Kim and

Goepfert, 1971). Alfalfa sprouts were the implicated source of an outbreak of gastrointestinal illness in the United States (Center for Disease Control, 1974, p. 23). There has also been some concern regarding the sale of sprouts in open bins due to the possible introduction of pathogens through handling.

There have been reports of disease outbreaks among humans as the result of consuming raw fresh vegetables. Geldreich and Bordner (1971) reviewed fecal contamination and specific pathogens reported on fresh vegetables which included Salmonella and Shigella. Also various common bacteria not usually regarded as pathogens such as fecal streptococci, Klebsiella, Enterobacter, Citrobacter, and Pseudomonas, have been suspected of causing illness when excessive numbers were present in foods (Center for Disease Control, 1977a).

In addition to the public health aspect, information concerning the flora on sprouts will provide a basis for further studies to determine which, if any, microorganisms are responsible for the spoilage of sprouts, which seems to be a problem to markets in Corvallis. One produce manager interviewed estimated a 33% weekly loss of sprouts due to spoilage.

Alfalfa and bean sprouts both contain adequate nutrients to sustain bacterial growth (Kylen and McCready, 1975). The surfaces of vegetables normally support a rich microflora. Sound vegetables

may yield extremely high aerobic plate counts because of contamination from soil or other natural sources, which would not necessarily be related to the safety or quality of the food. Therefore, in order to conduct a meaningful study on the flora of sprouts, specific organisms or groups of organisms which are of significance should be selected.

This study focused on bacteria of public health significance on alfalfa and bean sprouts. Products from various producers of both alfalfa and bean sprouts in Oregon and Washington were sampled from retail outlets. Samples were analyzed for total number of aerobic bacteria; psychrotrophic bacteria; acid-producing bacteria; the indicator groups of fecal streptococci, coliforms and fecal coliforms; and specific bacteria, including B. cereus, Klebsiella, Pseudomonas aeruginosa, Yersinia enterocolitica, Salmonella and Shigella. This information should answer questions regarding the safety of consuming raw sprouts.

REVIEW OF LITERATURE

Bacteria on Alfalfa and Bean Sprouts and Other Vegetables

There is not any information in the literature concerning the bacteria of public health significance on alfalfa and bean sprouts. However, a preliminary study on alfalfa and bean sprout flora was done at Oregon State University by Morita and Woodburn (unpublished data, 1976). The following organisms were found as determined by selective media: fecal streptococci, lactic acid bacteria, and Bacillus polymyxa. Total plate counts averaged 1×10^8 cells per g of sprouts and no fecal coliforms were detected on a limited number of samples. Morita and Woodburn concluded that B. cereus could have been present, but due to the overgrowth of B. polymyxa, detection was impossible. The Food and Dairy Division of the State Department of Agriculture in Salem, Oregon analyzed alfalfa and bean sprouts in 1973, 1974, and 1977 and found high aerobic and coliform plate counts and low numbers of E. coli on some samples (Bob Williams, personal communication).

Bean and alfalfa seeds are likely to have organisms common in soil, especially aerobic sporeformers, including Bacillus; Pseudomonas species; and Enterobacteriaceae members (Duckworth, 1966). Mundt and Hinkle (1976) studied the bacteria within ovules and seeds

of 27 plant varieties, including alfalfa. Of the 19 genera and 49 species found, those isolated in the greatest numbers were B. megaterium, B. cereus, Erwinia herbicola, Flavobacterium devorans, and P. aeruginosa. Less frequently recovered were in the genera Achromobacter, Acinetobacter, Alcaligenes, Brevibacterium, Corynebacterium, Cytophaga, Leuconostoc, Micrococcus, Proteus, Streptococcus, and Xanthomonas. Of the bacteria isolated, 42% were Gram negative rods and 33% were sporeformers.

Bacteria on the surfaces of vegetables normally include lactic acid bacteria, nonfecal coliforms and enterococci, and psychrotrophic organisms, such as Pseudomonas (Duckworth, 1966; Mundt, 1961; Nickerson and Sinskey, 1972, p. 93).

One might ask how pathogens could be introduced to sprouts. There could be pathogens on the seeds themselves, in the water used for growing, in unsanitary production practices, or perhaps in the handling of sprouts sold in open trays by the public.

The procedures used commercially to produce alfalfa and bean sprouts could not be found in the literature. To gain this background information, four producers in Oregon were visited by the investigator. These are discussed in the Results and Discussion Section.

The objective of this study was to identify the microbial flora of alfalfa and bean sprouts, especially those bacteria which may cause illness in humans. There are many tests and methods available for

the assessment of the microbiological safety and quality of foods. According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1978, p. 90), the purpose of the test and the normal history of the product must be considered in the choice and in the interpretation of results. Those microbial tests commonly used for foods are summarized with their applications to raw vegetables.

Total Plate Counts

The aerobic mesophilic (30-35°C) plate count (APC) is commonly used to indicate general microbial quality of foods. Plate counts are based on the assumption that each bacterial cell will form a visible, distinct colony, which is not always the case. Therefore, plate counts are an estimate, not an exact measure, of the total number of bacteria present (Gilliland et al., 1976). Recognized foodborne pathogenic bacteria are mesophiles. Certain genera of mesophilic bacteria normally considered harmless, such as Proteus or Pseudomonas, have been known to cause illness when present in food at high levels (ICMSF, 1978, p. 5). When high levels (10^8 per g) of microorganisms are present, foods, other than those produced by microbial action, are normally unacceptable because of odor, taste, or appearance. However, the surfaces of vegetables normally support a rich microflora, which would not necessarily be related to

safety or quality (Splittstoesser and Mundt, 1976). Therefore, an APC would not have much significance if used alone for vegetables. Aerobic plate counts do give an idea as to what proportion of the total, the organisms isolated represent.

Psychrotrophic bacteria, which grow well at low temperatures, even though their optimum growth temperatures might be 10-30°C higher (Eddy, 1960), include species of Pseudomonas, Flavobacterium, and Alcaligenes (Gilliland et al., 1976). When psychrotrophic bacteria occur in large numbers, the result is often off-flavors and physical deterioration. In refrigerated foods, the presence of high numbers of these bacteria indicates a potential for spoilage during storage and therefore increased perishability. Because sprouts are stored under refrigeration, this type of test could give useful information.

The temperature selection is arbitrary and although many laboratories currently use 7°C which makes comparison possible, it is important to consider the temperature at which the food will probably be stored. If the low temperatures used for psychrotrophic plate counts (PPC) are near the minimum growth temperature for the bacteria, a slight decrease in temperature could suppress growth or a slight increase in temperature could increase growth (Gilliland et al., 1976).

Lactic acid bacteria include the Lactobacillaceae and

Streptococcaceae families with genera Lactobacillus and Leuconostoc, Pediococcus, and Streptococcus, respectively in Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). The term "lactic acid bacteria" describes a group of organisms which are capable of producing lactic acid from a fermentable carbohydrate source (Stamer, 1979). Lactobacilli and streptococci are common to vegetables (Mundt, 1961; Nickerson and Sinskey, 1972, p. 93-94). Acid-producing bacteria are often considered beneficial in that they may lower the pH of the food, which in turn may inhibit the growth of pathogenic bacteria. Concentrated numbers of lactic acid bacteria generate hydrogen peroxide, which inhibits many microorganisms (Stamer, 1979). Lactics (10^8-10^9 cells per g) added to ground beef markedly inhibited psychrotrophic bacteria although effects could not be linked with acid production or pH reduction (Gilliland and Speck, 1975). On the other hand, food spoilage at refrigeration temperatures may be caused by four genera of lactics: Streptococcus, Leuconostoc, Pediococcus, and Lactobacillus (Nickerson and Sinskey, 1972, p. 94). A commonly used, but less specific,test than for lactics is for total acid-producers. Testing for lactics and total acid-producing bacteria will help determine the flora of sprouts.

Indicator Organisms

Pathogens in food are often difficult to enumerate, especially with the presence of competing organisms. This has resulted in the widespread use of "indicator groups" which are more easily isolated and indicate possible exposure to conditions that allowed the introduction and/or multiplication of pathogenic bacteria (ICMSF, 1978, p. 3-4).

Fecal streptococci counts have been used in the food industry to indicate sanitary quality in processed items and water. They are normally present in the intestinal tract of warm and cold-blooded animals as well as insects, but also occur widely in the environment (Deibel and Hartman, 1976). Fecal streptococci are a part of the naturally occurring flora of many plants (Mundt, 1961). Fecal streptococci are resistant to unfavorable conditions such as high and low temperatures, so that they may survive when other enteric pathogens, such as Salmonella or Shigella, would not. The potential pathogenicity of fecal streptococci is generally considered to be low, even though this group may cause infections in debilitated persons (Chordash and Insalata, 1978; Deibel and Hartman, 1976). The significance of this test as an indicator of sanitation may not be as important as some of the others mentioned previously for raw vegetables, but may be useful in determining types of bacteria present.

A second indicator group includes coliform bacteria. The coliform group is comprised of a variety of genera, including Escherichia, Enterobacter, Erwinia, Klebsiella, Aeromonas, Citrobacter, Serratia, and Salmonella. Coliforms are from intestinal and nonintestinal habitats (soil, plants, and water). Therefore, the presence of coliforms indicates possible fecal contamination. Some resistant coliforms, Enterobacter or Erwinia, for instance, may survive longer than enteric pathogens. Also the natural flora of raw vegetables includes nonfecal coliforms. Lettuce has been found an acceptable environment for the growth of coliforms (Maxcy, 1978). For these reasons, this test is generally not suggested to evaluate raw vegetables on a routine basis or as the sole test for possible enteric pathogens. However, the coliform test can be informative when used with other tests such as fecal coliforms, especially when the flora is unknown.

A more specific indicator group than coliforms is fecal coliforms. With the elevated temperatures that are used in the identification, there is more certainty that the bacteria are of fecal origin. Therefore, there is a higher probability that fecal coliforms are associated with enteric pathogens than for coliforms (Fishbein et al., 1976). Recently attention has been drawn to the fact that Klebsiella isolated from environmental sources (including vegetables and vegetable seeds) gives a positive fecal coliform response (Bagley and Seidler, 1977). This test is valid to use for vegetables which are

consumed uncooked.

E. coli is the classic indicator of fecal contamination. It is a normal inhabitant of the intestinal tract of warm-blooded animals and is often present in larger numbers in the environment than pathogens. E. coli has a resistance to environmental conditions similar to that of intestinal pathogens, is easy to isolate if present, and indicates a potential risk that pathogenic organisms are present. It is the best indicator of fecal contamination used at this time, so would be an appropriate test for raw vegetables (Gilliland et al., 1976).

Specific Bacteria of Public Health Importance

Bacillus cereus

Bacillus cereus is an aerobic sporeforming bacterium belonging to the Bacillaceae family, is widely distributed in nature and common to soil, vegetation, and raw and processed foods. B. cereus is considered part of the normal flora of grains such as alfalfa seeds (Mundt and Hinkle, 1976). Although B. cereus is generally regarded as harmless, it can cause gastrointestinal illness if ingested in large numbers (Turnbull et al., 1979). Spores must germinate, outgrow, and multiply to be of any potential danger (Goepfert, 1976). Multiplication of B. cereus has been observed primarily in foods (Buchanan and Gibbons, 1974, p. 534).

For over 25 years B. cereus has been known as an agent causing foodborne illness. B. cereus food poisoning is reported chiefly in European countries. Until 1970, there was not a single, well documented outbreak of B. cereus food poisoning in the United States, whereas in Hungary, it was ranked as the third most common cause of food poisoning (Goepfert et al., 1972). Kim and Goepfert (1971) thought that the infrequency of reported outbreaks in the United States could reflect a lack of examination of suspected foods and of a selective isolation medium rather than absence of occurrence. With the development of selective isolation media and increased awareness of this bacterium, outbreaks of foodborne illness have been confirmed in this country.

There are two distinct forms of B. cereus food poisoning which result in different symptoms. The classic or diarrheal type, documented since 1950, involves a 10-16 hour incubation period after ingestion and symptoms are characterized by profuse diarrhea and abdominal pain. The vomiting type, reported only since 1971, involves a short incubation period of 1-5 hours after ingestion and symptoms of vomiting prevail (Turnbull et al., 1979).

Foods that have been the source of B. cereus food poisoning include meats, chicken soup, gravy mixes, puddings, potatoes, fried rice, and sprouts (Gilbert and Parry, 1977; Portnoy et al., 1976). Sprouts have been the source of two outbreaks of B. cereus food

poisoning in the United States (Gilbert and Parry, 1977; Portnoy et al., 1976). Although only two cases have been reported which involved sprouts, it is probably significant. First of all, B. cereus is rarely identified in the United States as a food poisoning agent. Secondly, roughly 300 outbreaks and 20,000 cases of foodborne diseases are reported annually in the United States, but the actual incidence may be 10-1000 times higher than reported because many people do not seek treatment or health agencies may not report all cases (Sanders et al., 1976). Thirdly, the fact that sprouts are a food item that wasn't popular and widely consumed in the past. Portnoy et al. (1976) reported an outbreak of B. cereus food poisoning resulting from contaminated vegetable sprouts grown in a commercially available sprouting kit. Victims developed symptoms, which included nausea, vomiting, cramps and diarrhea, 6-15 hours after eating the sprouts. Total plate counts and B. cereus counts were made. The samples were also examined by standard techniques for the presence of Salmonella, Shigella and enteropathogenic E. coli. The seeds sold with the sprouting kit were soy, mustard, and cress. The implicated sprouts were found to contain large numbers ($>10^7$ per g) of aerobic spore forming bacteria and B. cereus (10^5-10^7 per g). Soy seeds from new kits contained only B. cereus, while on mustard and cress seeds, B. cereus represented a minor part of the flora. After germination, however, all the

sprouts had large numbers of B. cereus via cross contamination. Other Bacillus species were identified, but no other pathogenic type was isolated.

Since grains are established sources of Bacillus species, it is possible that alfalfa seeds and mung beans could contain B. cereus spores, which in turn, could multiply and ultimately cause gastro-intestinal illness. Therefore, it was concluded that B. cereus is an organism of public health significance.

Klebsiella pneumoniae

The genus Klebsiella belongs to the Enterobacteriaceae family. Species within are K. pneumoniae, K. ozaenae, and K. rhinoscler-matis. The latter two species have rarely been detected in clinical infections and are not usually associated with the environment (Buchanan and Gibbons, 1974, p. 323). K. pneumoniae is widely distributed in soil, water, grains, plants and normally found in the intestinal tract of man and animals (Buchanan and Gibbons, 1974, p. 323). Klebsiella is a recognized opportunistic human and animal pathogen (Martin et al., 1971; Smith et al., 1973; Umsawasdi et al., 1973), however any specific health hazard due to their presence in the environment has not been determined. There are many biotypes of K. pneumoniae, K. oxytoca being one more frequently isolated (Naemura and Seidler, 1978). In past literature Aerobacter aerogenes

was the name used for K. pneumoniae.

The occurrence of Klebsiella in the environment has been a puzzle for sanitary microbiologists. Klebsiella is classified as a coliform, or pollution indicator, although it has been recovered in large numbers in areas apparently devoid of warm-blooded contamination (Brown and Seidler, 1973; Dufour and Cabelli, 1976; Duncan and Razzell, 1972). Some environmental K. pneumoniae isolates produce a positive fecal coliform response, which is indicative of an original fecal origin (Bagley and Seidler, 1977). The actual significance of fecal coliform positive K. pneumoniae from the environment is currently under investigation.

The importance of Klebsiella in nosocomial infections is increasing due to its multiple-antibiotic resistance (Schaberg et al., 1976; Selden et al., 1971). Klebsiella, Pseudomonas, and E. coli account for the majority of the more than two million yearly nosocomial infections in the United States (Mulholland et al., 1975; Smith et al., 1973). Klebsiella is usually associated with urinary and respiratory and surgical wound infections (Martin et al., 1971; Montgomerie et al., 1970; Mulholland et al., 1975). The most severe Klebsiella infections exist in patients with stress or disease resulting in decreased resistance (Smith et al., 1973; Umsawasdi et al., 1973). Klebsiella is an important cause of severe septicemia in cancer patients with a reported mortality rate of 62% (Umsawasdi

et al., 1973). Acute burn patients are susceptible to frequently fatal Klebsiella infections (Smith et al., 1973). Infants in intensive care units are also particularly susceptible and mortality rates of up to 47% have been reported (Selden et al., 1971).

K. pneumoniae was isolated from radish, carrot, rutabaga, turnip, lettuce, and cucumber seeds and from white and red potatoes, cucumbers, radishes, carrots, sweet potatoes, and green onions (Brown and Seidler, 1973). Duncan and Razzell (1972) examined forest samples (water, soil, needles, and bark) and fresh produce (fruits and vegetables) for Klebsiella. Seventy-one percent of 144 isolates from forest and 64% of 152 isolates from fresh produce were classified as Klebsiella, with K. pneumoniae the predominant species. Klebsiella was isolated in salads obtained from hospitals, canteens, and schools (Shooter et al., 1971). In a study of intensive care unit food, Klebsiella was present in 45% of 47 salads as well as other foods (Casewell and Phillips, 1978). There was a correlation among food, fecal, clinical, and kitchen Klebsiella isolates over a four-week period. Serotyping data showed patients seldom acquired an infection or colonization with the type they ingested, but one that had been eaten by another patient. It is implied that transmission occurred via staff hands.

Bagley and Siedler (1977) tested 191 K. pneumoniae isolates of clinical and environmental origin for fecal coliform response.

Eighty-five percent of 58 clinical K. pneumoniae and 16% of 120 environmental K. pneumoniae were fecal coliform positive (FC +). The fecal coliform response was stable in three clinical Klebsiella isolates after 270 generations of growth in sterile pulp mill waste. This indicates that K. pneumoniae of fecal origin in the environment may retain its original fecal coliform response, in which case it would be of health significance. Matsen et al. (1974) compared 266 environmental isolates (natural waters of three states) with human isolates. The biochemical reactions based on 28 tests and virulence in mice were similar. Klebsiella isolates from the environment are indistinguishable phenotypically and serologically from clinical isolates (Bagley and Seidler, 1977). However, because environmental Klebsiella have been found to be more genetically diverse than clinical Klebsiella (Seidler et al., 1975), it is possible that they have never been associated with warm-blooded animals.

Bagley and Seidler (1978a) tested potential pathogenicity by mouse LD_{50} and found no significant difference between Klebsiella cultures of clinical and environmental origins. Also, no statistical difference was found in mean LD_{50} values for FC+ K. pneumoniae and FC- K. pneumoniae and K. oxytoca from either origin. From these findings, it seems that Klebsiella of any type are potentially pathogenic.

Prompted by the relationship between ingested Klebsiella and

nosocomial infection, Bagley and Siedler (1978a) studied the ability of Klebsiella to colonize the intestinal tract of mice. Mice were exposed to Klebsiella by a single oral inoculation or over a period of six days in drinking water, both of which led to colonization. The longer exposure resulted in a higher percentage of colonized animals and fecal densities and even higher values were observed with mice given antibiotics. Fecal coliform positive K. pneumoniae resulted in a higher percentage of colonized animals and fecal densities than FC- K. oxytoca, which generally had higher numbers than FC- K. pneumoniae. It was concluded that the higher colonization of FC+ Klebsiella warrants that they be regarded as a potential health hazard, with K. oxytoca to a lesser degree.

Thus, the fact that Klebsiella is an opportunistic pathogen and is a frequent agent for nosocomial infections has been documented. Vegetable seeds and vegetables have been established as a source of Klebsiella. K. pneumoniae has also been isolated from chlorinated water (Ptak et al., 1973) which could be another possible means by which alfalfa or bean sprouts could gain the organism.

Pseudomonas aeruginosa

Pseudomonas aeruginosa of the family Pseudomonadaceae is commonly found in soil, water, and plants (Buchanan and Gibbons, 1974, p. 222). Although it is harmless for healthy persons, the

organism is capable of causing infections in hospitalized patients who are in some way debilitated, and is therefore a potential pathogen. Woundor blood infections are most common, but P. aeruginosa can also cause urinary and respiratory tract infections (Kominos et al., 1972). Examples of debilitated persons are those with burns; patients receiving antibiotics, drugs, or therapy that are deleterious to cells or interfere with immune responses; patients with catheters; and those who require respiratory equipment (Kominos et al., 1972). P. aeruginosa is resistant to many antibiotics and is therefore a major agent of nosocomial infections (Davis et al., 1973). Treatment of opportunistic P. aeruginosa infections often fails, and the mortality rate in Pseudomonas septicemia may exceed 80% (Davis et al., 1973).

Shooter et al. (1971) isolated P. aeruginosa on raw vegetable salads from hospitals, canteens, and schools. It was suggested that ingestion of P. aeruginosa in food could lead to colonization of the intestine. Kominos et al. (1972) examined hospital vegetables for the presence of P. aeruginosa. Tomatoes, radishes, celery, carrots, endive, cabbage, cucumbers, onions, and lettuce and tomato salads were positive for P. aeruginosa. Tomatoes contained P. aeruginosa most frequently and were the source of the highest counts. Many P. aeruginosa types isolated from these vegetables were identical to those from clinical specimens. Wright et al. (1976) also recovered P. aeruginosa from hospital salad vegetables. These data suggest

vegetables as a source of P. aeruginosa, which may lead to colonization of debilitated persons and possibly cause infections.

The quantity of ingested P. aeruginosa required for intestinal colonization is unknown. One would suspect that persons receiving antibiotics so that an altered intestinal flora results, may not require large numbers for colonization to occur. The fate of ingested P. aeruginosa ($\geq 10^6$ per g) in normal persons was studied with colonization occurring only in conjunction with ampicillin therapy (Buck and Cooke, 1969). Even if the colonized patient does not acquire an infection, P. aeruginosa can be transmitted to other patients and therefore represents a potential danger (Kominos et al., 1972).

Green et al. (1974) found that conditions of high temperature and high relative humidity (27°C, 80-95% RH) favored multiplication of P. aeruginosa in lettuce and bean tissues. The frequent soaking and rinsing in water of alfalfa and bean sprouts during production provides a humid environment. Therefore, sprouts may favor the growth of P. aeruginosa.

Yersinia enterocolitica

Yersinia is a genus of the Enterobacteriaceae family, formerly placed as a species in the Pasturella genus. Pasturella X, P. pseudotuberculosis, and P. pseudotuberculosis-like organisms have been used as names in the past for Y. enterocolitica. Y.

enterocolitica is found in nature, often in association with water, as well as in feces and lymph nodes of sick and healthy animals and man (Buchanan and Gibbons, 1974, p. 332). This bacterium has also been isolated from various foods such as oysters, retail meat, vacuum packaged lamb and beef, well water, ice cream, milk, cheese, and vegetables (Caprioli et al., 1978; Center for Disease Control, 1977b; Hanna et al., 1976; Hanna et al., 1977; Highsmith et al., 1977; Morris and Feeley, 1976; Peterson and Cook, 1963; Schiemann, 1978a; Schiemann, 1978b; Toma and Lafleur, 1974). The increase in frequency of isolation in foods in recent years has caused concern.

Human Y. enterocolitica infections produce a variety of syndromes. A frequent form of infection is acute gastrointestinal illness with fever, abdominal pain, and diarrhea. Other clinical manifestations are pseudoappendicitis, polyarthritis, Reiter's disease, septicemia, meningitis, terminal ileitis, and abscesses of the spleen, colon, and neck (Hanna et al., 1977; Highsmith et al., 1977). An outbreak of intestinal illness among 218 school children in New York resulted from chocolate milk contaminated with Y. enterocolitica (Center for Disease Control, 1977b). Thirty-five children were hospitalized for suspected appenticitis as symptoms of Y. enterocolitica mimic appendicitis. In each of the 13 children that had appendectomies, the appendix was normal or only slightly inflamed. This was the first outbreak of illness from Y. enterocolitica in which

foodborne transmission was documented.

A unique characteristic of Y. enterocolitica, unlike most other enteric organisms, is that it thrives at refrigeration temperatures. Mature sprouts are stored at refrigeration temperatures and therefore may provide a favorable environment for Y. enterocolitica. Also since sprouts are usually consumed raw, the presence of Y. enterocolitica would be a health hazard. Feeley et al. (1976) concluded that any food consumed uncooked containing Y. enterocolitica is unfit for human consumption. Investigations involving food have been limited due to the failure of Yersinia to compete well in the presence of other organisms, special temperature requirements, and the absence or inadequate use of recovery methods (Highsmith et al., 1977).

Salmonella and Shigella

Salmonella and Shigella belong to the Enterobacteriaceae family. All Salmonella species should be regarded as potential pathogens of humans (ICMSF, 1978, p. 160). Three forms of salmonellosis occur in man: enteric fever, septicemia, and acute gastroenteritis (Davis et al., 1973). Mortality is generally low, although the illness is more severe in young and very old persons (ICMSF, 1978, p. 16-20).

Since the entry of Salmonella is almost exclusively via the oral route for humans, the examination of foods for their presence is important. Although foods of animal origin are the primary vehicles

for transmission of salmonellae to humans, non-animal foods have also been found to contain salmonellae (ICMSF, 1978, p. 16-20). Geldreich and Bordner (1971) reviewed the prevalence and survival of salmonellae and shigellae on market vegetables. Ercolani (1976) in Italy found that 68% of 120 lettuce samples and 72% of 89 fennel samples contained salmonellae. Inocula of S. typhimurium were able to grow on lettuce at room temperature (Maxcy, 1978).

Shigella species, like Salmonella, are almost exclusively transmitted to humans via the oral route, and therefore their presence in food is important. All species of Shigella cause dysentery; however, it is much less invasive than salmonellae and rarely causes bacteremia (Davis et al., 1973). The normal habitat of Shigella is the intestinal tract of man and higher monkeys (Buchanan and Gibbons, 1974, p. 319). This bacterium is transmitted to food or water from contaminated feces. Vegetables such as lettuce have been incriminated in shigellosis (Bottiger and Norling, 1974).

Shigella is difficult to isolate from foods. The organism is sensitive to adverse environmental conditions; thus isolation probably indicates recent contamination (Morris et al., 1976). Shigellae have an increased survival at temperatures lower than 25°C (ICMSF, 1978, p. 20-22), which would be the case with stored sprouts. The number of Shigella needed for an infective dose is very low, possibly ten organisms (Morris et al., 1976).

Salmonella and Shigella both are pathogenic to humans and animals, are indicators of fecal contamination in food, and their presence in food consumed raw should cause it to be considered unfit for human consumption. These two genera are of public health importance and have been isolated on fresh vegetables so were included in this study.

Overview

To summarize, tests used to assess the safety and microbial quality of foods, as well as the identification of bacteria which may cause foodborne illness, have been reviewed with emphasis on their applications to sprouts. High numbers of bacteria are expected for aerobic mesophilic counts, which will be useful primarily for comparison purposes. Psychrotrophic and acid-producing bacteria are common to vegetables, and their numbers may influence the keeping quality. Coliforms and fecal streptococci are also found on vegetables and may not be significant other than identifying the normal sprout flora. The presence of E. coli and fecal coliforms, however, is relatively rare and may have sanitary significance. Bacillus cereus in high numbers may be associated with food poisoning. Other pathogenic organisms isolated and observed differences between organisms from packaged and unpackaged sprouts and other

parameters will give information about the safety of sprouts. The data will also provide a starting point for further studies emphasizing microbial spoilage of sprouts.

MATERIALS AND METHODS

Samples

The sprouts analyzed were produced in four different towns in Oregon and in two towns in Washington. Alfalfa sprout samples were products from five companies. All producers marketed alfalfa sprouts in packaged form and one also in bulk form. Bean sprout samples were products from four companies, two of which marketed only in bulk form, one which marketed only in packaged form, and one which marketed both forms. Products from each producer of alfalfa and bean sprouts were analyzed with four replications with the exception of one product which became unavailable after three replications. When a producer's product was available in more than one retail outlet, samples were taken from various markets to avoid an effect by one particular store. Table I shows the products which were purchased from each store.

A summary of sample preparation is shown in Figure 1. The temperature of sprouts in retail display cases was recorded with a temperature probe (Reotemp Instrument Corp., Van Nuys, Cal.) one minute after placement near the center of the sprout mass. For packaged sprouts, one package was selected per sample. Bulk alfalfa and bean sprouts, which were displayed in rectangular trays, were sampled so that all positions (surface, middle, and bottom) were

TABLE I. Producers and Stores from Which Alfalfa and Bean Sprout Samples were Obtained, Number of Replications Per Store, and Whether Marketed Bulk (B) or Packaged (P).

Producer	Product		Stores (A-I) from which samples were obtained and number of replications (1-4)
	Alfalfa	Bean	
1	P		F^3, G^1
2	P		F^1, G^2
3	P		A^2, E^2
4	P		G^1, H^3
5		B	H^4
6		B,P	F^4; G^4
7		B	A^4
8	B,P	P	I^4; B^1, C^1, D^2; B^1, C^1, D^1, E^1

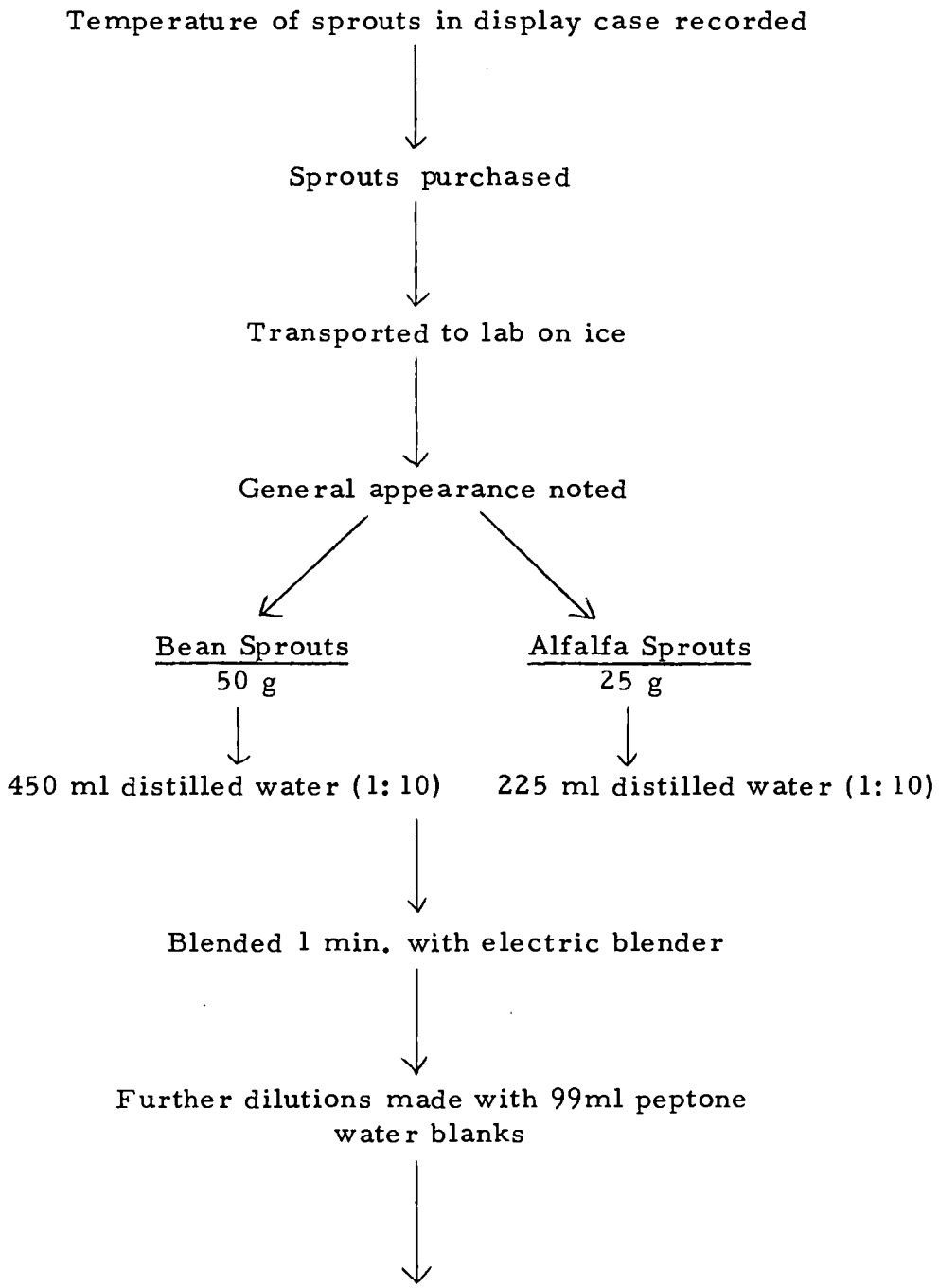

Figure 1. Summary of sample preparation for purchased alfalfa and bean sprouts.

included using the implement provided and placed in plastic bags also provided by the store. Approximately 80 g and 40 g of bean and alfalfa sprouts, respectively, were taken from various places in the tray starting with the center, then two diagonal corners, and finally the inner quadrants. The sprouts were purchased, transported to the laboratory on ice, and analyzed within one hour. The general appearance was noted by the investigator using a scale of excellent, good, fair, and poor quality.

Microbiological Analyses

Sample preparation and analyses were performed using aseptic techniques and standard microbiological methods as described in Compendium of Methods for the Microbiological Examination of Foods (Speck, 1976), Bacteriological Analytical Manual for Foods (Association of Official Analytical Chemists, 1976), Microorganisms in Foods 1 (ICMSF, 1978), and Biochemical Tests for Identification of Medical Bacteria (MacFaddin, 1976). For methods given in more than one source, the Compendium of Methods for the Examination of Foods was referred to as the reference. Bergey's Manual of Determanitive Bacteriology (Buchanan and Gibbons, 1974) was used as a guide for identification of isolates.

Bulk sprouts contained in plastic bags were mixed by shaking and samples were taken directly from each bag with a sterile

implement. The plastic wrap was removed from packaged alfalfa sprouts with the tray side up and portions were taken with sterile tweezers from various spots as described for the bulk samples. For packaged bean sprouts, the top of the bag was swabbed with 70% alcohol and cut open with sterile scissors. The contents were emptied onto plastic wrap and portions taken with sterile tongs as previously described. A double beam balance was used to weigh 25 g of alfalfa into a glass jar containing 225 ml sterile distilled water or 50 g bean sprouts into a glass jar containing 450 ml sterile distilled water. For blending, an Osterizer blender (John Oster Manufacturing Co., Milwaukee, Wisc.) was used. The blending time of one minute was divided into five seconds on low speed and 55 seconds on high speed. From each 1:10 blended dilution, progressive dilutions to 10^7 were prepared with peptone water diluent (Difco, Difco Laboratories, Detroit, Mich.).

Non-Selective and Selective Media

Enumeration media, source of media, incubation times and temperatures, typical positive reactions, and references are given in Table II. One milliliter for pour plates and 0.1 ml for spread plates of three appropriate dilutions were plated for each sample on the following agars: plate count agar (Difco) for total aerobic and total psychrotrophic counts, plate count agar with bromcresol

TABLE II. Media Used for Enumeration, Type of Plate, Incubation Time and Temperature, Typical Positive Appearance, and Reference for the Method.

Test	Medium	Spread (S) or Pour (P) plate	Incubation Time (hrs.)	Incubation Temperature (°C)	Typical Positive	References
total aerobic plate count	plate count agar (Difco)[1]	P	48	30	any colonies	CMMEF[2] p. 107-119
total psychrotrophs	plate count agar	S	240 (10 days)	7	any colonies	CMMEF p. 119-120
total acid-producers	plate count agar with bromcresol purple (Difco)	S	48	30	colonies with yellow halo	CMMEF p. 216-217
total lactobacilli	lactobacillus selection medium (BBL)[3]	P overlay	72	30	any colonies	CMMEF p. 43 Sandine[4] pers. comm.
fecal streptococci	KF streptococcus agar (Difco)	P	48	37	colonies with red or pink centers	CMMEF p. 372-373
total coliforms	violet red bile agar (Difco)	P overlay	24	37	purplish-red colonies with surrounding reddish zone, 0.5 mm diameter	CMMEF p. 386-287
	brilliant green bile broth, 2% (BBL)	-	24-48	37	tubes with gas	
fecal coliforms	lauryl sulfate tryptose broth (LST, Wilson Diagnostics)[5]	-	24-48	37	tubes with gas	CMMEF p. 282-284
	EC broth (Difco)	-	24	44.5 ± 0.5	tubes with gas	
Klebsiella	MacConkey-Inositol-Carbenicillin agar (MCIC)	P	24	37	pink to red colonies	Bagley & Seidler, 1978b
Pseudomonas aeruginosa	pseudosel agar (BBL)	P	48	37	fluorescent green colonies under ultraviolet light	Young, 1977 p. 4-5
Yersinia enterocolitica Salmonella Shigella	bismuth sulfite agar (BBL)	S	48	25	enamel black, black or green, and brown or green colonies	Hanna et al., 1977
Bacillus cereus	phenol-red egg-yolk polymyxin agar (MYP)	S	48	30	flat, dry, translucent to creamy white, wide zone of turbidity, alkaline reacting colonies	CMMEF p. 421-423 Mossel et al., 1967

1. Difco Laboratories, Detroit, Mich.
2. Compendium of Methods for the Microbiological Examination of Foods, Speck, 1976.
3. Division of Becton, Dickenson and Company, Cockeysville, Md.
4. Dr. William Sandine, Microbiology Department, Oregon State University, Corvallis, Ore.
5. Wilson Diagnostics, Inc., Glenwood, Ill.

purple (Difco) for total acid-producers, lactobacillus selection agar (BBL, Division of Becton, Dickenson, and Co., Cockeysville, Md.) for lactobacilli (lactics) count, KF streptococcus agar (Difco) for fecal streptococci count, violet red bile agar (Difco) for total coliform count, MacConkey-Inositol-Carbenicillin agar (Bagley and Seidler, 1978) for Klebsiella count, pseudosel agar (BBL) for Pseudomonas aeruginosa count, bismuth sulfite agar for Yersinia enterocolitica, Shigella or Salmonella counts and phenol-red egg-yolk polymyxin agar (Mossel et al., 1967) for Bacillus cereus count. For the fecal coliform test, one milliliter of each of three dilutions was inoculated into lauryl sulfate tryptose broth (Wilson Diagnostics, Inc., Glenwood, Ill.).

All media were prepared according to the manufacturers' instructions. The following agars were prepared as directed immediately prior to use and were not autoclaved: bismuth sulfite, violet red bile, lactobacillus selection, and pseudosel.

Some of the agars were used as spread plates to avoid possible death of temperature sensitive bacteria or to prevent the growth of spreading bacteria (Gilliland et al., 1976). Lactobacillus selection agar was overlayed to reduce the available oxygen (Etchells and Bell, 1976).

Twenty minutes was the maximum time from the blending of the sample until all media were inoculated. The 1:100 dilution of

each sample was refrigerated at 4°C to serve as an enrichment for Y. enterocolitica. At 14 and 21 days additional platings were made.

After incubation, colonies on agar plates were counted with a Quebec colony counter (Cenco, Central Scientific Co.) using standard methods. To isolate a pure culture, a single well defined colony was streaked on plate count or trypticase soy (Difco) agar plates. From this, a well isolated colony was then picked to a trypticase soy agar slant. For all biochemical testing, 18-24 hour pure cultures were used.

Controls

The following known organisms were obtained from Dr. Raymond Seidler, Department of Microbiology, Oregon State University, Corvallis, Oregon: Yersinia enterocolitica Center for Disease Control strains 75 and 105; Escherichia coli strains 2892 (human clinical) and E55-2 (drinking water); Enterobacter aerogenes ATCC (American Type Culture Collection) 13048; Pseudomonas aeruginosa; Klebsiella pneumoniae strains SL646 (human clinical), SL691 (human clinical, fecal coliform positive), E44.5-1 (redwood, fecal coliform positive) and V128 (vegetable, fecal coliform negative); and Klebsiella oxytoca strains JH1-3 and UOMS. Working cultures were kept and transferred periodically on brain heart infusion (BHI, Difco) or trypticase soy agar slants. The following organisms were obtained from Dr. William

Sandine, Department of Microbiology, Oregon State University, Corvallis, Oregon: Lactobacillus acidophilus N2P and Streptococcus faecalis 8043. These cultures were transferred weekly to skim milk broth (Galloway-West Co., Fond Du Lac, Wisc.). A Lactobacillus plantarum strain from pickles was obtained from Mr. Dan Gallager, Department of Food Science and Technology, Oregon State University, Corvallis, Oregon. This culture was transferred periodically to Rogosa MRS agar (Difco) slants. A known Bacillus cereus strain 158/73 from a food poisoning outbreak was obtained from Dr. J. M. Goepfert, Food Research Institute, University of Wisconsin, Madison, Wisc. This culture was transferred periodically to BHI or trypticase soy agar slants.

Two control plates, one with a known organism and one uninoculated were plated for each enumeration medium at each sampling day. A plate count agar plate was exposed for 15 minutes with each plating to check on bacteria in the air. The biochemical tests throughout the study were always incubated with a known positive, negative, and uninoculated control.

Total Coliforms Assay

To determine total coliforms, the solid medium method was used (Table II). After typical colonies were counted, ten colonies or fewer if ten were not present were picked to separate tubes of

brilliant green bile broth, 2% (BBL) containing fermentation tubes. These tubes were examined for gas production after 24-48 hours incubation at 37°C. The number of coliforms per g of sample was determined by multiplying the violet red bile agar count by the dilution factor used, and by the percentage of gas producing tubes.

Fecal Coliform Test

The method used for the most probable number (MPN) of fecal coliforms (FC) is summarized in Figure 2. Sample dilutions of 10^3, 10^4, 10^5 were found to be most satisfactory. One ml of each of the three dilutions was inoculated into three tubes of lauryl sulfate tryptose (LST) broth making up the nine tube MPN. Tubes were examined for gas production after 24-48 hours incubation at 37°C. All positive tubes were subcultured to EC broth (Difco) and incubated in a circulating water bath at 44.5°C for 24 hours with a known fecal coliform positive (FC+), a known FC-, and an uninoculated control. The MPN tables in Bacteriological Analytical Manual for Foods (AOAC, 1976) were used to determine numerical values for fecal coliforms. The confirming test for E. coli used in this study differs from the Association of Official Analytical Chemists (AOAC) method because only four gas positive tubes (two tubes each from the two highest positive dilutions) instead of the total number of positives

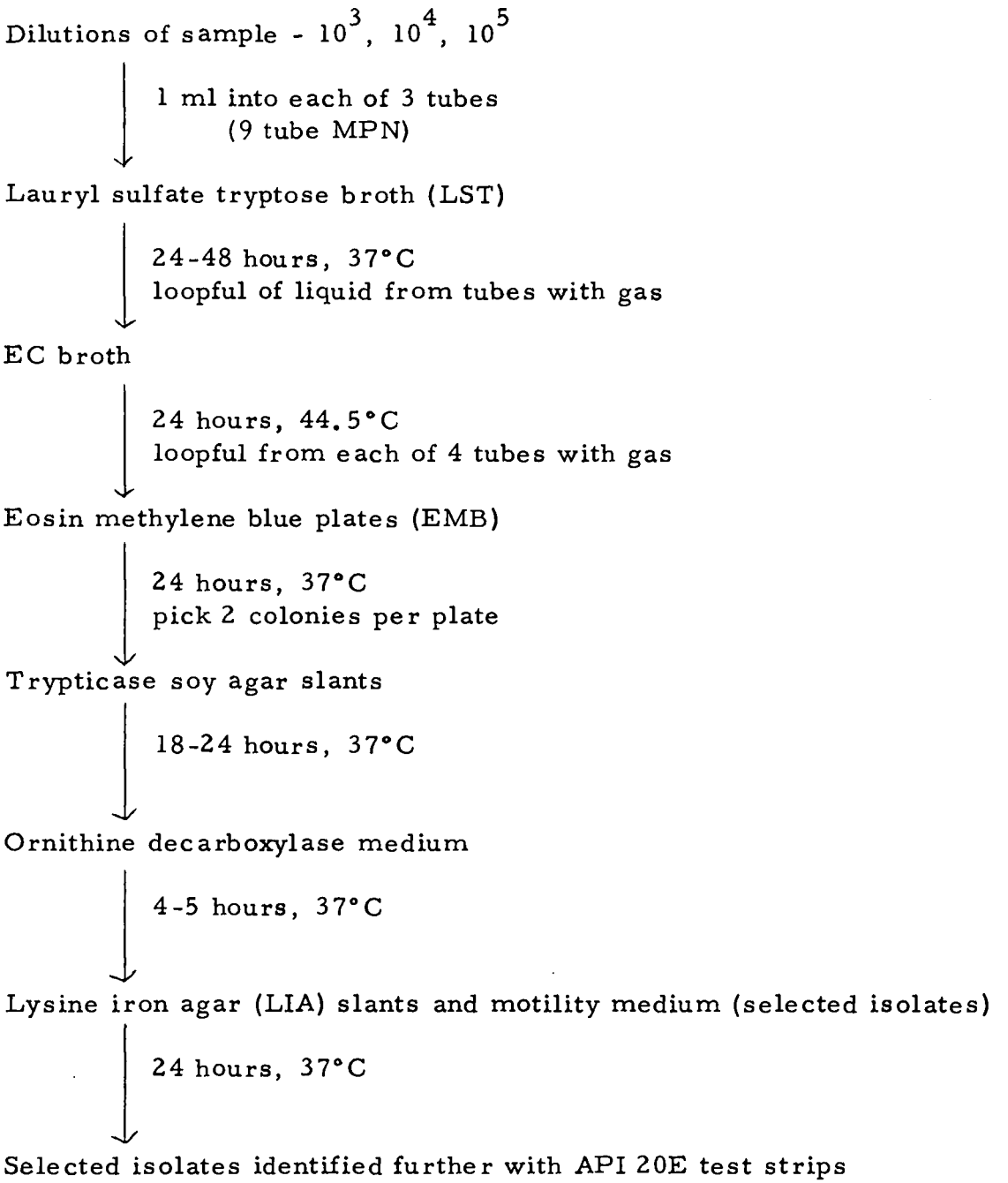

Figure 2. Method used to identify fecal coliforms.

were streaked onto eosin methylene blue (EMB, Inolex Corporation, Glendale, Ill.) plates. The plates were incubated at 37°C for 24 hours and then examined for typical colonies. According to standard methods, colonies are picked and "IMViC" designating Indole; Methyl Red; Voges-Proskauer; and Citrate tests are performed. E. coli typically gives a reaction of + + - - and E. aerogenes, a common nonfecal coliform, typically is - - + +. Early in this study, the IMViC tests were found unsatisfactory for reasons discussed later. Two colonies were picked from each EMB plate to trypticase soy agar slants and incubated at 37°C for 18-24 hours. If colonies were not well isolated on EMB plates, they were first streaked on plate count agar plates. To test for ornithine decarboxylase, a rapid method by Fay and Barry (1972) was used. Moeller's decarboxylase broth (Difco) with 1% ornithine hydrochloride (Difco) was inoculated with 18 hour cultures and incubated 4-5 hours at 37°C. Isolates which were negative for ornithine decarboxylase were then inoculated into lysine iron agar (LIA, BBL) slants and motility test medium (Difco) deeps for 24 hours at 37°C. Selected isolates were further identified with API 20E test strips (Analylab Products, Plainview, N. Y.) using the API 20E code Analytical Profile Index (Analylab Products, Plainview, N. Y.).

Klebsiella

Five colonies or fewer when five were not present from MacConkey-Inositol-Carbenicillin (MCIC) plates were isolated as pure cultures and inoculated into LIA slants and motility test medium deeps, both of which were incubated at 37°C for 24 hours. Klebsiella typically produces lysine decarboxylase and is nonmotile. Isolates that gave reactions typical for Klebsiella were then inoculated into ornithine decarboxylase broth as described for fecal coliform identification. Klebsiella are typically ornithine decarboxylase positive. Selected isolates were tested with API 20E test strips as a check on the biochemical tests used for confirmation.

Y. enterocolitica, Salmonella and Shigella

The refrigerated samples were streaked to several bismuth sulfite agar plates at 14 and 21 days. The plates were incubated at 25°C for 48-72 hours and examined for black colonies. The biochemical tests used for screening and typical reactions for the three organisms are shown in Figure 3. Suspected colonies from refrigerated and nonrefrigerated samples were isolated in pure culture form, then inoculated into triple sugar iron agar (TSI-Difco) slants for 24 hours at 25°C to test for fermentation and hydrogen sulfide production. Y. enterocolitica gives a reaction of acid slant, acid

Nonrefrigerated and
Refrigerated samples - streaked at 14 and 21 days, 4°C

↓

bismuth sulfite

↓ 48 hours, 25°C
black colonies

Confirmatory Tests and Typical Reactions

1. TSI[1]	24 hours, 25°C	Y. enterocolitica	A/A/-
		Salmonella	K/A/- or +
		Shigella	K/A/-
2. Urea[1]	24 hours, 37°C	Y. enterocolitica	+
		Salmonella	-
		Shigella	-
3. Motility[1]	24 hours, 37°C	Y. enterocolitica	-
		Shigella	-
		Salmonella	+
	24 hours, 25°C	Y. enterocolitica	+
		Shigella	-
		Salmonella	+
4. API 20E test strips[2]			

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[1] Difco Laboratories, Detroit, Mich.
[2] Analytab Products, Plainview, N. Y.

Figure 3. Identification scheme for possible Yersinia enterocolitica, Salmonella, or Shigella isolates enumerated from bismuth sulfite agar (BBL, Division of Becton, Dickenson, and Co., Cockeysville, Md.).

butt, no gas and no hydrogen sulfide produced. Salmonella and Shigella give a reaction of alkaline slant, acid butt, no gas, and may or may not produce hydrogen sulfide. Isolates which gave reactions typical for Y. enterocolitica were inoculated into urea broth (Difco) and incubated 24 hours at 37°C to test for urease. Motility test medium was also inoculated and incubated at both 25°C and 37°C for 24 hours. Y. enterocolitica typically produces urease and is motile at 25°C but nonmotile at 37°C. Selected positive isolates from bismuth sulfite plates were tested with API 20E test strips.

B. cereus

Depending upon the number of typical colonies present, up to ten colonies per sample were picked from the MYP (phenol-red egg-yolk polymyxin) plates. All media used for possible B. cereus isolates were incubated at 30°C for 24-48 hours. Glucose (Difco-1%), glycerol (Mallinckrodt reagent-1%), salicin (Difco-0.05%) and arabinose (Difco-1%) were added to phenol red carbohydrate broth base (Oxoid, Consolidated Lab., Inc., Chicago Heights, Ill.) containing fermentation tubes. B. cereus typically produces acid from glucose, glycerol, salicin, and no acid from arabinose. Nitrate broth (Difco) was inoculated to test for ability to reduce nitrate to nitrite, which B. cereus typically does. MR-VP broth (Difco) was inoculated to test for the presence of acetoin (acetylmethylcarbinol)

which is an end product of glucose fermentation. B. cereus is typically positive for acetoin (VP+). If an isolate gave reactions positive for B. cereus in the biochemical tests mentioned above, it was then inoculated into gelatin agar (BBL) deep tubes to test gelatinase activity and also into tubes of O-F medium (BBL) to test for the anaerobic utilization of glucose. B. cereus would typically be positive in both cases.

P. aeruginosa

Pseudosel agar (BBL) plates were examined under ultraviolet light for colonies with blue-green fluorescence typical of P. aeruginosa. Fluorescent colonies were streaked on pseudosel and trypticase agar plates for isolation, then transferred to trypticase soy agar slants. Growth at 42°C, motility at 37°C, and the presence of oxidase are typical of P. aeruginosa. The oxidase test used is described in API instructions. Selected isolates which gave positive reactions to the biochemical tests were tested with API 20E.

RESULTS AND DISCUSSION

Production of Alfalfa and Bean Sprouts

The production of alfalfa and bean sprouts involves the same basic principles, even though the four producers visited had their own individualized systems. The particular seed used will determine the general size of sprouts, the ease in which hulls are removed during growth and thus are an important factor for the quality of the end product. The beans or seeds are soaked in tap water at room temperature for four to twelve hours to initiate germination. Alternate cycles of rinsing and of soaking with water for a short period are employed every four to six hours to remove heat generated during growth. Alfalfa sprouts are exposed to sunlight after two to three days to develop their characteristic green color. After four to six days the sprouts are ready to market. Sprouts are packaged in plastic bags, placed in a styrofoam tray and covered with plastic wrap or in the case of sprouts to be marketed in bulk form, the sprouts are packaged in large (20 pound capacity) plastic sacks. At this point sprouts are refrigerated until transported to retail outlets in refrigerated or nonrefrigerated trucks. Retail produce managers usually display a limited quantity of sprouts which they replenish as needed and keep the remaining supply in a cold storage area.

The producers generally used different growing containers

(30 gallon glass jars, 10 gallon metal cylinders, 4 x 4 x 2 feet deep fiberglass tubs and 2 feet by 2 feet by 2 inches deep plastic trays), and time schedules for rinsing and soaking. At the end of each cycle the producers rinse the containers and room used for growth with a chlorine solution. No attempts were made to control the growth environment. Instead, operators adjusted their time schedules according to the weather (growth cycles are slower in the winter than summer). The production sites consisted of two garages, one house, and a restaurant. One particular site was situated on a dirt parking lot with several plants hung in the sprout growing room, both of which could contribute to the microbial population on sprouts.

Total Microbial Population

Total microbial numbers on selective and nonselective media (Tables III and IV) were generally consistent for alfalfa and bean sprouts regardless of producer, retail outlet, sample date, temperature of display case, and whether sprouts were marketed bulk or packaged.

The alfalfa and bean sprouts sampled had similar aerobic plate counts (APC); the combined range was 1.2-13 x 10^8 cells per g (Tables III and IV). In fresh vegetables, 10^8 cells per g is not considered a large population due to contact of seeds with soil nor is it indicative of detrimental quality or safety (Splittstoesser and Mundt,

TABLE III. Total Microbial Numbers Per Gram (except for fecal coliforms) on Selective and Nonselective Media, Sampling Date, Source, and Temperature of Display Case for Alfalfa Sprout Samples.

Date 1978	Producer-Store	Pkg. or Bulk	Temp. °C	APC[1] (x 10^8)	PPC[2] (x 10^6)	Lactics[3] (x 10^3)	Fecal Strep[4] (x 10^3)	Total Coliforms[5] (x 10^5)	Fecal Coliforms[6] (MPN per 100 g)		Klebsiella[7] (x 10^6)
7-10	1-F	P	16	3.4	< 0.1 Est[8]	< 1.0 Est	1.0 Est	88	≥ 2,400	(a)	3.4
9-12	1-F	P	16	9.5	160	< 1.0 Est	< 1.0 Est	24 Est	≥ 240,000	(c)	34
9-19	1-F	P	14	5.5	160	< 1.0 Est	< 1.0 Est	19	≥ 240,000	(c)	10
9-21	1-G	P	15	11	190	1.0 Est	< 1.0 Est	20 Est	46,000	(c)	4.7
7-10	2-G	P	18	7.2	20 Est	< 1.0 Est	2.0 Est	48	≥ 2,400	(a)	32
8-22	2-G	P	10	7.5	50 Est	6.0 Est	1.0 Est	90	≥ 24,000	(b)	20
9-21	2-F	P	18	5.9	210	1.0 Est	1.0 Est	1.0 Est	≥ 240,000	(c)	34
8-15	3-A	P	14	4.4	190 Est	< 1.0 Est	< 1.0 Est	24 Est	2,400	(b)	9.4
8-23	3-A	P	14	1.5	39 Est	< 1.0 Est	< 1.0 Est	11 Est	≥ 24,000	(b)	11
9-12	3-E	P	22	13	620	5.0 Est	< 1.0 Est	13 Est	110,000	(c)	5.3
9-21	3-E	P	20	3.9	140	1.0 Est	< 1.0 Est	4.5	≥ 9,300	(c)	1.5 Est
7-17	4-H	P	13	LA[9]	20 Est	3.0 Est	4.0 Est	1400 Est	12,400	(a)	< 0.1 Est
8-23	4-H	P	15	3.5	15 Est	< 1.0 Est	< 1.0 Est	82	≥ 24,000	(b)	12
9-14	4-H	P	16	12	190	< 1.0 Est	< 1.0 Est	300	≥ 240,000	(c)	36
9-19	4-G	P	17	11	190	< 1.0 Est	4.0 Est	460	≥ 240,000	(c)	29
7-17	8-D	P	16	2.3 Est	< 10 Est	< 1.0 Est	1.0 Est	21	≥ 2,400	(a)	0.1 Est
8-15	8-C	P	16	3.6	150 Est	< 1.0 Est	2.0 Est	7.2 Est	≥ 24,000	(b)	1.1
8-22	8-B	P	19	3.1	100 Est	8.0 Est	< 1.0 Est	10 Est	LA		11
9-14	8-D	P	17	1.4	120	< 1.0 Est	< 1.0 Est	1.4 Est	24,000	(c)	1.7 Est
9-12	8-I	B	10	1.2	120	1.0 Est	1.0 Est	10 Est	24,000	(c)	3.0
9-19	8-I	B	9	4.3	310	< 1.0 Est	< 1.0 Est	3.3 Est	21,000	(c)	1.1 Est
9-21	8-I	B	11	4.8	320	< 1.0 Est	< 1.0 Est	18 Est	110,000	(c)	5.5
9-27	8-I	B	13	2.3	160	< 1.0 Est	64	7.0 Est	≥ 240,000	(c)	4.8
Range				1.2-13	< 0.1-620	< 1.0-8.0	< 1.0-64	1.0-1400	≥ 2,400-≥ 240,000		< 0.1-36
Median				4.4	140	< 1.0	< 1.0	19			5.4

(a) 10^1, 10^2, 10^3, 10^4
(b) 10^2, 10^3, 10^4, 10^5
(c) 10^3, 10^4, 10^5

1. Aerobic plate count, 30°C, 48 hr, plate count agar (Difco, Difco Laboratories, Detroit, Mich.)
2. Psychrotrophic plate count, 7°C, 10 days, plate count agar (Difco)
3. Total lactobacilli, 30°C, 72 hr, lactobacillus selection medium (BBL, Division of Becton, Dickenson and Company, Cockeysville, Md.)
4. Fecal streptococci count, 37°C, 48 hr, KF streptococcus agar (Difco)
5. Total coliforms - standard methods, violet red bile agar (Difco); brilliant green bile broth, 2% (BBL)
6. Fecal coliforms - standard methods (44.5°C, 24 hr), lauryl sulfate tryptose broth (LST, Wilson Diagnostics, Inc., Glenwood, Ill.); EC broth (Difco)
7. Klebsiella count, 37°C, 24 hr - confirmed biochemically, MacConkey-Inositol-Carbenicillin agar (MCIC, Bagley and Seidler, 1978b)
8. Estimated (Est) counts: if the sign < precedes the number there were no colonies on the lowest plated dilution; Est. counts represent countable colonies, but not within range of 30-300 colonies.
9. Laboratory accident

TABLE IV. Total Microbial Numbers Per Gram (except for fecal coliforms) on Selective and Nonselective Media, Sampling Date, Source, and Temperature of Display Case for Bean Sprout Samples.

Date 1978	Producer-Store	Pkg. or Bulk	Temp. °C	APC[1] (x 10^8)	PPC[2] (x 10^6)	Lactics[3] (x 10^3)	Fecal Strep[4] (x 10^3)	Total Coliforms[5] (x 10^5)	Fecal Coliforms[6] (MPN per 100 g)		Klebsiella[7] (x 10^6)
7-17	5-H	B	7	2.5 Est[8]	6.6	240	47	52	≥ 2,400	(a)	0.1 Est
8-23	5-H	B	22	3.7	20	12 Est	3.7	28	≥ 24,000	(b)	5.7
9-14	5-H	B	9	6.5	23	< 1.0 Est	6.4	8.0 Est	≥ 240,000	(c)	4.1
9-27	5-H	B	9	1.8	16	18 Est	1300	24 Est	≥ 240,000	(c)	6.1
7-10	6-F	P	16	3.1	2.5 Est	< 1.0 Est	< 1.0 Est	43	1,100	(a)	5.5
9-12	6-F	P	17	7.8	30	1200	150	180	≥ 240,000	(c)	12
9-14	6-F	P	15	1.2	67	73	5.6	9.0 Est	≥ 240,000	(c)	3.0
9-19	6-F	P	15	4.2	250 Est	< 1.0 Est	4.9	3.0	≥ 240,000	(c)	5.6
7-10	6-G	B	16	12	< 0.1 Est	130000 Est	1600	70	≥ 2,400	(a)	9.8
8-22	6-G	B	14	13	1.4 Est	68	230	49	≥ 24,000	(b)	72
9-12	6-G	B	16	5.7	20	3000	710	200	≥ 240,000	(c)	45
9-19	6-G	B	14	7.0	110	4200	960	32	≥ 240,000	(c)	37
8-15	7-A	B	14	4.9	7.9	1800 Est	240	320 Est	≥ 24,000	(b)	2.1 Est
8-23	7-A	B	14	11	170 Est	6100	630	44	≥ 24,000	(b)	35
9-14	7-A	B	14	5.9	18 Est	5000 Est	620	67	≥ 240,000	(c)	43
9-19	7-A	B	10	7.3	24 Est	29000	1600 Est	30	≥ 240,000	(c)	18
7-17	8-D	P	13	3.9	6.8	5.0	4.8	36	≥ 2,400	(a)	0.3
8-15	8-C	P	16	6.2	210	4.0	42	30	≥ 24,000	(b)	23
8-22	8-B	P	14	1.2	1.6	< 1.0	54	18	≥ 24,000	(b)	8.6
9-12	8-E	P	20	2.3	28	210	6.3	32	≥ 240,000	(c)	6.3
Range				1.2-13	< 0.1 250	< 1.0-29000	< 1.0-1600	< 3.0-320	≥ 2,400-≥ 240,000		0.1-72
Median				4.9	20	68	54	32			8.6

(a) 10^1, 10^2, 10^3
(b) 10^3, 10^4, 10^5
(c) 10^5, 10^6, 10^7

1. Aerobic plate count, 30°C, 48 hr, plate count agar (Difco, Difco Laboratories, Detroit, Mich.)
2. Psychrotrophic plate count, 7°C, 10 days, plate count agar (Difco)
3. Total lactobacilli, 30°C, 72 hr, lactobacillus selection medium (BBL, Division of Becton, Dickenson and Company, Cockeysville, Md.)
4. Fecal streptococci count, 37°C, 48 hr, KF streptococcus agar (Difco)
5. Total coliforms - standard methods, violet red bile agar (Difco); brilliant green bile broth, 2% (BBL)
6. Fecal coliforms - standard methods (44.5°C, 24 hr), lauryl sulfate tryptose broth (LST, Wilson Diagnostics, Inc., Glenwood, Ill.); EC broth (Difco)
7. Klebsiella count, 37°C, 24 hr - confirmed biochemically, MacConkey-Inositol-Carbenicillin agar (MCIC, Bagley and Seidler, 1978b)
8. Estimated (Est.) counts: If the sign < precedes the number there were no colonies on the lowest plated dilution; Est. counts represent countable colonies, but not within range of 30-300 colonies.

1976). Studies by others on fresh vegetables show high total counts. Plate counts on fresh lettuce were 10^3 cells per g (Bolin et al., 1977) and 10^5 cells per g (Ercolani, 1976; Maxcy, 1978). Fresh cabbage had approximately 10^5 cells per g (King et al., 1976).

Psychrotrophic counts (PPC) were approximately 10-fold lower than APC's (Table III and IV). The combined range was 0.66-62 x 10^7 cells per g. From these data it appears that the bacterial flora on sprouts include a substantial population of psychrotrophs, which by definition are able to grow at refrigeration temperatures. However, many psychrotrophic organisms can grow at 32°C and mesophilic organisms are able to grow at 7°C, so there is not an absolute dividing line between the two. High numbers of psychrotrophs could contribute to the perishability of sprouts. The history of each sample is not known so it is possible that the sprouts were held under conditions permitting growth of mesophilic organisms. The consistency in PPC among producers and retail outlets suggests that differences in storage temperatures or history did not influence the numbers of psychrotrophs. It is interesting that even though alfalfa and bean sprouts had the same number of psychrotrophs per gram, bean sprouts have been observed by several retail produce managers to have a shorter shelf life than alfalfa sprouts. Therefore, the significance of high numbers of psychrotrophs on sprouts is not clear. One might compare the PPC's of spoiled sprouts to nonspoiled to

determine whether or not psychrotrophs actually contribute to spoilage. Another approach would be inoculation studies. Inoculum from spoiled sprouts could be inoculated to fresh sprouts and spoilage rates compared to uninoculated controls. No data on PPC's for similar vegetables were found in the literature.

The general appearance of samples as rated by the investigator did not show any trends with regard to microbial numbers. Alfalfa sprout sample (3-E, 9-12) rated as excellent quality, had a PPC of 62×10^7 cells per g (APC of 13×10^8 cells per g); alfalfa sprout sample (3-E, 9-21) rated as good quality, had a PPC of 14×10^7 cells per g (APC of 3.9×10^8 cells per g); alfalfa sprout sample (2-F, 9-21) rated as fair quality had a PPC of 21×10^7 cells per g (APC of 5.9×10^8 cells per g); and another alfalfa sprout sample (4-H, 9-14) rated as poor quality, had a PPC of 19×10^7 cells per g (APC 12×10^8 cells per g). In the examples cited above, the sample rated as excellent had a slightly higher PPC than those rated as good, fair, and poor which resulted in similar PPC's. The excellent and poor rated samples above had equivalent APC's.

The temperature of sprouts in retail outlets was included to determine if this parameter had an effect on the microbial counts. From the data, an effect is not apparent (Tables III and IV). The temperature range was 7-22°C. For example, bean sprout sample (5-H, 9-27) at 9°C, had an APC of 1.8×10^8 cells per g and bean

sprout sample (5-H, 8-23) at 22°C, had an APC of 3.7×10^8 cells per g. In general, stores displayed a limited quantity of sprouts and replaced them as needed from supplies held in a refrigerated storage area. Thus, display temperature effects were often short term.

Five psychrotrophic colony types from bulk alfalfa sprout sample (8-I, 9-19) were identified using API 20E test strips as follows: 2, Pseudomonas; 1, Alcaligenes; 1, Flavobacterium; and 1, Moraxella. The first three genera are common psychrotrophs.

Pseudomonas aeruginosa

No P. aeruginosa were recovered on pseudosel agar from the alfalfa and bean sprout samples. One sample, which had fluorescent colonies under ultraviolet light, was identified as P. fluorescens, a common spoilage psychrotroph, with API 20E. Pseudosel agar is comparable to other media used for P. aeruginosa isolation (Grant and Holt, 1977; Hart and Kite, 1977; Lambe, Jr. and Stewart, 1972). Lambe, Jr. and Stewart (1972) compared pseudosel agar to another agar selective for P. aeruginosa and reported no significant difference between the growth of 304 P. aeruginosa strains on the two agars, but pseudosel was a more satisfactory medium to detect the production of pigments which aids in the identification of P. aeruginosa. Grant and Holt (1977) have proposed a new medium which they have found to be superior to pseudosel and three other Pseudomonas agars.

However, because their proposed medium is not specifically selective for P. aeruginosa, the greater cost and preparation time, and its lack of use by others, it was not chosen for this experiment. Hart and Kite (1977) compared four selective agars for the isolation of Pseudomonas (including pseudosel) and concluded the four selective agars were not suitable for the isolation of small numbers of Pseudomonas and suggested an enrichment in soybean casein digest broth. An enrichment step might have aided in the recovery of P. aeruginosa in this study. There is a need for a better selective medium for P. aeruginosa.

Total Acid-Producers and Lactobacilli

Fewer than 10^3 acid-producing bacteria per g were identified on the bromcresol purple plates from all of the samples. In preliminary studies, however, positive plates were observed, which was one reason this test was included. Perhaps a lower dilution would have enumerated acid-producers. Due to the large population that grew on this nonselective and noninhibitory agar, the higher dilutions were necessary.

Both alfalfa and bean sprouts had low total lactobacilli (lactics) counts. Two-thirds of the alfalfa sprout samples gave no colonies on the lowest plated dilution (10^3) and the others only very low numbers ($1\text{-}8 \times 10^3$ cells per g). On the other hand, two-thirds of the bean

sprout samples had over 1 x 10^3 lactics per g. Alfalfa sprouts had 8 x 10^3 cells or fewer per g and 5000 x 10^3 cells or fewer per g for bean sprouts. The low number of lactics was in agreement with the negative bromcresol purple plates since the lowest dilution (10^3) plated for lactics was 100-fold lower than plated for acid-producres.

It appears that lactobacilli or acid-producing bacteria do not represent a major part of the flora on alfalfa or bean sprouts, but are recovered more frequently from bean sprouts. Without high numbers of these organisms to lower the pH, sprouts may be a suitable food for the multiplication of pathogens if mishandled.

Indicator Organisms

There is a general trend for slightly higher numbers of indicator organisms on bean sprouts than on alfalfa sprouts (Tables III and IV). This trend is observed with fecal streptococci, total coliform, and fecal coliform counts. The difference is too small to support a conclusion that bean sprouts are of lower sanitary quality than alfalfa sprouts.

The presumptive fecal streptococci counts were low for alfalfa sprouts: 64 x 10^2 cells or fewer per g (Table III). Over half of the samples had no colonies on the lowest dilution (10^2) plated. Bean sprouts, however, had a range of 1-1600 x 10^3 cells per g with

a median of 54 x 10^3 cells per g (Table IV). Only one sample resulted in no colonies on the lowest dilution (10^2) plated. Ercolani (1976) found a mean of 2 x 10^1 fecal streptococci per g of fresh lettuce over a two year period in Italy. The significance of this test for vegetables is questionable since streptococci are common to plants (Mundt, 1961). For this reason, the colonies were not confirmed to determine whether they were fecal streptococci.

Alfalfa and bean sprouts had similar total coliform counts with a combined range of 1-1400 x 10^5 cells per g (Tables III and IV). Bean sprouts and alfalfa sprouts had medians of 32 x 10^5 and 19 x 10^5 cells per g, respectively. Findings on related vegetables included 10^3 coliforms per g (Maxcy, 1978) and 6 x 10^2 coliforms per g (Ercolani, 1976) on fresh lettuce. Geldreich et al. (1964) concluded that coliforms from the intestines of warm-blooded animals contributed a small percentage (14%) of the flora on vegetation.

The fecal coliform numbers were relatively high (10^5 cells per g) for both alfalfa and bean sprouts (Tables III and IV). When comparing values in Tables III and IV, the dilutions used must be noted as they were not the same throughout. It appears that high numbers of fecal coliforms were present as part of the normal flora. Therefore, this test would not be an appropriate means to monitor sprouts. Tests for specific bacteria may better indicate the presence of pathogens, as found in

the study. Again, the bean sprout samples exhibited slightly higher numbers than the alfalfa sprouts. All MPN tubes for the three dilutions were positive for 19 of 20 bean sprout samples, but for only half of the alfalfa sprout samples.

It is important to determine what bacteria are causing the fecal coliform response. The standard test to identify fecal coliforms consists of streaking from positive EC tubes to EMB plates, observing incubated plates for typical E. coli colonies, picking colonies to agar slants and performing the IMViC tests (AOAC, 1976). Isolates picked from EMB plates from fecal coliform positive tubes may not be those giving the positive response since there may be a mixed population therein. Some bacteria, Enterobacter aerogenes for example, survive and multiply at the elevated temperatures, even though they do not contribute to gas production from lactose. For this reason, colonies from EMB plates are referred to as EMB isolates rather than fecal coliform isolates.

Eosin methylene blue plates streaked from positive EC tubes rarely had colonies with the typical green-metallic sheen of E. coli. When colonies on EMB plates were typical of E. coli, they were identified via API 20E. Only one such isolate was indeed E. coli and therefore was not the major fecal coliform on sprouts.

The predominant colony type was a pink mucoid colony with a dark center. A sub-study to identify these isolates was conducted.

For samples analyzed on 8-15, isolates from EMB gave an acid slant, acid butt, and gas production as the predominant reaction (11 of 12 isolates) on TSI slants so none of the TSI reactions were typical of salmonellae, shigellae or other pathogens. Two of these isolates were identified with API 20E test strips as Enterobacter cloacae and Klebsiella pneumoniae. Eosin methylene blue isolates from eight sprout samples (8-22, 8-23) were tested by the standard methods previously described. All isolates (18) gave IMViC reactions typical of E. aerogenes. However, 14 of the 18 isolates were confirmed as Klebsiella via biochemical tests for lysine and ornithine decarboxylase and motility. Eight of the these confirmed Klebsiella isolates were further identified as K. pneumoniae with API 20E test strips (Tables V and VI). One isolate which did not give typical reactions for Klebsiella was identified with API 20E as Citrobacter freundii.

The IMViC reactions alone were not sufficient to determine what bacteria were responsible for the fecal coliform positive (FC+) response and therefore were discontinued for the remainder of this investigation. During a study of coliforms in chlorinated water supplies, the IMViC reactions were also found to be unsatisfactory due to misleading or erroneous results (Ptak et al., 1973). Samples analyzed after 8-23 were streaked from positive EC tubes as before and isolates in pure culture were tested for ornithine decarboxylase. Selected isolates were identified with API 20E test strips. The

TABLE V. Identification of Isolates from MacConkey-Inositol-Carbenicillin[1] (MCIC) and eosin methylene blue[2] (EMB) plates of alfalfa sprouts using API 20E[3].

Producer-Store	Pkg. or Bulk	Date 1978	API Identification MCIC Isolates	EMB Isolates
1-F	P	9-19	1 K. pneumoniae 1 K. oxytoca 1 Klebsiella sp.	ND
2-F	P	9-21	1 K. pneumoniae 1 K. oxytoca 1 Klebsiella sp.	ND
2-G	P	7-10	1 K. pneumoniae	ND
2-G	P	8-22	ND	1 C. freundii
3-A	P	8-15	ND	1 E. cloacae
3-A	P	8-23	1 K. oxytoca	ND
3-E	P	9-12	ND	2 K. pneumoniae
4-H	P	8-23	ND	2 K. pneumoniae
8-C	P	8-15	1 K. oxytoca	1 K. pneumoniae
8-B	P	8-22	1 K. pneumoniae	2 K. pneumoniae
8-I	B	9-12	ND	3 K. pneumoniae
8-I	B	9-21	ND	2 K. pneumoniae 1 C. freundii
8-I	B	9-27	1 K. pneumoniae 2 K. oxytoca 1 Klebsiella sp.	ND
			5 K. pneumoniae 6 K. oxytoca 3 Klebsiella sp.	12 K. pneumoniae 2 C. freundii 1 E. cloacae

[1] Bagley and Seidler, 1978b

[2] Inolex Corporation, Glenwood, Ill.

[3] Analytab Products, Plainview, N. Y.

TABLE VI. Identification of Isolates from MacConkey-Inositol-Carbenicillin[1] (MCIC) and eosin methylene blue[2] (EMB) plates of bean sprouts using API 20E[3].

Producer-Store	Pkg. or Bulk	Date 1978	API Identification MCIC Isolates	EMB Isolates
5-H	B	9-14	ND	1 C. freundii 2 K. pneumoniae 1 Klebsiella sp.
5-H	B	9-27	4 K. pneumoniae	4 E. cloacae
6-F	P	9-14	ND	1 C. freundii
6-F	P	9-12	ND	1 K. pneumoniae 1 Klebsiella sp.
6-G	B	7-10	2 K. pneumoniae	ND
6-G	B	8-22	ND	2 K. pneumoniae
6-G	B	9-19	2 K. pneumoniae 1 K. oxytoca	ND
7-A	B	8-15	ND	1 E. coli
7-A	B	8-23	ND	2 K. pneumoniae
7-A	B	9-14	ND	1 C. freundii
8-B	P	8-22	1 K. pneumoniae	ND
			9 K. pneumoniae 1 K. oxytoca	7 K. pneumoniae 2 Klebsiella sp. 4 E. cloacae 3 C. freundii

[1] Bagley and Seidler, 1978b.

[2] Inolex Corporation, Glenwood, Ill.

[3] Analytab Products, Plainview, N. Y.

ornithine decarboxylase test is of critical value to distinguish between Enterobacter and Klebsiella. Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) lists Escherichia and Shigella as variable with regard to ornithine decarboxylase, while Salmonella, Enterobacter, and Yersinia enterocolitica are positive and Klebsiella and Citrobacter are negative. All but one of 38 isolates from EMB were ornithine decarboxylase negative, typical of Klebsiella. Twenty of the 37 ornithine decarboxylase negative isolates were tested further with API 20E test strips and identified as follows: 18, K. pneumoniae; 2, Klebsiella species; and 1, C. freundii (Tables V and VI). Based on these findings, it can be reasonably assumed that the rest of the 37 isolates were predominately K. pneumoniae as well. Citrobacter and Enterobacter which were isolated on occasion, are not capable of eliciting the fecal coliform response. Klebsiella, the predominate bacterium isolated from EMB plates, is known to include FC+ strains. The significance of FC+ K. pneumoniae will be discussed in conjunction with Klebsiella data.

Klebsiella

Alfalfa and bean sprouts had similar confirmed Klebsiella counts (Tables III and IV). Median values were 5.4×10^6 and 8.6×10^6 cells per g, respectively. Selected isolates from MCIC (MacConkey-Inositol-Carbenicillin) plates, which had been identified as Klebsiella via lysine

and ornithine decarboxylase and motility, were tested with API 20E strips. The three biochemical reactions listed above adequately identified MCIC isolates as Klebsiella since all isolates (24) were confirmed as Klebsiella with API 20E (Tables V and VI). The combined alfalfa and bean sprout API data resulted in 14 K. pneumoniae, 7 K. oxytoca and 3 Klebsiella species not specified. It is interesting to note the lower incidence of K. oxytoca on bean sprouts (1 of 10) than on alfalfa sprouts (6 of 14). It is also interesting that K. oxytoca was not recovered as EMB isolates along with other nonfecal coliforms such as Enterobacter and Citrobacter.

Due to the high levels of Klebsiella present, the predominance of K. pneumoniae in EMB and MCIC isolates, and the fact that K. pneumoniae was the only organism capable of a positive fecal coliform response isolated in significant numbers, it is concluded that K. pneumoniae is responsible for the high densities of fecal coliforms present on the sprouts in this study. In order to prove this statement, MCIC or EMB isolates that were identified as K. pneumoniae would have to be tested for the fecal coliform response. This was performed on a small scale with two of six K. pneumoniae isolates tested FC+. Brown and Seidler (1973) reported 64% of the K. pneumoniae isolates from vegetables were FC+. The fecal coliform MPN levels found on sprouts in this study were much higher than those previously reported for a variety of similar vegetables, which ranged from <200

17,000 MPN per 100 g wet weight, with the exception of 5.4×10^6 per 100 g for green onions (Duncan and Razzell, 1972).

The actual health significance of FC+ K. pneumoniae is currently being studied and has drawn attention to the elevated fecal coliform test. One author notes an increased use of this test as an indicator of environmental quality during the 1970's (Dockins and McFeters, 1978), which may account for recent reports of FC+ K. pneumoniae. The reliability of this elevated temperature test has been challenged (Dockins and McFeters, 1978). The validity of the test when K. pneumoniae is present and E. coli is absent in a positive fecal coliform sample has been questioned (Dufour and Cabelli, 1976). When a sample contains both E. coli and K. pneumoniae, fecal contamination is considered to be recent (Bagley and Seidler, 1977; Knittel, 1975). Occurrence of FC+ K. pneumoniae probably indicates fecal pollution at some time (Bagley and Seidler, 1977), but does not imply that other pathogenic bacteria are present. Perhaps the FC+ K. pneumoniae are of intestinal origin and survive in the environment more successfully than E. coli. Pathogenic Klebsiella have been shown experimentally to multiply on vegetable surfaces and therefore plants may serve as a reservoir (Brown and Seidler, 1973). Klebsiella have also been found to survive chlorine treatment of drinking water better than E. coli (Ptak et al., 1973). However, even if Klebsiella may not indicate recent fecal contamination, they

are still opportunistic pathogens and do not appear to lose virulence in the environment (Bagley and Seidler, 1977).

Approximately one-fourth of Klebsiella isolates from sprouts were identified as K. oxytoca, the indole positive pectin liquifying biotype of K. pneumoniae. One-third (18 of 53) vegetable, seed, and tree K. pneumoniae isolates were reported as indole positive (Seidler et al., 1975). No environmental indole positive K. pneumoniae have been found to be FC+ (Bagley and Seidler, 1977; Dufour and Cabelli, 1976; Knittel, 1975). The health importance of fecal coliform negative K. oxytoca cannot be disregarded since 34% of fecal Klebsiella isolates (Davis and Matsen, 1974) and 17% of human clinical strains (Martin et al., 1971) have been reported as indole positive. It should be noted that not all E. coli of environmental and clinical origin are FC+ (Bagley and Seidler, 1977). Klebsiella is present in the intestinal tract of 30-40% of humans and animals (Davis and Matsen, 1974; Buttiaux, 1959). In one particular study, one-third of the Klebsiella strains from 400 persons were indole positive (K. oxytoca) (Davis and Matsen, 1974).

In the past, Klebsiella has not been considered pathogenic when present in the intestinal tract of man. Montgomerie et al. (1970) acknowledged an association between the intestinal colonization of Klebsiella and increased infection rates in humans. The intestinal colonization of humans has been attributed to ingestion of foods, in-

including raw vegetables, contaminated with Klebsiella (Shooter et al., 1971). Montgomerie et al. (1970) showed that a single ingestion of 10^5 to 10^7 Klebsiella per ml of milkshake resulted in temporary intestinal colonization of humans. When vegetables are consumed uncooked, colonization may occur and ultimately result in a reservoir for future nosocomial infections (Selden et al., 1971).

K. pneumoniae strains isolated from the intestinal tract of persons with tropical sprue were shown to produce an enterotoxin which induced secretion of water and electrolytes, impaired xylose absorption and caused structural abnormalities of intestinal mucosa in rats (Klipstein et al., 1975; Klipstein and Schenk, 1975). Klipstein and Engert (1976) found similarities between the low molecular weight, heat stable enterotoxins of E. coli and K. pneumoniae. It has yet to be determined if these toxins as well as those produced by other coliforms such as E. cloacae (isolated on sprouts) are single, specific entities. A heat stable enterotoxin would also warrant concern with cooked sprouts containing high numbers of Klebsiella.

Although the debate is continuous, evidence has shown environmental FC+ Klebsiella to be equally as pathogenic as clinical FC+ Klebsiella and the general conclusion would be that FC+ Klebsiella of environmental origin should be regarded as potential pathogens of public health significance, with K. oxytoca perhaps to a lesser degree (Bagley and Seidler, 1977; Bagley and Seidler, 1978a).

Pathogenic Microorganisms

No Bacillus cereus, Yersinia enterocolitica, Salmonella or Shigella were isolated from the samples tested. The selective medium for B. cereus enumeration permitted the growth of other Bacillus species at numbers of 10^7 to 10^8 cells per g of sample. B. cereus may have been present in lower numbers which were not within the range of dilutions required for a countable plate. Attempts to develop a more selective agar (unpublished data) were not successful. Bismuth sulfite may have been too inhibitory for low numbers of Y. enterocolitica. Hanna et al. (1977) used bismuth sulfite for inoculation studies of Y. enterocolitica on raw and cooked meat with success. Several other media have been shown to be successful (Lee, 1977; Mehlman et al., 1978). Other workers have also found the cold enrichment in phosphate buffer unsatisfactory (Lee, 1977; Mehlman et al., 1978). Salmonella and Shigella were included in the study because they could be easily screened for with biochemical tests used for Y. enterocolitica isolates and because their presence would be of public health significance. Lettuce at room temperature supported the growth of Salmonella typhimurium (Maxcy, 1978). There are superior selective agars available for these bacteria if they had been the focus of the study.

Significance of Study and Future Directions

Sprouts contain high numbers of aerobic and psychrotrophic bacteria which may contribute to spoilage problems. Further studies are needed to determine if the large population of psychrotrophs contribute to the spoilage of sprouts.

The findings do not show pathogenic organisms to be present. Klebsiella pneumoniae, a potential pathogen, is a public health concern. This bacterium could cause illness in persons who have a decreased resistance, including antibiotic therapy, but would not harm healthy individuals. Since some may be at a risk in consuming sprouts, a follow-up study might include an investigation to determine by what means the numbers of Klebsiella could be lowered. A treatment that could be applied by consumers would be helpful for those who grow their own.

Fecal coliforms were apparently part of the normal flora on alfalfa and bean sprouts. An appropriate means to test for the presence of pathogens on sprouts must be found. Tests for specific bacteria such as E. coli or Salmonella may better indicate fecal contamination.

Further investigations are needed to determine the conditions required for Bacillus cereus to multiply on sprouts and result in foodborne outbreaks. A more selective isolation medium would aid in this type of study.

SUMMARY

Alfalfa and bean sprouts from eight producers in Oregon and Washington were purchased in nine retail outlets for a study of their microbial flora, with an emphasis on bacteria of public health significance. Four replications from each product, with one exception, were sampled for a total of 23 alfalfa and 20 bean sprout samples. Microbiological assays were performed using methods recommended for foods.

There appeared to be no effect on the microbial populations from producers, sample date, retail outlet, temperature of display case, and whether marketed in packaged or bulk form. The aerobic plate counts for alfalfa and bean sprout samples were fairly high (10^8 cells per g) with psychrotrophs (10^7 cells per g), total coliforms (10^6 cells per g) and Klebsiella (10^6 cells per g) contributing significantly to the total numbers of bacteria. Total acid-producing bacteria were low ($< 10^3$ cells per g) and lactobacilli counts were low ($< 10^3$ to 10^4 cells per g). Fecal streptococci numbers were low for sprout samples (10^2 to 10^3 cells per g). The high fecal coliform densities recovered ($\geq 240,000$ per 100 g) resulted from K. pneumoniae. Laboratories finding high fecal coliforms and IMViC reactions typical of Enterobacter should include a test such as ornithine decarboxylase to distinguish Klebsiella. No pathogenic bacteria were isolated,

although Klebsiella are considered by many to be of important public health significance.

Further studies are needed to determine if the large population of psychrotrophs contributes to the spoilage of sprouts, a means by which the numbers of Klebsiella could be lowered, and the conditions required for Bacillus cereus to multiply on sprouts and result in foodborne outbreaks.

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