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The present investigation was undertaken to characterize a number of strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* intended for use by a commercial starter supply company. Thorough characterization of each culture was required in order to combine compatible strains so that their usefulness in Mozzarella cheese manufacture would be maximized. In this regard, cocci were assayed for formate and carbon dioxide production, rods for proteolysis, and both types for salt and phosphate tolerance as well as rate of acid production. In addition, certain combinations of cocci and rods were assayed as mixtures for these characteristics.

Analyses of the various strains of lactobacilli and *S. thermophilus* were performed. Proteolysis, as determined by the Church method, for the rods (*L. bulgaricus* , *L. helveticus* and *L. lactis*) varied from as low as 11.3 to as high as 34.7 mM when incubated for six hours. Proteolysis analyses for *S. thermophilus* also revealed a wide range of values from a low of 18.5 to a high of 46.4 mM. However, when strains were incubated for 16 hours, rods

were shown to be nearly twice as proteolytic as cocci. When mixed cultures were tested for proteolysis, results were dependent on strain synergism. Values ranged from a low of 5.1 mM to 70.5 mM in mixed cultures.

Various strains of *S. thermophilus* and mixed cultures were assayed for formate production. The *S. thermophilus* strain values were from a low of 4.2 to as high as 20.3 mg/L. Formate production in mixed cultures varied from traces of formate in one culture to quantities two and a half times that produced by the single *S. thermophilus* strains tested.

Carbon dioxide production for the rods (*L. bulgaricus* , *L. helveticus* , and *L. lactis*) varied from as low as 0 μl to as high as 376 μl when incubated for six hours at 44°C. Carbon dioxide production for *S. thermophilus* ranged from 5 μl to 1259 μl. Also, *S. thermophilus* strains produced significantly more carbon dioxide than rod cultures, with only three exceptions. All mixtures were weak producers of carbon dioxide.

Nine of 19 *L. bulgaricus* strains were stimulated by 0.1% phosphate ion and one strain showed stimulation at 0.3% phosphate ion. Thirteen of 19 strains were severely inhibited by 0.5% phosphate. Three of 10 *L. helveticus* strains were stimulated by 0.1% phosphate and another three strains were unaffected. All strains were inhibited by 0.5% phosphate. Two *L. lactis* strains showed stimulation at 0.1% phosphate, but inhibition at 0.3% and 0.5%. Acid production by strains of *S. thermophilus* was inhibited in 11 of 13 cases at 0.1% phosphate. The two strains not inhibited were slightly stimulated by 0.1% and 0.3% phosphate and unaffected by

0.5% phosphate. The mixed cultures of *L. bulgaricus* CR 14/ *S. thermophilus* 2 and *L. bulgaricus* Q/ *S. thermophilus* 2 were not inhibited by 0.1% phosphate, but inhibition occurred at higher concentrations. Mixed cultures of *L. bulgaricus* C, E/ *S. thermophilus* 7, 12 and *L. bulgaricus* C, G/ *S. thermophilus* 4, 12 were stimulated by all three concentrations of phosphate salts tested.

Sodium chloride produced toxic effects on the rods at concentrations ranging from 2.5% to 3.0%, and acid production was stimulated 7 of 32 strains by low salt concentrations(0.5%). In general, cocci were more sensitive to NaCl, with 6 of 13 strains showing sensitivity at 0.5%. Sensitivity to salt was a more gradual effect in the cocci as revealed by a gradual reduction in rate of acid production as NaCl concentrations increased. Mixed cultures were more tolerant to NaCl with no inhibition occurring at concentrations of 1.0%. Culture *L. bulgaricus* C, G/*S. thermophilus* 4, 7 were stimulated at concentrations through 1.5%. The synergistic properties of the mixed strains increased NaCl tolerance.

CHARACTERIZATION OF THERMOPHILIC ROD AND COCCUS STARTER STRAINS USED IN MOZZARELLA CHEESE MANUFACTURE

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To my parents, Charles and Geraldine,
without their love and support I never would have made it.

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Table of Contents

	Page
INTRODUCTION	1
MATERIALS AND METHODS	11
Proteolysis	11
Formate Production	13
Carbon Dioxide Production	16
Phosphate Sensitivity	19
Salt Tolerance	20
Carbohydrate Fermentation	21
RESULTS	22
Proteolysis	22
Formate Production	30
Carbon Dioxide Production	33
Phosphate Sensitivity	39
Salt Tolerance	45
Carbohydrate Fermentation	96
CONCLUSIONS	98
BIBLIOGRAPHY	103

List of Figures

Figure		Page
1.	Proteolysis standard curve showing linear increase in absorbance at 340 nm in response to increasing concentration of the peptide Leucine-Glycine..	12
2.	Formate standard curve (Δ) showing linear increase in absorbance at 340 nm in response to increasing concentrations of sodium formate. Control 1a, 2a, 3a, represents these same concentrations extracted from 11% NFDM..	15
3.	Drawing showing design of laboratory gasometer for measuring gas production..	17
4.	Proteolytic activity of *S. thermophilus* strain 3 grown from a 1% inoculum in 11% NFM and incubated at 38°C at controlled pH. Values are mM concentration of liberated amino acids expressed as a function of pH as determined by the Church assay..	28
5.	Proteolytic activity of *L. bulgaricus* strain J grown from a 1% inoculum in 11% NFM and incubated at 44°C at controlled pH. Values are mM concentration of liberated amino acids expressed as a function of pH as determined by the Church assay..	29
6.	Salt sensitivity of *L. bulgaricus* strain A grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically..	47
7.	Salt sensitivity of *L. bulgaricus* strain B grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically..	48

8. Salt sensitivity of *L. bulgaricus* strain C grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 49

9. Salt sensitivity of *L. bulgaricus* strain D grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 50

10. Salt sensitivity of *L. bulgaricus* strain E grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 51

11. Salt sensitivity of *L. bulgaricus* strain F grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 52

12. Salt sensitivity of *L. bulgaricus* strain G grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 53

13. Salt sensitivity of *L. bulgaricus* strain H grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 54

14. Salt sensitivity of *L. bulgaricus* strain H grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 55

15. Salt sensitivity of *L. bulgaricus* strain J grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 56

16. Salt sensitivity of *L. bulgaricus* strain K grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 57

17. Salt sensitivity of *L. bulgaricus* strain L grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 58

18. Salt sensitivity of *L. bulgaricus* strain M grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 59

19. Salt sensitivity of *L. bulgaricus* strain N grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 60

20. Salt sensitivity of *L. bulgaricus* strain O grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 61

21. Salt sensitivity of *L. bulgaricus* strain P grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 62

22. Salt sensitivity of *L. bulgaricus* strain Q grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 63

23. Salt sensitivity of *L. bulgaricus* strain R grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 64

24. Salt sensitivity of *L. bulgaricus* strain S grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 65

25. Salt sensitivity of *L. helveticus* strain A grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 66

26. Salt sensitivity of *L. helveticus* strain B grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 67

27. Salt sensitivity of *L. helveticus* strain C grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 68

28. Salt sensitivity of *L. helveticus* strain D grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 69

29. Salt sensitivity of *L. helveticus* strain E grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 70

30. Salt sensitivity of *L. helveticus* strain F grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 71

31. Salt sensitivity of *L. helveticus* strain G grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 72

32. Salt sensitivity of *L. helveticus* strain H grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 73

33. Salt sensitivity of *L. helveticus* strain I grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 74

34. Salt sensitivity of *L. helveticus* strain J grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 75

35. Salt sensitivity of *L. lactis* strain A grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 76

36. Salt sensitivity of *L. lactis* strain B grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 77

37. Salt sensitivity of *L. lactis* strain C grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.. 78

38. Salt sensitivity of *S. thermophilus* strain 1 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.. 79

39. Salt sensitivity of *S. thermophilus* strain 2 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.. 80

40. Salt sensitivity of *S. thermophilus* strain 3 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.. 81

41. Salt sensitivity of *S. thermophilus* strain 4 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.. 82

42. Salt sensitivity of *S. thermophilus* strain 5 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.. 83

43. Salt sensitivity of *S. thermophilus* strain 6 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.. 84

44. Salt sensitivity of *S. thermophilus* strain 7 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.. 85

45. Salt sensitivity of *S. thermophilus* strain 8 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.. 86

46. Salt sensitivity of *S. thermophilus* strain 9 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.. 87

47. Salt sensitivity of *S. thermophilus* strain 10 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.. 88

48. Salt sensitivity of *S. thermophilus* strain 11 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.. 89

49. Salt sensitivity of *S. thermophilus* strain 12 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.. 90

50. Salt sensitivity of *S. thermophilus* strain 13 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.. 91

51. Salt sensitivity of *L. bulgaricus* CR 14 and *S. thermophilus* 2 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed graphically.. 92

52. Salt sensitivity of *L. bulgaricus* Q and *S. thermophilus* 2 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed graphically.. 93

53. Salt sensitivity of *L. bulgaricus* C and E, and *S. thermophilus* 7 and 12 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed graphically.. 94

54. Salt sensitivity of *L. bulgaricus* C and G, and *S. thermophilus* 4 and 7 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed graphically.. 95

List of Tables

Table		Page
1.	Amounts of added phosphates and final solution concentrations used in making milk for phosphatetolerance determinations..	20
2.	Proteolytic activity of *L. bulgaricus* strains grown from a 1% inoculum in 11% NFM and incubated at 44°C for 6 hours. Values are the average of three trials expressed as absorbance at 340 nm and as mM concentration of amino acids liberated from milk protien as determined by the Church assay..	23
3.	Proteolytic activity of *L. helveticus* strains grown from a 1% inoculum in 11% NFM and incubated at 44°C for 6 hours. Values are the average of three trials expressed as absorbance at 340 nm and as mM concentration of amino acids liberated from milk protien as determined by the Church assay..	24
4.	Proteolytic activity of *L. lactis* strains grown from a 1% inoculum in 11% NFM and incubated at 44°C for 6 hours. Values are the average of three trials expressed as absorbance at 340 nm and as mM concentration of amino acids liberated from milk protien as determined by the Church assay..	25
5.	Proteolytic activity of *S. thermophilus* strains grown from a 1% inoculum in 11% NFM and incubated at 38°C for 6 hours. Values are the average of three trials expressed as absorbance at 340 nm and as mM concentration of amino acids liberated from milk protien as determined by the Church assay..	26
6.	Proteolytic activity of *L. bulgaricus* and *S. thermophilus* strains grown from a 1% inoculum in 11% NFM and incubated at 42°C for 6 hours. Values are the average of three trials expressed as absorbance at 340 nm and as mM concentration of amino acids liberated from milk protien as determined by the Church assay..	27

7. Formate production of *S. thermophilus* strains grown from a 1% inoculum in 11% NFM and incubated at 38°C for 6 hours. Values are the average of three trials expressed as milligrams per liter.. 31

8. Formate production of *L. bulgaricus* and *S. thermophilus* strains grown from a 1% inoculum in 11% NFM and incubated at 42°C for 6 hours. Values are the average of three trials expressed as milligrams per liter.. 32

9. Carbon dioxide production of *L. bulgaricus* strains grown from a 1% inoculum in 11% NFM and incubated at 44°C for 6 hours. Values are the average of three trials expressed as microliters of carbon dioxide generated.. 34

10. Carbon dioxide production of *L. helveticus* strains grown from a 1% inoculum in 11% NFM and incubated at 44°C for 6 hours. Values are the average of three trials expressed as microliters of carbon dioxide generated.. 35

11. Carbon dioxide production of *L. lactis* strains grown from a 1% inoculum in 11% NFM and incubated at 44°C for 6 hours. Values are the average of three trials expressed as microliters of carbon dioxide generated.. 36

12. Carbon dioxide production of *S. thermophilus* strains grown from a 1% inoculum in 11% NFM and incubated at 44°C for 6 hours. Values the average of three trials are expressed as microliters of carbon dioxide generated.. 37

13. Carbon dioxide production of *L. bulgaricus* and *S. thermophilus* strains grown from a 1% inoculum in 11% NFM and incubated at 42°C for 6 hours. Values are the average of three trials expressed as microliters of carbon dioxide generated.. 38

14. Phosphate sensitivity of *L. bulgaricus* strains grown from a 1% inoculum in 11% NFM supplemented with phosphate salts in concentrations of 0.1%, 0.3% and 0.5% and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 40

15. Phosphate sensitivity of *L. helveticus* strains grown from a 1% inoculum in 11% NFM supplemented with phosphate salts in concentrations of 0.1%, 0.3% and 0.5% and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 41

16. Phosphate sensitivity of *L. lactis* strains grown from a 1% inoculum in 11% NFM supplemented with phosphate salts in concentrations of 0.1%, 0.3% and 0.5% and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 42

17. Phosphate sensitivity of *S. thermophilus* strains grown from a 1% inoculum in 11% NFM supplemented with phosphate salts in concentrations of 0.1%, 0.3% and 0.5% and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.. 43

18. Phosphate sensitivity of *L. bulgaricus* and S. thermophilus strains grown from a 1% inoculum in 11% NFM supplemented with phosphate salts in concentrations of 0.1%, 0.3% and 0.5% and incubated at 42°C for 6 hours. Values are the average of three trials expressed as final pH.. 44

19. Salt sensitivity of *L. bulgaricus* strain A grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 47

20. Salt sensitivity of *L. bulgaricus* strain B grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 48

21. Salt sensitivity of *L. bulgaricus* strain C grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 49

22. Salt sensitivity of *L. bulgaricus* strain D grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 50

23. Salt sensitivity of *L. bulgaricus* strain E grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 51

24. Salt sensitivity of *L. bulgaricus* strain F grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 52

25. Salt sensitivity of *L. bulgaricus* strain G grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 53

26. Salt sensitivity of *L. bulgaricus* strain H grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 54

27. Salt sensitivity of *L. bulgaricus* strain I grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 55

28. Salt sensitivity of *L. bulgaricus* strain J grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 56

29. Salt sensitivity of *L. bulgaricus* strain K grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 57

30. Salt sensitivity of *L. bulgaricus* strain L grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 58

31. Salt sensitivity of *L. bulgaricus* strain M grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 59

32. Salt sensitivity of *L. bulgaricus* strain N grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 60

33. Salt sensitivity of *L. bulgaricus* strain O grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 61

34. Salt sensitivity of *L. bulgaricus* strain P grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 62

35. Salt sensitivity of *L. bulgaricus* strain Q grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 63

36. Salt sensitivity of *L. bulgaricus* strain R grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 64

37. Salt sensitivity of *L. bulgaricus* strain S grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 65

38. Salt sensitivity of *L. helveticus* strain A grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 66

39. Salt sensitivity of *L. helveticus* strain B grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 67

40. Salt sensitivity of *L. helveticus* strain C grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 68

41. Salt sensitivity of *L. helveticus* strain D grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 69

42. Salt sensitivity of *L. helveticus* strain E grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 70

43. Salt sensitivity of *L. helveticus* strain F grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 71

44. Salt sensitivity of *L. helveticus* strain G grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 72

45. Salt sensitivity of *L. helveticus* strain H grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 73

46. Salt sensitivity of *L. helveticus* strain I grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 74

47. Salt sensitivity of *L. helveticus* strain J grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 75

48. Salt sensitivity of *L. lactis* strain A grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 76

49. Salt sensitivity of *L. lactis* strain B grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 77

50. Salt sensitivity of *L. lactis* strain C grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.. 78

51. Salt sensitivity of *S. thermophilus* strain 1 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.. 79

52. Salt sensitivity of *S. thermophilus* strain 2 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.. 80

53. Salt sensitivity of *S. thermophilus* strain 3 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.. 81

54. Salt sensitivity of *S. thermophilus* strain 4 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.. 82

55. Salt sensitivity of *S. thermophilus* strain 5 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.. 83

56. Salt sensitivity of *S. thermophilus* strain 6 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.. 84

57. Salt sensitivity of *S. thermophilus* strain 7 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.. 85

58. Salt sensitivity of *S. thermophilus* strain 8 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.. 86

59. Salt sensitivity of *S. thermophilus* strain 9 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.. 87

60. Salt sensitivity of *S. thermophilus* strain 10 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.. 88

61. Salt sensitivity of *S. thermophilus* strain 11 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.. 89

62. Salt sensitivity of *S. thermophilus* strain 12 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.. 90

63. Salt sensitivity of *S. thermophilus* strain 13 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.. 91

64. Salt sensitivity of *L. bulgaricus* CR 14 and *S. thermophilus* 2 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed as final pH.. 92

65. Salt sensitivity of *L. bulgaricus* Q and *S. thermophilus* 2 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed as final pH.. 93

66. Salt sensitivity of *L. bulgaricus* C and E, and *S. thermophilus* 7 and 12 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed as final pH.. 94

67. Salt sensitivity of *L. bulgaricus* C and G, and *S. thermophilus* 4 and 7 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed as final pH.. 95

CHARACTERIZATION OF THERMOPHILIC ROD AND COCCUS STARTER STRAINS USED IN MOZZARELLA CHEESE MANUFACTURE

INTRODUCTION

Mozzarella cheese production in the U. S. has risen 10% annually for the past ten years (USDA, 1989). Production of Mozzarella cheese now ranks second to Cheddar cheese. This trend has been predicted to continue through the year 2000 (Olson, 1973).

Growth of the Mozzarella cheese industry has placed increased demands on the cheese manufacturer. These demands include maximizing efficiency and producing a consistent quality product, while trying to increase profit margin (Sanders, 1991).

Microorganisms used in the manufacture of Mozzarella cheese, and other dairy foods such as yogurt and Swiss cheeses, are *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) and *Streptococcus salivarious* subsp. *thermophilus* (*S. thermophilus*). In the cheese industry these are commonly referred to as rod-coccus cultures in reference to the morphology of the two bacteria, *L. bulgaricus* the rod and *S. thermophilus* the coccus. It is known that these cultures form a symbiotic relationship during growth, though synergism is not uniform between all strains. Not all strains are compatible and an imbalance in growth may occur (Nielsen, 1975; Pette and Lolkema, 1951; Tramer, 1973). Moon and Reinbold, (1974a; 1974b), found some rod/coccus culture combinations to be inhibitory, stimulatory, or neutral with regard to rate of lactic acid production as compared to single strain cultures. Researchers have

often found that mixed strains of *L. bulgaricus* and *S. thermophilus* have resulted in increased rates of lactic acid production and growth (Bautista et al., 1966; Moon and Reinbold, 1976; Moon et al. 1975; Pette and Lolkema, 1950a).

The basis for the relationship has not been thoroughly defined, though certain facts about it have been identified. Pette and Lolkema, (1950b), found the stimulants for *S. thermophilus* produced by *L. bulgaricus* to be eleven amino acids, with the most stimulatory being valine. Bautista et al., (1966), identified the stimulatory factors to be glycine and histidine only. Bracquart et al., (1978), found that a combination of glutamine, methionine, histidine, arginine, phenylalanine and tryptophan were needed for stimulation. Shanker and Davies, (1977), found the amino acids glutamine, histidine, valine, methionine and leucine to be stimulatory, and this finding was supported by Higashio et al., (1977a).

Another study by Shanker and Davies, (1976), indicated certain peptides released from milk casein by *L. bulgaricus* were stimulatory for growth of *S. thermophilus*. Desmazeud and Hermier, (1972), isolated stimulatory peptides of variable amino acid composition from casein hydrolysates. Only the lysyl residue was common to all peptides and tryptophan was not present in any. They suggested that *S. thermophilus* possesses a peptide-transport system with specific length requirements and also requiring the presence of a lysyl residue. This active transport system is dependent on temperature and pH and is inhibited by L-cysteine (Akpemado and Bracquart, 1983). The group of amino acids present

in the stimulatory peptide were also found to be stimulatory. They suggested that these peptides acted as the amino acid source.

Stimulation of *L. bulgaricus* by *S. thermophilus* has been studied to a lesser degree (Radke-Mitchell and Sandine, 1984). Formate has been found to stimulate the growth of *L. bulgaricus* (Galesloot et al., 1968). This stimulatory factor was produced only under conditions of low oxygen concentration. Veringa et al., (1968), later determined that formic acid was stimulatory in concentrations of 32 milligrams per liter of milk. Higashio et al., (1977b), demonstrated that formic and pyruvic acids produced by *S. thermophilus* together exert a synergistic effect on stimulating growth and acid production in *L. bulgaricus*. It was also shown that in this synergistic combination, pyruvate could be replaced by oxaloacetic, furmaric, L-malic, or α-ketoglutaric acids; however, formic acid was irreplaceable (Higashio et al., 1977b).

More recent research suggests that stimulation of *L. bulgaricus* cannot be explained by production of formate alone (Driessen et al., 1982). Experimental evidence has shown that *Lactobacillus bulgaricus* need > 31 mg of carbon dioxide per kilogram of milk, in addition to formate, to receive stimulation.

An important metabolic trait of dairy lactic cultures is their ability to degrade casein. Lactic starter bacteria have limited biosynthetic capabilities, so they require most amino acids to be added exogenously for growth. The concentration of free amino acids and peptides in milk are insufficient to support rapid growth of lactic acid bacteria to produce high cell densities. Consequently, the cells

secrete a compliment of proteinases and peptidases to hydrolyze casein (Konings et al., 1991; Rajagopal and Sandine, 1990).

The initial step in casein proteolysis is performed by a proteinase which is attached to the cell membrane or released in the medium. Proteolysis of casein by the proteinase results in the release of peptides which are too large to be taken up by the organism (Konings et al., 1991). Further degradation of these oligopeptides occurs by various peptidases with different specificities and activities (Laan et al., 1989) The resulting peptides and amino acids can then be translocated across the membrane where they are further hydrolyzed by the organism.

Some disagreement exists about casein hydrolysis. Ohmiya and Sato, (1968; 1969), initially reported that α_S-casein was more easily hydrolyzed than β-casein by *L. bulgaricus* intracellular proteases. Creamer, (1976), reported that more α_{S1}-casein remained in Mozzarella cheese than either of the other types. This indicates more κ- and β–casein hydrolysis. This is supported by Ohmiya and Sato, (1978), whose findings indicate that the hydrolytic rates of *L. bulgaricus* intracellular proteases decrease going from κ-casein to β-casein to α_S-casein. However, during ripening Ohmiya and Sato, (1978), found α_S-casein more readily hydrolyzed than β-casein by *L. bulgaricus* intracellular proteases.

The process of cheese ripening results from activities of natural milk proteinase, plasmin, rennet, and proteinases of the starter culture (Thomas and Mills, 1981). Since proteinases and peptidases play a critical role in cheese flavor and body development, it is important to characterize these enzyme activities used for cheese

making (Fox, 1989). Researchers have shown that changes in cheese flavor during ripening are related to breakdown of proteins (Aston et al., 1983; Edwards and Kosikowski, 1983). Studies have also correlated protein breakdown with texture changes in cheese (Creamer et al., 1985; deJong, 1977). Levels of intact casein have been suggested as a significant determinant of stretching properties of melted Mozzarella cheese (Creamer, 1976).

Although lactic acid bacteria are weakly proteolytic compared to other groups of bacteria such as *Bacillus, Proteus, Pseudomonas,* and coliforms, they do cause a significant degree of proteolysis in many fermented dairy products (Tamine and Deeth, 1980). Proteolysis in cheese is affected by several factors including ripening, temperature, pH, activities of residual coagulant, indigenous milk proteases as well as starter protienase:peptidase, casein:moisture and salt:moisture ratios (Farkye et al., 1991).

Bitter flavor in cheese is a major concern of industry. Bitter compounds were first identified as peptides in Gouda cheese (Raadsveld, 1953) and more recently in Cheddar cheese (Harwalker and Elliot, 1971; Richardson and Creamer, 1973; Hamilton et al., 1974). Two hypothesis have been proposed to explain development of bitterness in Cheddar cheese. The first suggested that all bitter peptides were produced solely by residual rennet in the cheese, and none by the starter (Czulak, 1959). The second hypothesis proposed that bitter peptides were produced via rennet hydrolyses of casein to high molecular weight, non-bitter peptides which then are hydrolyzed to low molecular weight bitter peptides by starter (Lowrie and Lawrence, 1972). Mills and Thomas, (1980), found that

bitter flavor could be manipulated by varying the ratio of Prt^+ (proteinase positive) and Prt^- (proteinase negative) cells, providing evidence that the level of starter proteinase has a direct role in bitterness development in cheese. Yun et al., (1992), observed that increased rod to coccus ratios increased proteolysis.

Several methods are available for measuring proteolysis. The Hull method (Hull, 1947), relies on the chemical characteristics of released tryptophan and tyrosine to reflect proteolysis. The method lacks sensitivity and specificity, especially in complex protein mixtures low in tryptophan and tyrosine (Church et al., 1983). To overcome undesirable characteristics of the Hull method, the trinitrobenzene sulfonic acid, (TNBS), procedure was developed by Fields, (1971). This method uses total soluble amino nitrogen to estimate proteolysis (Samples et al., 1984). Other recently introduced methods to detect proteolysis include ^{14}C (Exterkate, 1979), spectrophotmetric *O* -phtaldialdehyde (OPA) (Svedas, 1980), flourometric OPA (Porter et al., 1982), laser flourometry (Coburn et al., 1986), reflectance colorimetry (Richardson et al., 1988), and use of flourescamine (Pierce, 1979; Richardson et al., 1988), azocasein (Christian and Marshall, 1984) casein agar (Singh and Sharma, 1983), and resorufin-labeled casein (Twining, 1984).

Church et al., (1983), applied a method using OPA to determine proteolysis caused by lactic acid bacteria in nonfat dry milk. This procedure measures culture ability to produce primary amines from nonfat dry milk (Weimer et al., 1989).

The stimulation of *L. bulgaricus* by a factor produced by *S. thermophilus* was reported over 20 years ago (Galesloot et al., 1968;

Veringa et al., 1968). This factor was determined to be formate. The stimulatory action of formate on *L. bulgaricus* is important for reducing fermentation time in yogurt (Rasic and Kurmann, 1978; Tamine and Robinson, 1985; Terre, 1986). This property, however, has not yet been taken into account in the selection of strains for cheese starters (Bouillane and Desmazeaud, 1980).

Formate production by *S. thermophilus* takes place during late exponential and early stationary growth phases and is dependent on strain, culture medium, and temperature (Perez et al., 1991). Suzuki et al., (1986), observed that *L. bulgaricus* exhibited abnormal elongation when grown in milk with low formate concentrations. Bottazzi et al., (1971), and Stolk, (1956) recorded similar cell elongation in *L. bulgaricus* in yogurt cultures with extremely low numbers of *S. thermophilus* which would naturally be low in formate.

Suzuki et al., (1986), reported that formate would be the only substance to act as a carbon donor in *L. bulgaricus*. Formate is needed for the synthesis of purines and pyrimidines incorporated into ribonucleic acid and deoxyribonucleic acid.

Several methods are used for formate determination in lactic cultures. These include chromatographic methods (Rhee and Pack, 1980; Thomas et al., 1980; Veringa et al., 1968), colormetric methods (Moletta and Albagnal, 1984), and enzymatic methods (Fordyce et al., 1984). Due to the sensitivity and specificity of the enzymatic method using formate dehydrogenase (Hopner and Knapp, 1974) it is the best procedure for determining formate concentration in milk samples (Perez et al.; 1990).

Driessen et al., (1981), concluded that formic acid was not the only limiting substrate for *L. bulgaricus*. Later it was determined that carbon dioxide also was needed for optimum growth (Driessen et al., 1982). Evidence was found that *L. bulgaricus* needs > 31 mg of CO_2 per kg of milk.

Whitehead et al., (1958), first reported carbon dioxide as a stimulant of acid production in lactic streptococci. Driessen et al., (1982), found that *S. thermophilus* produces large amounts of carbon dioxide. This carbon dioxide is not formed from lactose metabolism since *Streptococcus thermophilus* is strictly homofermentative. Carbon dioxide production is due to urease activity which breaks down urea into CO_2 and NH_3 (Miller and Kandler, 1967; Tinson et al., 1982; Juilliard et al., 1988). This leads to alkalization of the growth medium and directly affects acidification rate measurements in milk (Spinnler and Corrieu, 1989; Famelart and Maubois, 1988; Zourari et al., 1991a). Zourari et al., (1991b), advocated use of urease activity as a method of quantitating thermophilic streptococcal numbers in a mixed culture with *L. bulgaricus* by measuring carbon dioxide produced.

Bacteriophage contamination of starter cultures is one of the main causes of starter culture failure during cheese and cultured dairy product manufacture (Wright and Klaenhammer, 1984). It has been demonstrated that bacteriophage require divalent ions , in particular calcium, for adsorption and subsequent proliferation (Cherry and Watson, 1949; Collins et al., 1950; Babel, 1958). Studies by Hargrove et al., (1961), demonstrated that addition of phosphate

to starter milk would chemically bind the calcium and inhibit phage proliferation.

Hargrove et al., (1961), reported that between 2% and 3% phosphate suppressed phage, but also reduced acid producing activity in several mixed strain cultures. Zottola and Marth, (1966), reported on a phage inhibitory medium containing 1% each of Na_2HPO_4 and KH_2PO_4 that was not inhibitory to growth of starter cultures. Ausavanodom et al., (1977), showed that significantly lower levels of phosphate could be used in phage inhibitory media if the pH was continuously held between 6.0 and 6.2 during culture growth. Ledford and Speck, (1979) found injury to cultures grown in commercial phage inhibitory media containing high levels of phosphates. Also, Wright and Klaenhammer, (1984), found decreased acid production, growth inhibition, and change in morphology of *L. bulgaricus* grown in media containing 1% to 2% phosphate.

Salting is an essential step in making all cheese except for a few unripened varieties. The purposes of salting are to suppress growth of unwanted microorganisms, retard growth of lactic acid and other wanted microorganisms, to assist the physicochemical changes in the curd, and to give cheese an appetizing taste (Nilson, 1968). Regions of low salt are focal points of flavor defects and even putrefication (Davies et al., 1937).

It has been found that sodium chloride in small portions acts as a stimulant to lactic cultures, while in larger portions it exhibits toxic properties (McDowell and Whelan, 1933). Walter et al., (1958), found strain inhibition in Cheddar cheese curd varying from slight at

1.4% to almost complete at 2.0% salt. Kosikowski, (1968), found *L. bulgaricus* to be retarded by more than 2.0% salt concentration. By increasing the salt:moisture ratio, lactose metabolism was adversely affected (Thomas and Pierce, 1981, Lawrence and Gilles, 1982). Turner and Thomas, (1980) found lactose to be completely fermented at 4% salt:moisture, and metabolism completely inhibited at 6% salt:moisture. Commercial mixed strain cultures displayed reduced acid development in skim milk in the presence of 1.5% to 2.0% salt (Irvine and Price, 1961; Marth and Hussong; 1963).

The present investigation was undertaken to characterize a number of strains of *L. bulgaricus* and *S. thermophilus* intended for use by a commercial starter supply company. Thorough characterization of each culture was required in order to combine compatible strains so that their usefulness in Mozzarella cheese manufacture would be maximized. In this regard cocci were assayed for formate and carbon dioxide production, rods for proteolysis, and both types for salt and phosphate tolerance as well as rate of acid production. In addition, certain combinations of cocci and rods were assayed as mixtures for these characteristics.

MATERIALS AND METHODS

Proteolysis

Samples were taken from an appropriate 10-12 hour, 37°C, milk culture. To 1.0-ml culture in a microfuge tube, 0.5-ml Reagent A (Promega Corp., Madison WI) was added. Mixing was performed by inversion of the microfuge tubes 10 times. The mixture was centrifuged at 13000 rpm for 5 minutes. The supernatant was poured off and the cell pellet resuspended in 1.0 ml of pH 7.0, 0.1 M phosphate buffer. Sample was again centrifuged at 13000 rpm for 5 minutes and the supernatant poured off. The cell pellet was then resuspended in 1.0 ml of pH 7.0 phosphate buffer. A 1% inoculum then was pipetted into 11% (wt/vol) reconstituted NFDM, previously pasteurized at 63°C for 60 minutes.

The inoculated milk tubes were then incubated in a water bath for six hours. Temperature used was dependent upon the culture. *Streptococcus thermophilus* cultures were incubated at 38°C; *L. bulgaricus, L. lactis,* and *L. helveticus* were incubated at 44°C; and rod-coccus mixed cultures were incubated at 42°C. These temperatures were optimum for the respective culture types as determined by Radke-Mitchell and Sandine (1986).

The method used to determine proteolysis was the Church assay (Church et al., 1983). Cultures were sampled aseptically after gentle mixing. To a 5.0-ml sample, 1.0 ml of sterile distilled water was added. While vortexing, 5-ml .75 N trichloracetic acid (TCA) was added. After 5 minutes, a 1.5 ml representative sample was centrifuged at 13000 for 15 minutes. Supernatant (1.0-ml) was then transferred to a microfuge tube and frozen at -60°C until assayed.

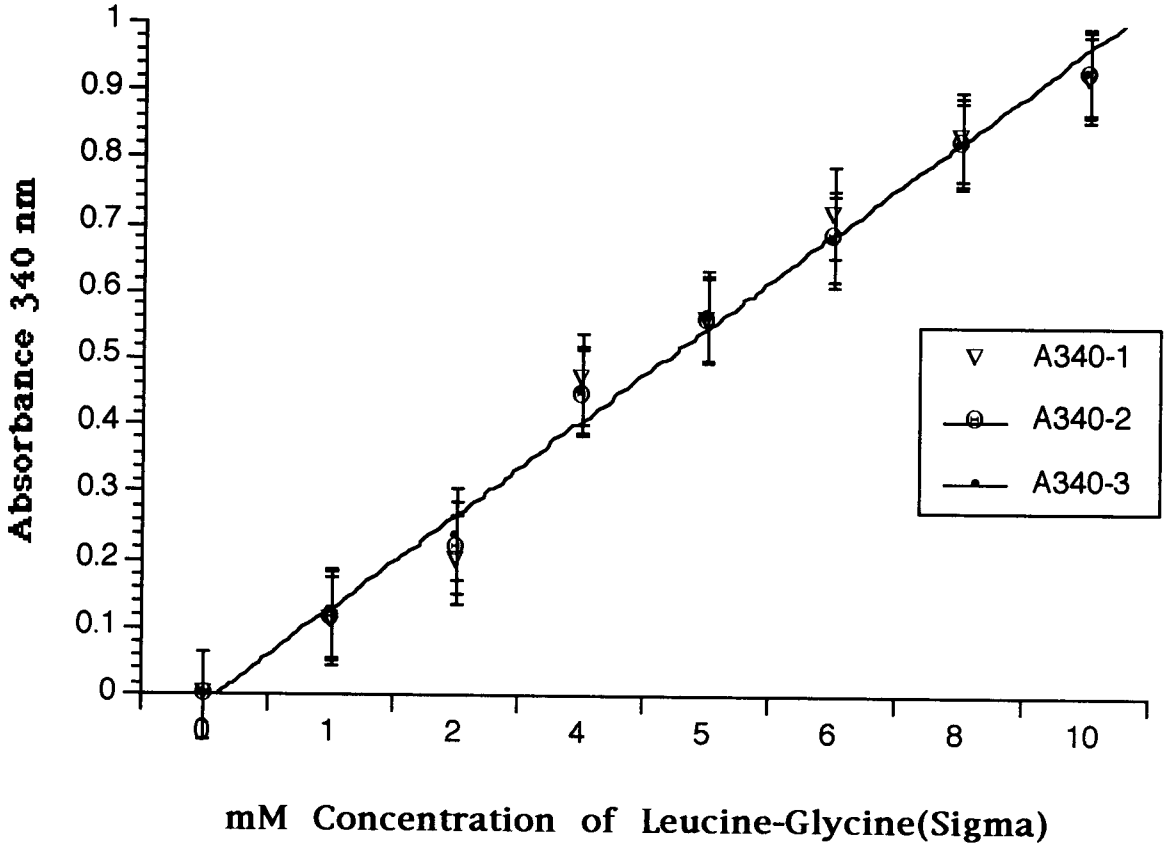


Figure 1. Proteolysis standard curve showing linear increase in absorbance at 340 nm in response to increasing concentration of the peptide Leucine-Glycine.

The *O* - phthaldialdehyde (OPA) reagent must be prepared daily for the assay. It was made by combining the following reagents and diluting to a final volume of 50 ml with water: 25 ml sodium tetraborate; 2.5 ml of 20% (wt/wt) sodium dodecyl sulfate; 40 mg of OPA (dissolved in 1.0 ml of methanol); and 100 µl of ß-mercaptoethanol.

In a plastic cuvette, 1.0 ml of OPA reagent and 50 µl of sample were mixed by inversion and incubated at ambient temperature for two minutes. Absorbance was then measured at 340 nm using a Beckman Model DU-40 spectrophotometer and the amount of proteolysis determined from a standard curve (Figure 1.) using Leucine-Glycine as a reference substrate. The standard curve was constructed using Delta Graph software (Delta Point Inc. Monterey, CA).

Formate Production

Samples were taken from an appropriate 10-12 hour, 37°C, milk culture. To 1.0-ml culture in a microfuge tube, 0.5-ml Reagent A (Promega) was added. Mixing was performed by inversion of the microfuge tubes 10 times. The mixture was centrifuged at 13000 rpm for 5 minutes. The supernatant was poured off and the cell pellet resuspended in 1.0 ml of pH 7.0, 0.1 M phosphate buffer. Sample was again centrifuged at 13000 rpm for 5 minutes and the supernatant poured off. The cell pellet was then resuspended in 1.0 ml of pH 7.0 phosphate buffer. A 1% inoculum then was pipetted into 11% (wt/vol) reconstituted NFDM, previously pasteurized at 63°C for 60 minutes.

The inoculated milk tubes were then incubated in a water bath for six hours. Temperature was dependent upon the culture. *Streptococcus thermophilus* cultures were incubated at 38°C; and rod-coccus mixed cultures were incubated at 42°C. These temperatures were optimum for the respective culture types as determined by Radke-Mitchell and Sandine (1986).

The method used to determine formate production was the formic acid detection kit(Boehringer Mannheim, Indianapolis, IN), the principle of the assay being oxidation of formate in the presence of formate dehydrogenase (FDH) by nicotinamide-adenine dinucleotide (NAD) quantitatively to carbon dioxide.

$$\text{Formate} + NAD^+ + H_2O \xrightarrow{FDH} HCO_3^- + NADH + H^+$$

The amount of NADH formed is stoichiometric to the amount of formic acid. The increase in NADH was measured by means of its absorbance at 340nm.

Cultures were sampled aseptically after gentle mixing. To a 5.0-ml sample, 1.0-ml of sterile distilled water was added. While vortexing, 10-ml .75 N trichloroacetic acid (TCA) was added. After 10 minutes at 0°C, a 5-ml representative sample was centrifuged at 1500*g* for 15 minutes. The supernatant was then transferred to a sterile test tube and neutralized to a final pH of 6.0 to 7.0 with solid $KHCO_3$(Sigma Chemical Co., St. Louis, MO). The supernatant, 1.5-ml, was transferred to a microfuge tube and frozen at -60° C until assayed.

Samples were thawed at room temperature and centrifuged at 1000*g* for 15 minutes. In a cuvette 1.9-ml water, 0.1-ml sample, and 1.0-ml reaction mixture 2(NAD) were mixed by inversion.

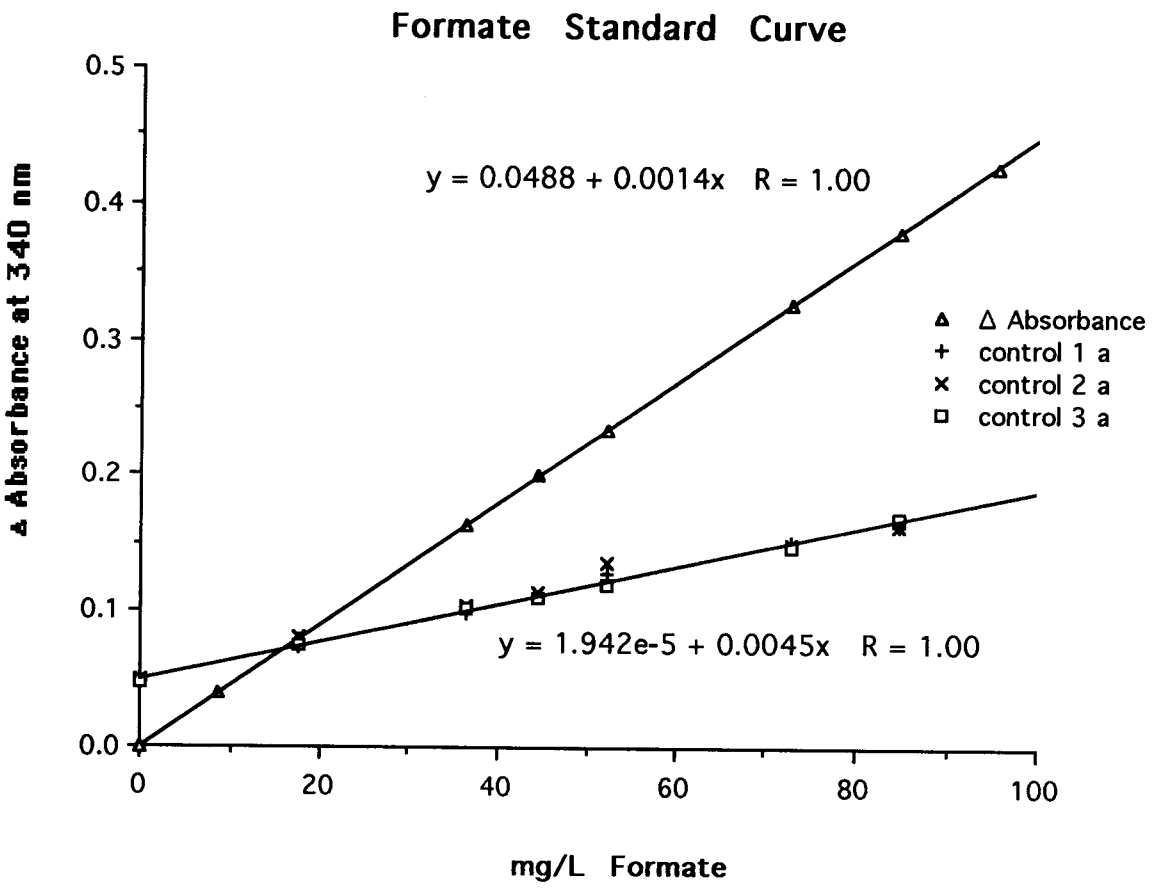


Figure 2. Formate standard curve (Δ) showing linear increase in absorbance at 340 nm in response to increasing concentrations of sodium formate. Control 1a, 2a, 3a, represents these same concentrations extracted from 11% NFDM.

After 5 minutes at room temperature the absorbance was measured at 340nm using a Beckman Model DU-40 spectrophotometer. To the cuvette, 0.05-ml of solution 3(FDH) was added and mixed by inversion. After 20 minutes at room temperature the absorbance was recorded at 340 nm using a Beckman Model DU-40 spectrophotometer and formate production was determined from a standard curve (Figure 2) using sodium formate(Sigma Chemical Co., St. Louis, MO) as a reference substrate. The standard curve was constructed using Cricket Graph Software (Computer Associates, Islandia, NY).

Carbon Dioxide Production

To measure carbon dioxide production, a gasometer as described by Sandine et al., (1957), was used (Figure 3). Each gasometer was calibrated as follows: the distance from the middle of the second bend to the top of the gas-measuring tube was etched with a file at 1 cm. intervals. It then was inverted and mercury drawn into the side arm. The volume of this side arm then was determined by delivering the mercury from a known distance along the etched segment of the tube into a tared container. The weight of the mercury delivered per cm. of tube length then was noted and this value converted to microliters (μl.) volume per cm. using the density of mercury at that temperature. Gasometers calibrated in this manner were found to contain approximately 20 μl. volume per cm. on the gas-measuring arm.

Brodie's solution was used as the liquid in the gas-measuring tubes. It was made as follows: 23 grams of NaCl and 5 grams of

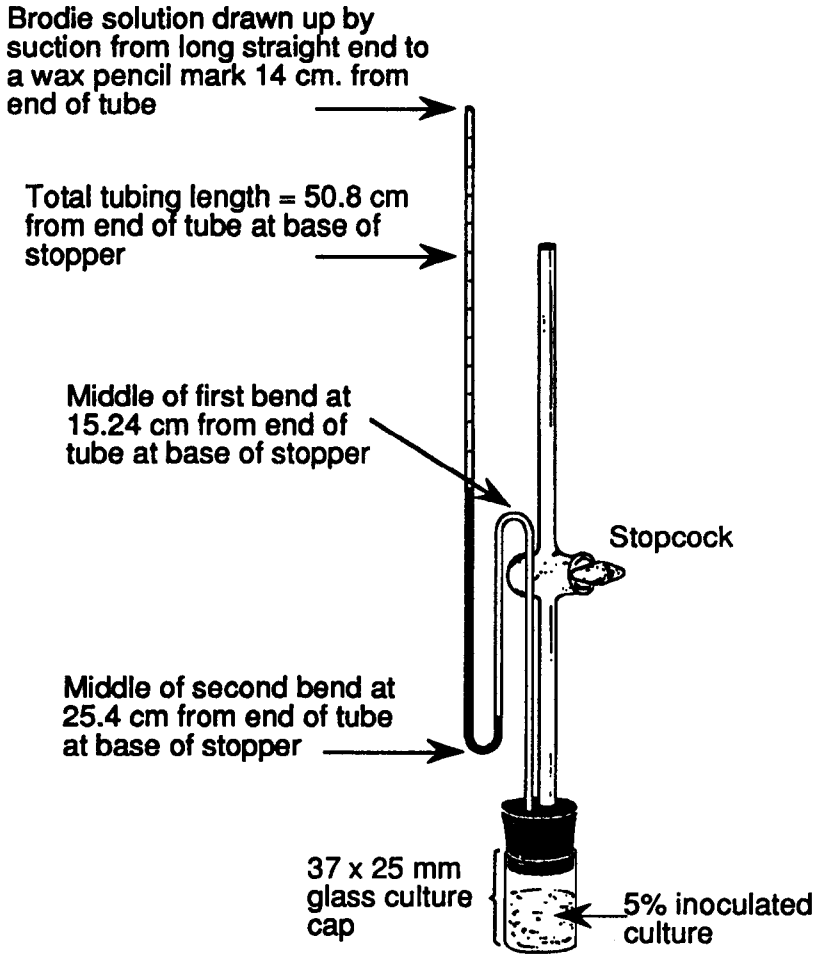


Figure 3. Drawing showing design of laboratory gasometer for measuring gas production.

sodium choleate (ox-gall) were dissolved in 500 ml. of distilled water and the solution colored red with fuchsin basic (Sigma). Specific gravity of fresh Brodie's solution was approximately 1.033. The best method for filling the gas-measuring tubes with equal amounts of Brodies solution was to invert the apparatus, open the stopcock, and apply suction to the non measuring arm to draw fluid up to the 14 cm. mark from the tip of the tube. The apparatus was then turned right side up and the solution allowed to settle in the measuring arm.

The medium used was 11% (wt/vol) reconstituted NFDM inoculated with 5% 10-12 hour, 37°C milk culture. Sterile, dry flasks were filled with 15 ml. of inoculated milk. This volume, 15 ml., was used to fill the flask and reduce head space. One flask was filled with 15 ml. of uninoculated milk to serve as a control. The measuring arm and stopcock vent were inserted into the flask and placed in a water bath equipped with an agitator. The stopcock vent was open and remained so for 15 minutes to allow temperature equilibrium. The stopcocks were then closed and initial readings recorded by reading the position of the meniscus of the Brodie's solution in the gas measuring arms. The gasometers were then incubated in a water bath for six hours. Temperature was dependent upon the culture. *Streptococcus thermophilus* cultures were incubated at 38°C; *L. bulgaricus*, *L. lactis*, and *L. helveticus* were incubated at 44°C; and rod-coccus mixed cultures were incubated at 42°C. These temperatures were optimum for the respective culture types as determined by Radke-Mitchell and Sandine (1986).

The gasometers were closely monitored since very high gas producing cultures may evolve sufficient gas to force the level of

fluid over the top of the measuring arm. In this case it was necessary to record the level of liquid as it approached the top (at the same time reading the control flask), open the vent to reset the solution, then close the vent to allow the measurement to continue for the remaining time.

The microliters of gas evolved for each culture were calculated by subtracting the net increase of the control flask from the net increase observed in the test flask and multiplying by 20 to obtain microliters of carbon dioxide produced. Occasionally a decrease in atmospheric pressure resulted in a negative value for a control. This value was then added to each net test reading.

Phosphate Sensitivity

Phosphate milk was prepared as described by Thunell and Sandine, (1983). Solutions were prepared by reconstituting 62 grams NFDM in 400 grams water. Phosphate salt concentrations as derived from Table 1, were brought to a volume of 100 ml(wt/vol) with distilled water. Phosphate solutions and reconstituted milk were autoclaved separately, and cooled in an ice bath. Phosphate-milk solutions were then prepared by combining sterile NFDM and individual phosphate solutions (total wt 562 g). Phosphate concentrations (as PO_4 ion, rather than total phosphate salt) for phosphate-milk solutions were 0.1, 0.3, and 0.5 percent.

Phosphate milk aliquots of 10 ml were pipetted into tubes and then inoculated with 1% 10-12 hour, 37°C, milk culture. Tubes were then incubated in a water bath for six hours, and then the pH determined. Temperature was dependent upon the culture.

Streptococcus thermophilus cultures were incubated at 38°C; *L. bulgaricus, L. lactis,* and *L. helveticus* were incubated at 44°C; and rod-coccus mixed cultures were incubated at 42°C. These temperatures were optimum for the respective culture types as determined by Radke-Mitchell and Sandine (1986).

Table 1. Amounts of added phosphates and final solution concentrations used in making milk for phosphate-tolerance determinations.

	Combined wt Phosphate		
Final % PO_4 ion	g K_2HPO_4	g NaH_2PO_4	% total salt final conc.
0.1	0.68	0.41	0.19
0.3	2.03	1.23	0.58
0.5	3.39	2.05	0.97

[a]Approximately 50% of the PO_4 ion at each concentration was derived from each phosphate salt used.

Salt Tolerance

Salted milk was prepared as described by Thunell and Sandine, (1983). Reagent grade sodium chloride was added to sterile reconstituted 11% NFDM in half percent increments ranging from 0% to 5% (w/v) and thoroughly mixed. Milk was pipetted in 10 ml quantities into sterile tubes and inoculated with 1% 10-12 hour, 37°C, milk culture. Tubes were then incubated in a water bath for six hours. Temperature was dependent upon the culture. *Streptococcus thermophilus* cultures were incubated at 38°C; *L. bulgaricus, L. lactis,* and *L. helveticus* were incubated at 44°C; and rod-coccus mixed cultures were incubated at 42°C. These temperatures were

optimum for the respective culture types as determined by Radke-Mitchell and Sandine (1986). After six hours, pH was recorded from each tube.

Carbohydrate Fermentation

Metabolic determinations were done with 49 carbohydrates using the API Rapid CH 50 system(Analytab Products, Plainview, NY). The procedure involved plating on MRS (Difco), (rods), or M17 (Difco), (cocci), agar medium to confirm a pure culture. Strains were then incubated at 37°C for 24 hours, centrifuged 10 minutes at 3000 RPM and the supernatant discarded. The pellet was then homogenized in 5 ml distilled water and centrifuged again for 10 minutes at 3000 RPM. The supernatant was poured off and the pellet homogenized in 2 ml distilled water. This solution was considered the inoculum added to the CHL medium, (rods), or CHS medium, (cocci). Medium sized drops of inoculum were added to 5 ml distilled water until an optical density of 0.5 to 0.75 was reached. This number was doubled and added to the CHL or CHS medium. This medium then was pipetted into each individual carbohydrate cupule. The opening was covered with sterile mineral oil to produced anaerobic conditions and placed in an incubator at 37°C. Readings were made at 3, 6, 24, and 48 hours. A color change from purple to yellow in CHL, and red to yellow in CHS indicated carbohydrate utilization.

RESULTS

Proteolysis

Results of analyses of the various strains of lactobacilli and *S. thermophilus* are shown in Tables 2 through 6. Table 2 shows data for the *L. bulgaricus* strains which vary from as low as 15.2 (strain B) to as high as 32.6 mM (strain N). Table 3 shows data for the *L. helveticus* strains which vary from 12.5 to as high as 34.7 mM. The data for *L. lactis*, table 4, ranged from 11.3 to 32.3 mM, though only three strains were tested.

Proteolytic analyses for *S. thermophilus* appear in Table 5. Again a wide range of values were seen from the low of 18.5 to as high as 46.4 mM. However strains were more similar in proteolytic ability than was true for rod cultures.

While only four multiple strain cultures were tested for proteolysis, the data appear in table 6. One mixture was weakly proteolytic while another was very high, infact moreso than any of the single strains of lactobacilli and *S. thermophilus* tested.

Graphs showing the effect of various pH values on proteolysis of *S. thermophilus* 3 and *L. bulgaricus* J appear in Figures 4 & 5 respectively. It is clear that incubation for at least 15 hours is necessary to determine the magnitude of the pH effect. Also for *S. thermophilus* 3 the optimum pH for proteolysis is 6.0, while for *L. bulgaricus* J it is also pH 6.0.

Table 2. Proteolytic activity of *L. bulgaricus* strains grown from a 1% inoculum in 11% NFM and incubated at 44°C for 6 hours. Values are the average of three trials expressed as absorbance at 340 nm and as mM concentration of amino acids liberated from milk protein as determined by the Church assay.

Strain	A340	STDEV	mM
A	0.182	0.12	17.0
B	0.165	0.15	15.2
C	0.206	0.18	19.5
D	0.182	0.06	17.0
E	0.178	0.15	16.5
F	0.197	0.04	18.5
G	0.215	0.06	20.5
H	0.289	0.16	28.1
I	0.333	0.09	32.7
J	0.337	0.12	33.1
K	0.291	0.03	28.3
L	0.285	0.01	27.7
M	0.332	0.12	32.6
N	0.275	0.08	26.6
O	0.205	0.06	19.4
P	0.232	0.09	22.2
Q	0.237	0.12	22.7
R	0.224	0.18	21.4
S	0.214	0.10	20.3

Table 3. Proteolytic activity of *L. helveticus* strains grown from a 1% inoculum in 11% NFM and incubated at 44°C for 6 hours. Values are the average of three trials expressed as absorbance at 340 nm and as mM concentration of amino acids liberated from milk protein as determined by the Church assay.

Strain	A340	STDEV	mM
A	0.169	0.06	15.60
B	0.223	0.07	21.20
C	0.212	0.02	20.10
D	0.224	0.04	21.40
E	0.263	0.12	25.40
F	0.139	0.07	12.50
G	0.213	0.06	20.20
H	0.308	0.15	30.10
I	0.352	0.15	34.70
J	0.258	0.03	24.90

Table 4. Proteolytic activity of *L. lactis* strains grown from a 1% inoculum in 11% NFM and incubated at 44°C for 6 hours. Values are the average of three trials expressed as absorbance at 340 nm and as mM concentration of amino acids liberated from milk protein as determined by the Church assay.

Strain	A340	STDEV	mM
A	0.128	0.06	11.3
B	0.329	0.14	32.3
C	0.279	0.18	27.1

Table 5. Proteolytic activity of *S. thermophilus* strains grown from a 1% inoculum in 11% NFM and incubated at 38°C for 6 hours. Values are the average of three trials expressed as absorbance at 340 nm and as mM concentration of amino acids liberated from milk protein as determined by the Church assay.

Strain	A340	STDEV	mM
1	0.223	0.04	21.3
2	0.198	0.06	18.7
3	0.284	0.08	27.6
4	0.222	0.10	21.1
5	0.199	0.09	18.7
6	0.231	0.08	22.1
7	0.212	0.01	20.1
8	0.223	0.03	21.3
9	0.229	0.00	21.9
10	0.238	0.01	22.9
11	0.464	0.06	46.4
12	0.182	0.03	16.9
13	0.197	0.23	18.5

Table 6. Proteolytic activity of *L. bulgaricus* and *S. thermophilus* strains grown from a 1% inoculum in 11% NFM and incubated at 42°C for 6 hours. Values are the average of three trials expressed as absorbance at 340 nm and as mM concentration of amino acids liberated from milk protein as determined by the Church assay.

Strain	A340	STDEV	mM
L. b. CR 14/S. t. 2	0.068	0.11	5.1
L. b. Q/S. t. 2	0.696	0.04	70.5
L. b. C, E/S. t.7,12	0.506	0.16	50.8
L. b. C, G/S. t. 4, 7	0.583	0.07	58.7

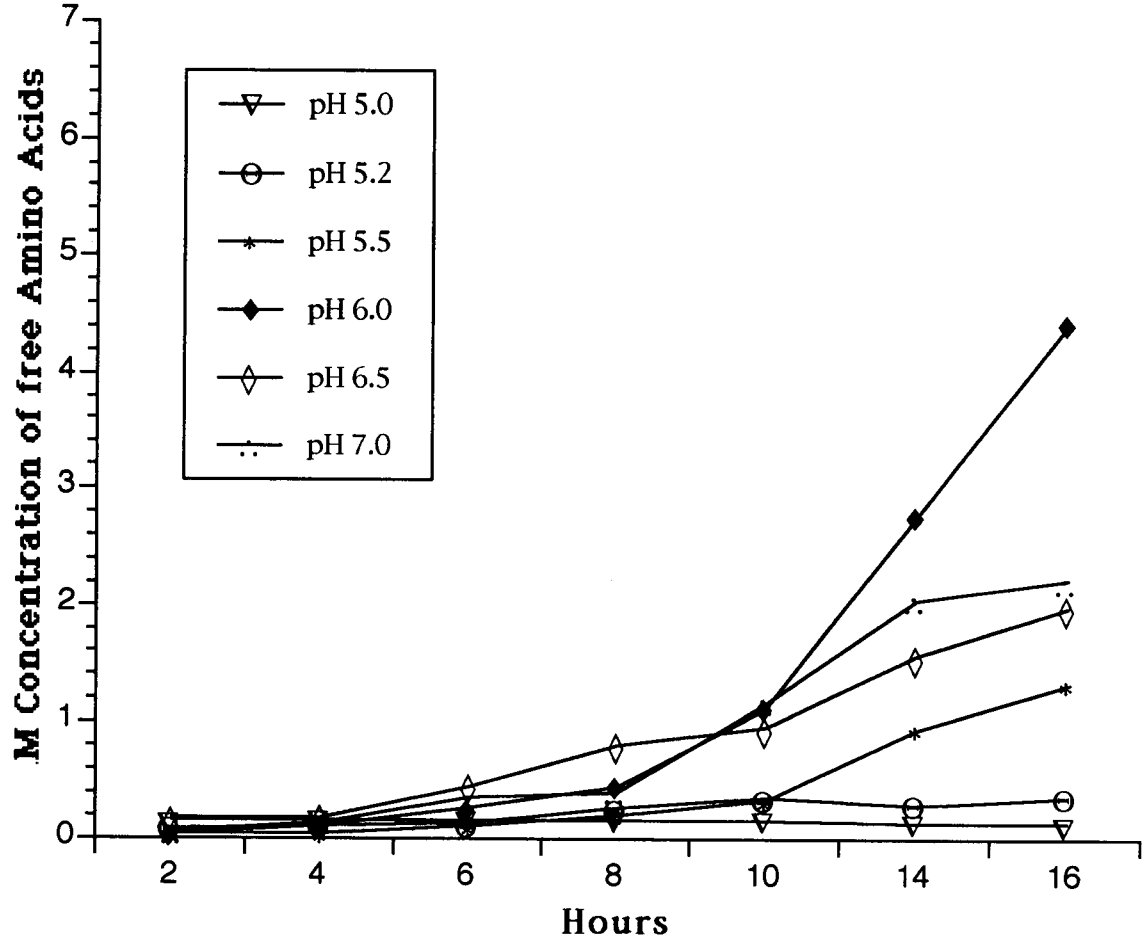


Figure 4. Proteolytic activity of *S. thermophilus* strain 3 grown from a 1% inoculum in 11% NFM and incubated at 38°C at controlled pH. Values are M concentration of liberated amino acids expressed as a function of pH as determined by the Church assay.

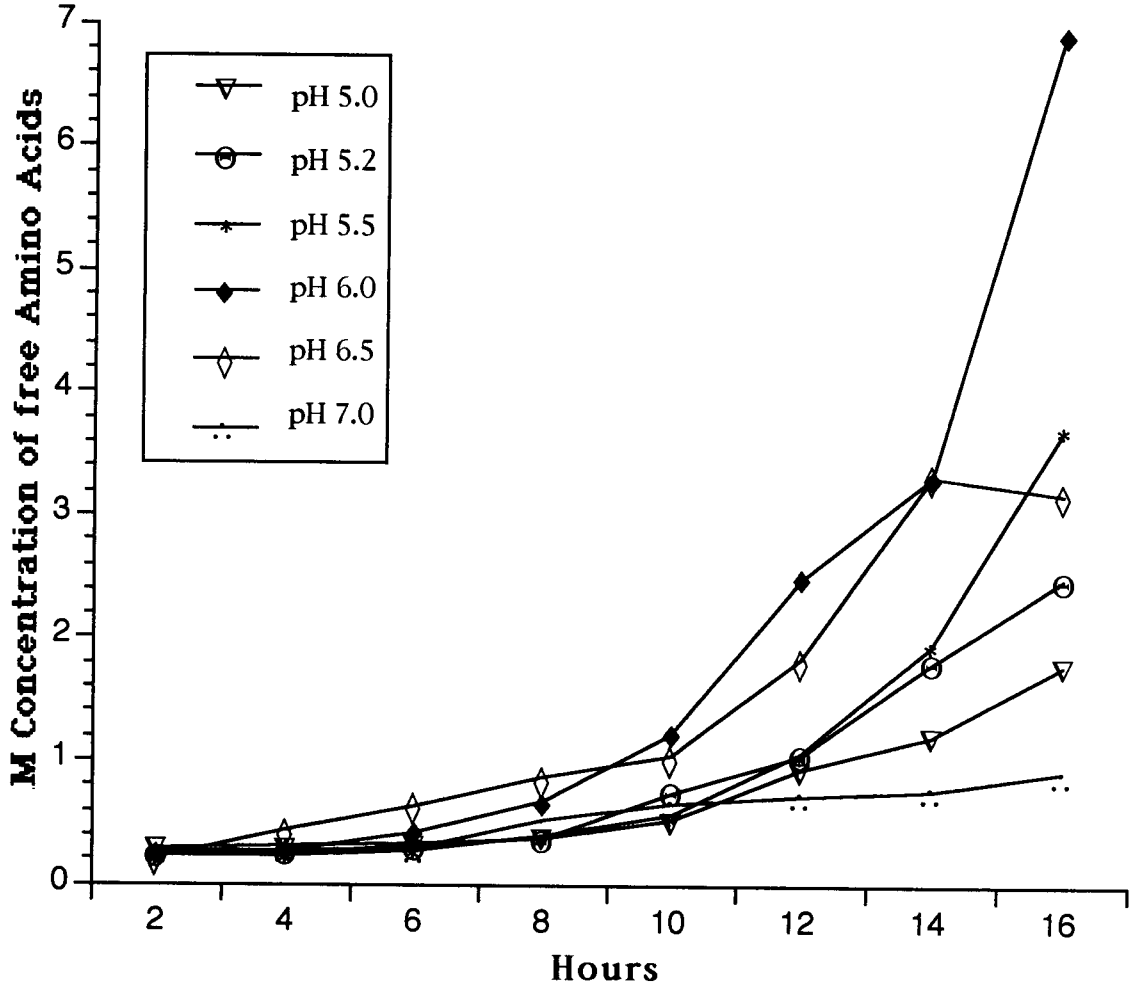


Figure 5. Proteolytic activity of *L. bulgaricus* strain J grown from a 1% inoculum in 11% NFM and incubated at 44°C at controlled pH. Values are M concentration of liberated amino acids expressed as a function of pH as determined by the Church assay.

Formate Production

Results of formate production analyses on the various strains of *S. thermophilus* and mixed cultures are shown in Tables 7 and 8. Formate production data for *S. thermophilus* appear in Table 7. A wide range of values are seen from the low of 4.2 to as high as 20.3 mg/L.

While only four multiple strain cultures were tested for formate production, the data appear in Table 8. One mixture produced very little formate while the others were very high, infact, two and a half times as much produced as by the single *S. thermophilus* strains tested.

Table 7. Formate production of *S. thermophilus* strains grown from a 1% inoculum in 11% NFM and incubated at 38°C for 6 hours. Values are the average of three trials expressed as milligrams per liter.

Strain	mg/L	STDEV
1	13.4	6.96
2	18.1	9.85
3	6.7	2.04
4	9.1	1.79
5	20.3	3.25
6	4.0	1.02
7	17.8	0.64
8	5.4	1.68
9	8.7	0.77
10	8.3	1.48
11	8.9	2.38
12	4.2	2.07
13	10.3	1.48

Table 8. Formate production of *L. bulgaricus* and *S. thermophilus* strains grown from a 1% inoculum in 11% NFM and incubated at 42°C for 6 hours. Values are the average of three trials expressed as milligrams per liter.

Strain	mg/L	STDEV
L. b. CR 14/S. t. 2	6.4	3.11
L. b. Q/S. t. 2	50.5	0.84
L. b. C, E/S. t.7,12	54.6	2.18
L. b. C, G/S. t. 4, 7	47.6	1.07

Carbon Dioxide Production

Results of analyses of the various strains of lactobacilli and *S. thermophilus* for CO_2 production are shown in Tables 9 through 13. Table 9 shows data for the *L. bulgaricus* strains which vary from as low as zero (strain S) to as high as 376 µl (strain A). Table 10 shows data for the *L. helveticus* strains which vary from 6 to as high as 212 µl. The data for *L. lactis*, Table 11, ranged from 56 to 94 µl, though only three strains were tested.

Carbon dioxide production analyses for *S. thermophilus* appear in Table 12. Again a wide range of values were seen from the low of 5 to as high as 1259 µl. However, *S. thermophilus* strains produced significantly more carbon dioxide, with three exceptions, than the rod cultures.

Only four multiple strain cultures were tested for carbon dioxide production, and the data appear in Table 13. All mixtures were weak producers of carbon dioxide.

Table 9. Carbon dioxide production of *L. bulgaricus* strains grown from a 1% inoculum in 11% NFM and incubated at 44°C for 6 hours. Values are expressed as microliters of carbon dioxide generated.

Strain	µl CO_2
A	376
B	162
C	110
D	344
E	142
F	92
G	160
H	86
I	148
J	16
K	18
L	36
M	50
N	362
O	52
P	4
Q	50
R	2
S	0

Table 10. Carbon dioxide production of *L. helveticus* strains grown from a 1% inoculum in 11% NFM and incubated at 44°C for 6 hours. Values are expressed as microliters of carbon dioxide generated.

Strain	µl CO_2
A	212
B	120
C	5.6
D	140
E	108
F	64
G	140
H	140
I	16
J	10

Table 11. Carbon dioxide production of *L. lactis* strains grown from a 1% inoculum in 11% NFM and incubated at 44°C for 6 hours. Values are expressed as microliters of carbon dioxide generated.

Strain	µl CO_2
A	56
B	76
C	94

Table 12. Carbon dioxide production of *S. thermophilus* strains grown from a 1% inoculum in 11% NFM and incubated at 44°C for 6 hours. Values the average of three trials are expressed as microliters of carbon dioxide generated.

Strain	µl CO_2
1	109
2	1117
3	1164
4	1259
5	911
6	1164
7	798
8	5
9	13
10	913
11	1196
12	987
13	912

Table 13. Carbon dioxide production of *L. bulgaricus* and *S. thermophilus* strains grown from a 1% inoculum in 11% NFM and incubated at 42°C for 6 hours. Values are the average of three trials expressed as microliters of carbon dioxide generated.

Strain	µl CO_2
L. b. CR14/S. t. 2	71
L. b. Q/S. t. 2	56
L. b. C, E/S. t.7,12	386
L. b. C, G/ S.t.4,12	266

Phosphate Sensitivity

Results of testing the various strains of lactobacilli and *S. thermophilus* for phosphate sensitivity are shown in Tables 14 through 18. Table 14 shows data for the *L. bulgaricus* strains in which 9 of 19 strains were stimulated by 0.1% phosphate, after corrections for pH change due to phosphate addition. Strain F showed stimulation at 0.3% phosphate. Thirteen of 19 strains were severely inhibited by 0.5% phosphate. Table 15 shows data for the *L. helveticus* strains in which three of 10 strains were stimulated by 0.1% phosphate, and another three strains were unaffected. All stains were inhibited by 0.5% phosphate, although strain J was inhibited only slightly. The data for *L. lactis*, Table 16, show stimulation for strains B and C at 0.1%, but inhibition at 0.3% and 0.5% phosphate.

Phosphate sensitivities for *S. thermophilus* appear in Table 17. Acid production was inhibited in 11 of 13 strains at 0.1% phosphate. Strains 5 and 7 were the two strains not inhibited; in fact they were slightly stimulated by 0.1% and 0.3% phosphate and unaffected by 0.5% phosphate.

Four multiple strain cultures were tested for phosphate sensitivity and data appear in Table 18. Mixed cultures of L. b. CR 14/ S. t. 2 and L. b. Q/ S. t. 2 were not inhibited by 0.1% phosphate, but inhibition occured at higher concentrations. The mixed cultures of L. b. C, E/ S. t. 7, 12 and L. b. C, G/ S. t. 4, 12 were stimulated by all three concentrations of phosphate salts tested.

Table 14. Phosphate sensitivity of *L. bulgaricus* strains grown from a 1% inoculum in 11% NFM supplemented with phosphate salts in concentrations of 0.1%, 0.3% and 0.5% and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Strain	Phosphate [ ] 0.00%	0.10%	0.30%	0.50%
A	4.08	4.07	4.49	6.33
B	4.56	4.56	5.54	6.23
C	4.52	4.57	5.52	6.23
D	4.70	4.69	4.95	5.03
E	4.66	4.64	5.44	6.21
F	4.30	4.12	4.39	5.24
G	4.29	4.16	4.67	5.24
H	4.78	4.91	5.95	6.28
I	4.52	4.42	5.51	6.10
J	4.01	4.13	4.50	5.18
K	4.43	4.78	5.67	6.18
L	4.51	4.58	4.99	6.29
M	4.28	4.28	5.07	6.09
N	4.14	4.14	4.46	5.60
O	4.88	5.00	6.05	6.36
P	4.23	4.28	5.13	6.15
Q	3.96	4.08	4.30	5.39
R	4.33	4.43	5.14	6.12
S	4.47	4.50	5.53	6.23

Table 15. Phosphate sensitivity of *L. helveticus* strains grown from a 1% inoculum in 11% NFM supplemented with phosphate salts in concentrations of 0.1%, 0.3% and 0.5% and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

	Phosphate [ ]			
Strain	0.00%	0.10%	0.30%	0.50%
Neg. Control	6.28	6.41	6.50	6.55
A	4.73	4.75	5.34	6.03
B	4.33	4.46	5.53	6.32
C	4.74	4.69	5.60	6.38
D	4.19	4.35	5.03	6.41
E	4.58	4.72	5.68	6.46
F	4.85	4.79	5.25	6.10
G	4.54	4.72	5.66	6.41
H	4.63	4.72	5.51	6.18
I	4.08	4.30	4.72	5.98
J	3.96	3.95	4.20	4.50

Table 16. Phosphate sensitivity of *L. lactis* strains grown from a 1% inoculum in 11% NFM supplemented with phosphate salts in concentrations of 0.1%, 0.3% and 0.5% and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

	Phosphate [ ]			
Strain	0.00%	0.10%	0.30%	0.50%
Neg. Control	6.28	6.41	6.50	6.55
A	5.08	5.30	6.05	6.33
B	4.78	4.66	5.84	6.05
C	4.73	4.68	5.95	6.24

Table 17. Phosphate sensitivity of *S. thermophilus* strains grown from a 1% inoculum in 11% NFM supplemented with phosphate salts in concentrations of 0.1%, 0.3% and 0.5% and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.

Strain	Phosphate [ ] 0.00%	0.10%	0.30%	0.50%
Neg. Control	6.28	6.41	6.50	6.55
1	5.02	5.19	5.63	6.23
2	5.10	5.37	5.79	6.06
3	5.12	5.22	5.51	6.00
4	5.10	5.31	5.61	5.96
5	4.52	4.38	4.47	4.72
6	5.04	5.21	5.65	5.90
7	4.51	4.40	4.51	4.73
8	4.96	5.11	5.48	5.84
9	4.86	5.05	5.41	5.76
10	4.97	5.15	5.36	5.71
11	5.12	5.27	5.59	5.83
12	4.97	5.09	5.38	5.71
13	4.95	5.08	5.38	5.61

Table 18. Phosphate sensitivity of *L. bulgaricus* and *S. thermophilus* strains grown from a 1% inoculum in 11% NFM supplemented with phosphate salts in concentrations of 0.1%, 0.3% and 0.5% and incubated at 42°C for 6 hours. Values are the average of three trials expressed as final pH.

Strain	Phosphate [ ] 0.00%	0.10%	0.30%	0.50%
Neg. Control	6.28	6.41	6.50	6.55
L. b. CR 14/S. t. 2	4.47	4.46	4.99	6.00
L. b. Q/S. t. 2	3.69	3.72	3.95	4.36
L. b. C, E/S. t. 7, 12	3.63	3.26	3.51	3.70
L. b. C, G/S. t. 4, 12	3.92	3.13	3.27	3.45

Salt Tolerance

Results of testing various strains of lactobacilli and *S. thermophilus* for salt sensitivity are shown in Tables 19 through 67 and Figures 4 through 52. Tables 19 through 37 and Figures 4 through 22 show data for the *L. bulgaricus* strains. Acid production by most *L. bulgaricus* strains was completely inhibited at 2.5% sodium chloride; strains P and Q were exceptions, exhibiting slight amounts of acid production at 2.5% sodium chloride. Seven of 19, (B, C, E, J, K, M, and O), strains demonstrated significant decreases in acid production with the addition of 0.5% NaCl. Strains F, G, I, and S showed significant decreases at 1.0% salt. Strains D, N, and P demonstrated slight stimulation in acid production at lower salt concentrations.

Tables 38 through 47 and Figures 23 through 32 show data for the *L. helveticus* strains. Acid production in *L. helveticus* was completely inhibited in most strains at 3.0% sodium chloride; strains E and F were the exceptions, exhibiting slight amounts of acid production at 3.0% sodium chloride. Four of 10, (C, D, I, and J), strains demonstrated significant decreases in acid production with the addition of 0.5% NaCl. Strains A and H showed significant decreases at 1.0% salt. Strains B, E, F, and G demonstrated slight stimulation in acid production at lower salt concentrations.

The data for *L. lactis* strains are presented in Tables 48 through 50 and Figures 33 through 35. Strain A showed no inhibition up to 1.0% NaCl, and B no inhibition at 0.5% NaCl. All three strains formed a gradual even slope as inhibition occurred, and no salt concentration caused a sudden inhibitory effect.

Tables 51 through 63 and Figures 36 through 48 show data for the *S. thermophilus* strains. Six of 13 strains (1, 2, 9, 10, 11, and 12) showed inhibition at 0.5% sodium chloride. Strains 5, 6, and 7 were stimulated by low concentrations of salt. Inhibition was more gradual with the *S. thermophilus* strains, although this was in part due to the slower rate of acid production in general by *S. thermophilus* strains.

Tables 64 through 67 and Figures 49 through 52 show data for mixed strains which were tolerate to salt at concentrations up to 1.0%, Mixed culture L. b. C, G/ S. t. 4, 7 was actually stimulated by salt up to 1.5%.

Table 19. Salt sensitivity of *L. bulgaricus* strain A grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	3.82	4.65	4.15	0.42	4.21
0.50%	4.18	4.29	4.23	0.06	4.23
1.00%	4.35	4.53	4.38	0.10	4.42
1.50%	4.99	5.11	5.01	0.06	5.04
2.00%	5.75	5.74	5.73	0.01	5.74
2.50%	5.91	5.86	5.89	0.03	5.89
3.00%	5.91	5.88	5.91	0.02	5.90
3.50%	5.91	5.96	5.92	0.03	5.93
4.00%	5.92	5.94	5.93	0.01	5.93
4.50%	5.91	5.94	5.92	0.02	5.92
5.00%	5.91	5.93	5.92	0.01	5.92

Figure 6. Salt sensitivity of *L. bulgaricus* strain A grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

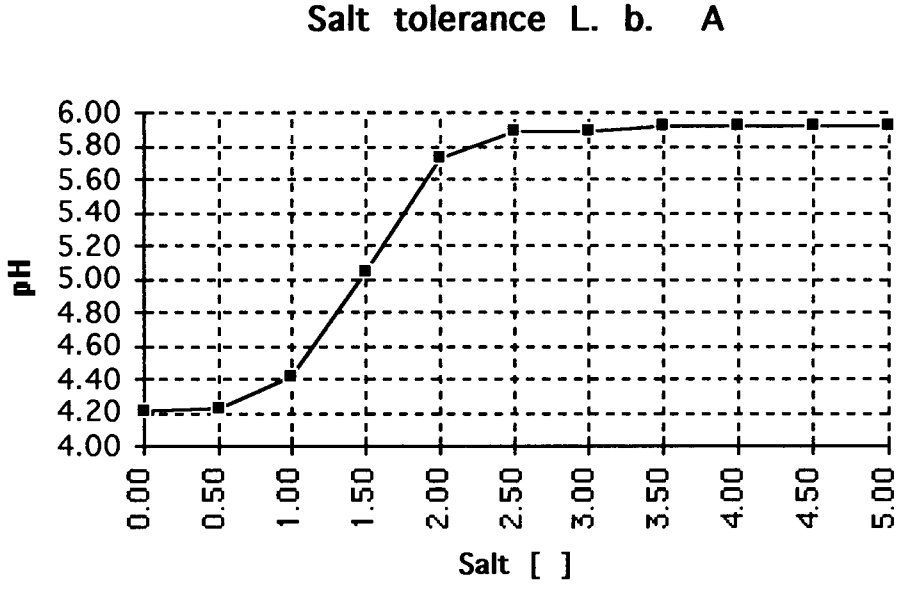


Table 20. Salt sensitivity of *L. bulgaricus* strain B grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.31	4.46	4.40	0.08	4.39
0.50%	4.72	4.87	4.70	0.09	4.76
1.00%	5.23	5.17	5.16	0.04	5.19
1.50%	5.36	5.16	5.30	0.10	5.27
2.00%	5.68	5.55	5.61	0.07	5.61
2.50%	5.89	5.74	5.85	0.08	5.83
3.00%	5.93	5.74	5.91	0.10	5.86
3.50%	5.92	5.84	5.92	0.05	5.89
4.00%	5.91	5.82	5.91	0.05	5.88
4.50%	5.88	5.82	5.89	0.04	5.86
5.00%	5.88	5.82	5.89	0.04	5.86

Figure 7. Salt sensitivity of *L. bulgaricus* strain B grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

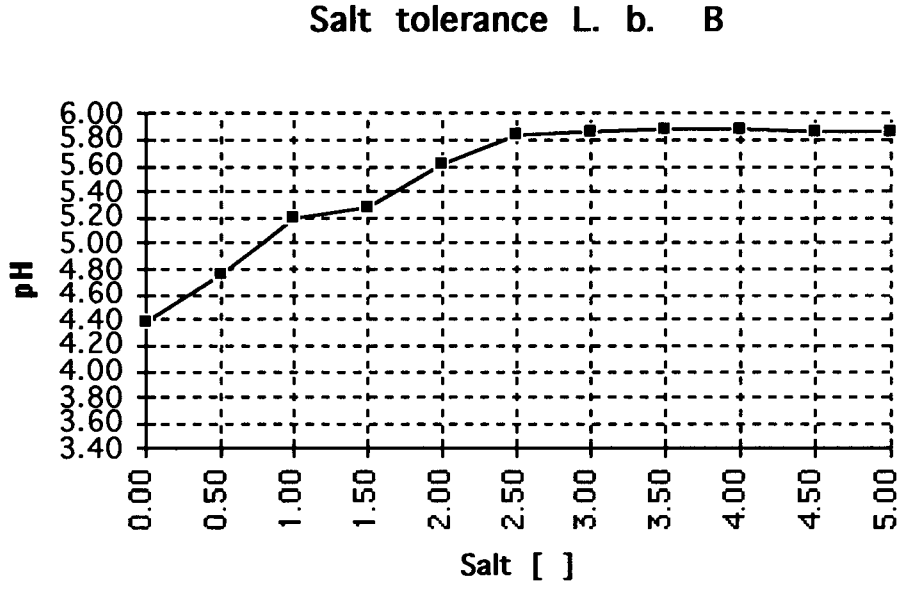


Table 21. Salt sensitivity of *L. bulgaricus* strain C grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.39	4.23	4.18	0.11	4.27
0.50%	4.66	4.50	4.52	0.09	4.56
1.00%	5.16	4.96	5.07	0.10	5.06
1.50%	5.37	5.25	5.24	0.07	5.29
2.00%	5.48	5.38	5.41	0.05	5.42
2.50%	5.80	5.57	5.67	0.12	5.68
3.00%	5.89	5.66	5.81	0.12	5.79
3.50%	5.89	5.74	5.81	0.08	5.81
4.00%	5.89	5.71	5.80	0.09	5.80
4.50%	5.87	5.70	5.80	0.09	5.79
5.00%	5.87	5.69	5.78	0.09	5.78

Figure 8. Salt sensitivity of *L. bulgaricus* strain C grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

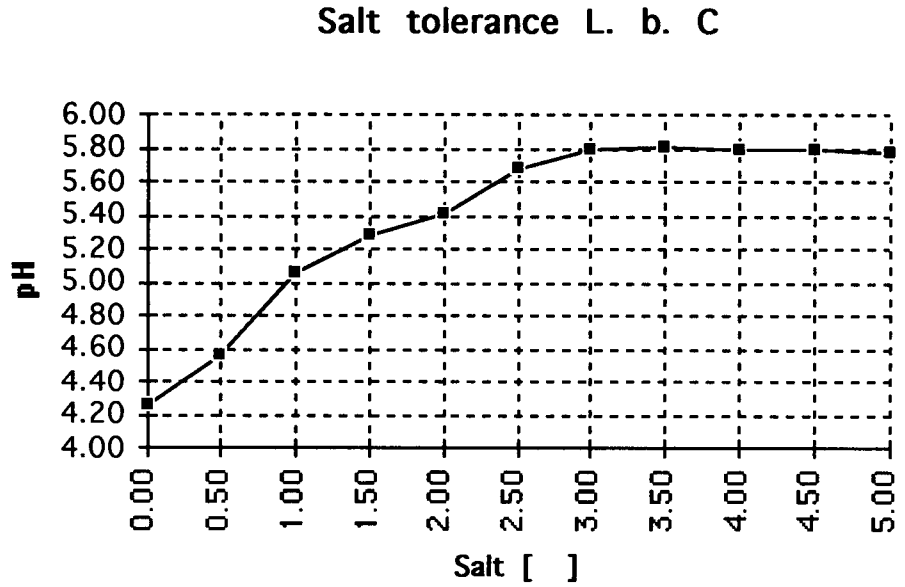


Table 22. Salt sensitivity of *L. bulgaricus* strain D grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.38	4.37	4.40	0.02	4.38
0.50%	4.56	4.24	4.32	0.17	4.37
1.00%	4.56	4.20	4.30	0.19	4.35
1.50%	4.86	4.21	4.41	0.33	4.49
2.00%	5.25	4.61	4.80	0.33	4.89
2.50%	5.74	5.69	5.70	0.03	5.71
3.00%	5.85	5.72	5.75	0.07	5.77
3.50%	5.89	5.80	5.83	0.05	5.84
4.00%	5.91	5.81	5.85	0.05	5.86
4.50%	5.91	5.82	5.87	0.05	5.87
5.00%	5.91	5.82	5.94	0.06	5.89

Figure 9. Salt sensitivity of *L. bulgaricus* strain D grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

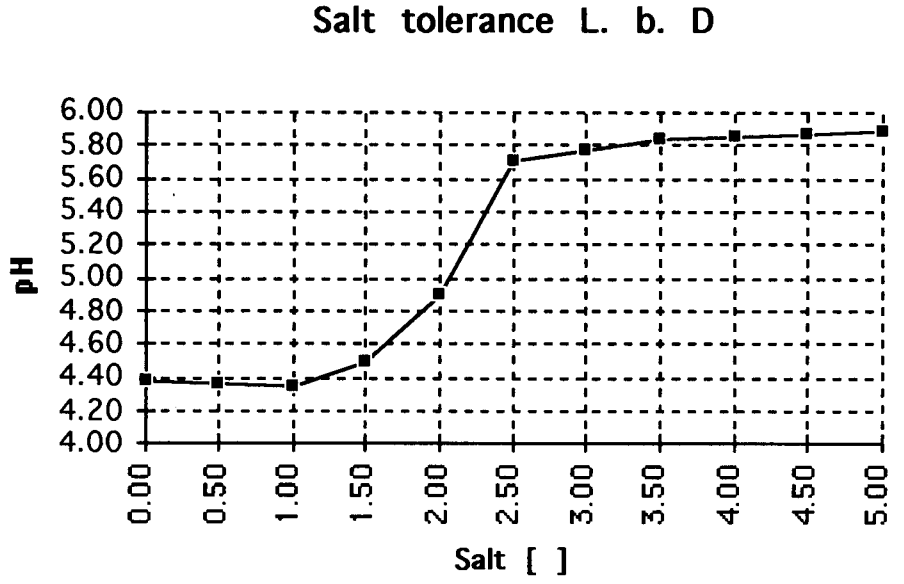


Table 23. Salt sensitivity of *L. bulgaricus* strain E grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.39	4.48	4.68	0.15	4.52
0.50%	4.75	5.01	5.10	0.18	4.95
1.00%	5.20	5.32	5.35	0.08	5.29
1.50%	5.30	5.38	5.40	0.05	5.36
2.00%	5.58	5.78	5.80	0.12	5.72
2.50%	5.77	5.90	5.88	0.07	5.85
3.00%	5.82	5.91	5.88	0.05	5.87
3.50%	5.89	5.91	5.89	0.01	5.90
4.00%	5.90	5.89	5.89	0.01	5.89
4.50%	5.91	5.88	5.90	0.02	5.90
5.00%	5.92	5.90	5.89	0.02	5.90

Figure 10. Salt sensitivity of *L. bulgaricus* strain E grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

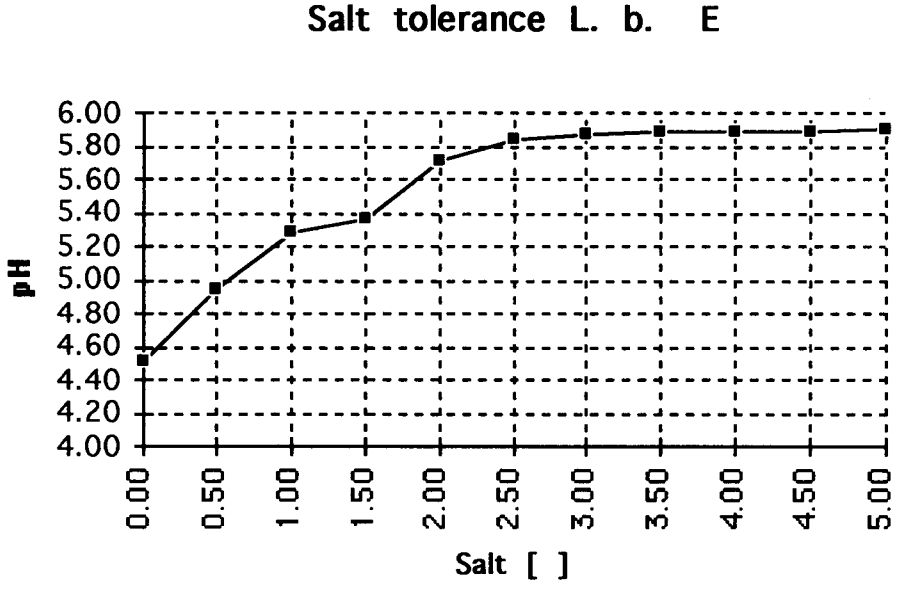


Table 24. Salt sensitivity of *L. bulgaricus* strain F grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.06	4.06	4.07	0.01	4.06
0.50%	4.22	4.38	4.24	0.09	4.28
1.00%	4.50	4.52	4.55	0.03	4.52
1.50%	5.40	5.44	5.39	0.03	5.41
2.00%	5.73	5.69	5.65	0.04	5.69
2.50%	5.82	5.77	5.74	0.04	5.78
3.00%	5.85	5.77	5.83	0.04	5.82
3.50%	5.89	5.83	5.84	0.03	5.85
4.00%	5.91	5.84	5.84	0.04	5.86
4.50%	5.91	5.84	5.83	0.04	5.86
5.00%	5.91	5.90	5.83	0.04	5.88

Figure 11. Salt sensitivity of *L. bulgaricus* strain F grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

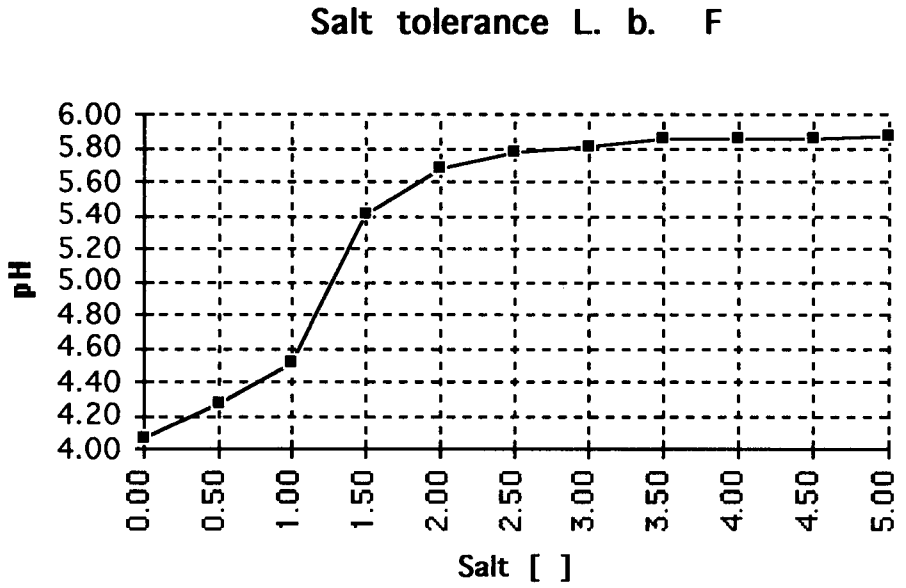


Table 25. Salt sensitivity of *L. bulgaricus* strain G grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	3.87	4.08	4.02	0.11	3.99
0.50%	3.95	3.98	3.96	0.02	3.96
1.00%	4.20	4.28	4.25	0.04	4.24
1.50%	5.18	5.44	5.31	0.13	5.31
2.00%	5.78	5.77	5.77	0.01	5.77
2.50%	5.81	5.83	5.85	0.02	5.83
3.00%	5.76	5.85	5.86	0.06	5.82
3.50%	5.83	5.86	5.84	0.02	5.84
4.00%	5.82	5.86	5.85	0.02	5.84
4.50%	5.80	5.85	5.86	0.03	5.84
5.00%	5.80	5.84	5.86	0.03	5.83

Figure 12. Salt sensitivity of *L. bulgaricus* strain G grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

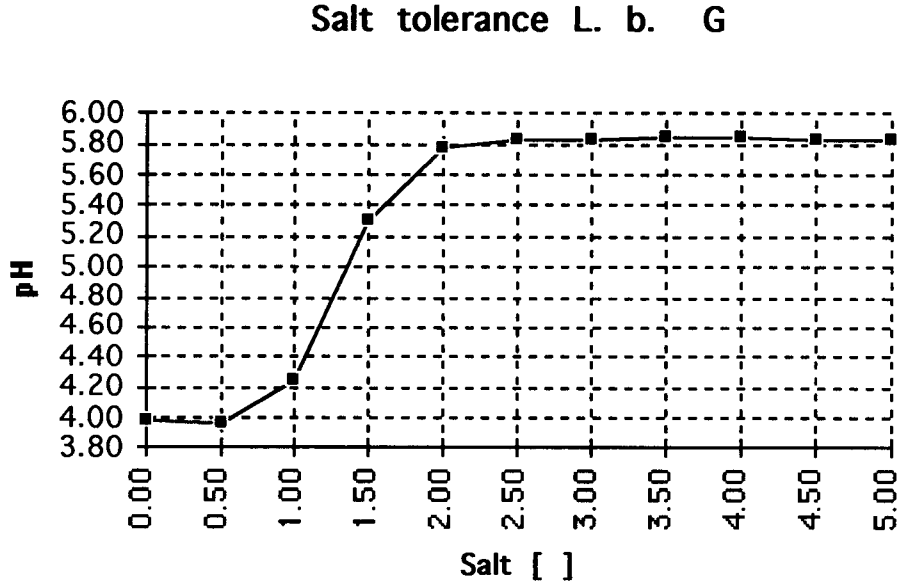


Table 26. Salt sensitivity of *L. bulgaricus* strain H grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.73	4.58	4.56	0.09	4.62
0.50%	4.88	4.89	4.86	0.02	4.88
1.00%	5.08	4.82	5.01	0.13	4.97
1.50%	5.25	4.89	5.12	0.18	5.09
2.00%	5.55	5.27	5.38	0.14	5.40
2.50%	5.72	5.65	5.69	0.04	5.69
3.00%	5.74	5.66	5.72	0.04	5.71
3.50%	5.71	5.78	5.74	0.04	5.74
4.00%	5.71	5.78	5.75	0.04	5.75
4.50%	5.71	5.77	5.74	0.03	5.74
5.00%	5.71	5.78	5.73	0.04	5.74

Figure 13. Salt sensitivity of *L. bulgaricus* strain H grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

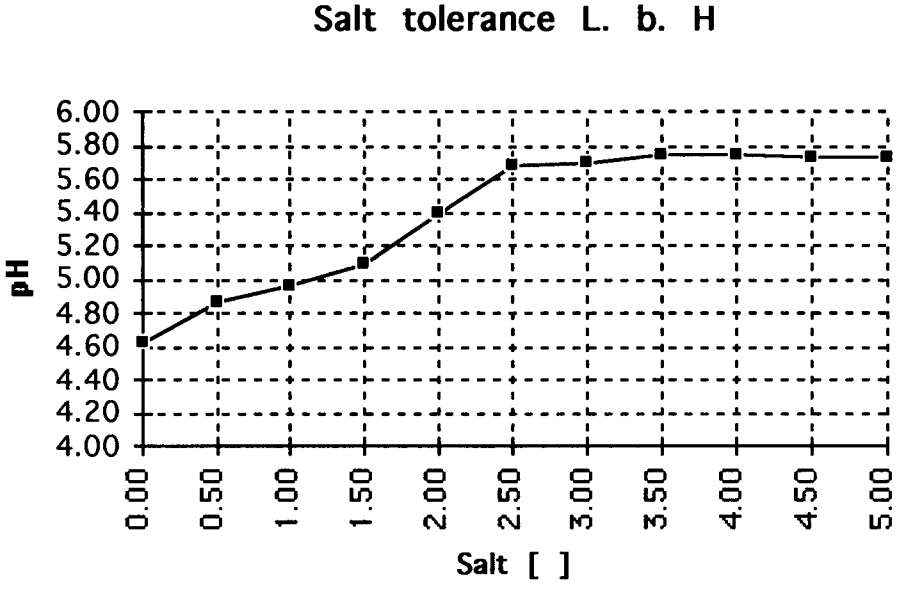


Table 27. Salt sensitivity of *L. bulgaricus* strain I grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.39	4.31	4.35	0.04	4.35
0.50%	4.33	4.38	4.29	0.05	4.33
1.00%	5.01	4.83	4.91	0.09	4.92
1.50%	5.23	5.17	5.24	0.04	5.21
2.00%	5.50	5.49	5.52	0.02	5.50
2.50%	5.67	5.57	5.64	0.05	5.63
3.00%	5.72	5.62	5.73	0.06	5.69
3.50%	5.70	5.79	5.78	0.05	5.76
4.00%	5.71	5.82	5.81	0.06	5.78
4.50%	5.68	5.81	5.81	0.08	5.77
5.00%	5.68	5.69	5.79	0.06	5.72

Figure 14. Salt sensitivity of *L. bulgaricus* strain H grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

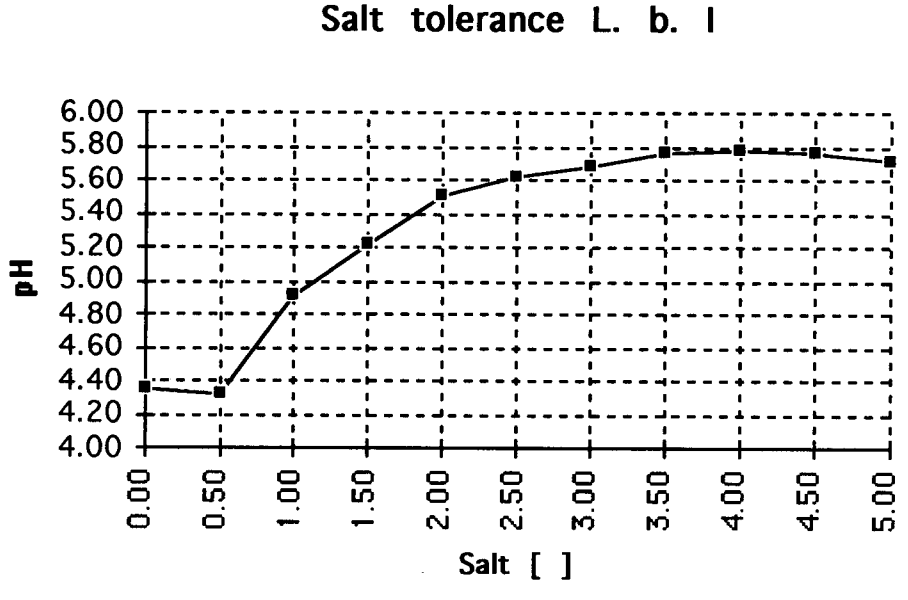


Table 28. Salt sensitivity of *L. bulgaricus* strain J grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.07	3.99	4.08	0.05	4.05
0.50%	4.41	4.37	4.35	0.03	4.38
1.00%	4.61	4.50	4.61	0.06	4.57
1.50%	4.77	4.83	4.71	0.06	4.77
2.00%	5.30	5.22	5.25	0.04	5.26
2.50%	5.78	5.67	5.47	0.16	5.64
3.00%	5.80	5.74	5.79	0.03	5.78
3.50%	5.87	5.84	5.83	0.02	5.85
4.00%	5.88	5.83	5.82	0.03	5.84
4.50%	5.85	5.81	5.81	0.02	5.82
5.00%	5.85	5.83	5.81	0.02	5.83

Figure 15. Salt sensitivity of *L. bulgaricus* strain J grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

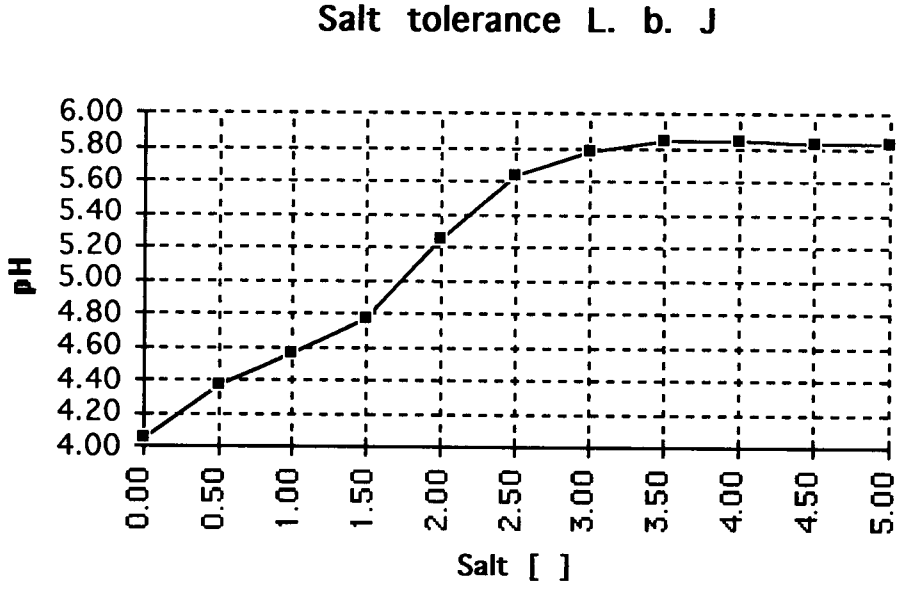


Table 29. Salt sensitivity of *L. bulgaricus* strain K grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.21	4.32	4.27	0.06	4.27
0.50%	4.92	4.96	4.90	0.03	4.93
1.00%	5.13	5.05	5.15	0.05	5.11
1.50%	5.35	5.19	5.27	0.08	5.27
2.00%	5.73	5.69	5.62	0.06	5.68
2.50%	5.84	5.78	5.79	0.03	5.80
3.00%	5.87	5.79	5.80	0.04	5.82
3.50%	5.83	5.80	5.81	0.02	5.81
4.00%	5.84	5.77	5.82	0.04	5.81
4.50%	5.84	5.73	5.82	0.06	5.80
5.00%	5.84	5.72	5.81	0.06	5.79

Figure 16. Salt sensitivity of *L. bulgaricus* strain K grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

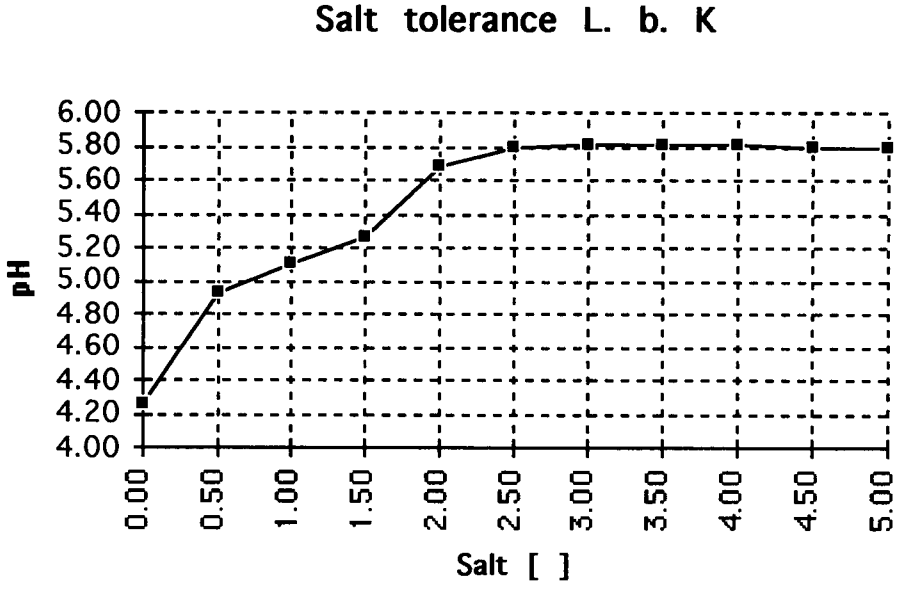


Table 30. Salt sensitivity of *L. bulgaricus* strain L grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.21	4.17	4.32	0.08	4.23
0.50%	4.28	4.26	4.40	0.08	4.31
1.00%	4.30	4.28	4.45	0.09	4.34
1.50%	4.92	4.90	5.02	0.06	4.95
2.00%	5.58	5.56	5.60	0.02	5.58
2.50%	5.76	5.90	5.84	0.07	5.83
3.00%	5.75	5.89	5.87	0.08	5.84
3.50%	5.79	5.88	5.88	0.05	5.85
4.00%	5.75	5.87	5.87	0.07	5.83
4.50%	5.73	5.85	5.84	0.07	5.81
5.00%	5.72	5.85	5.84	0.07	5.80

Figure 17. Salt sensitivity of *L. bulgaricus* strain L grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

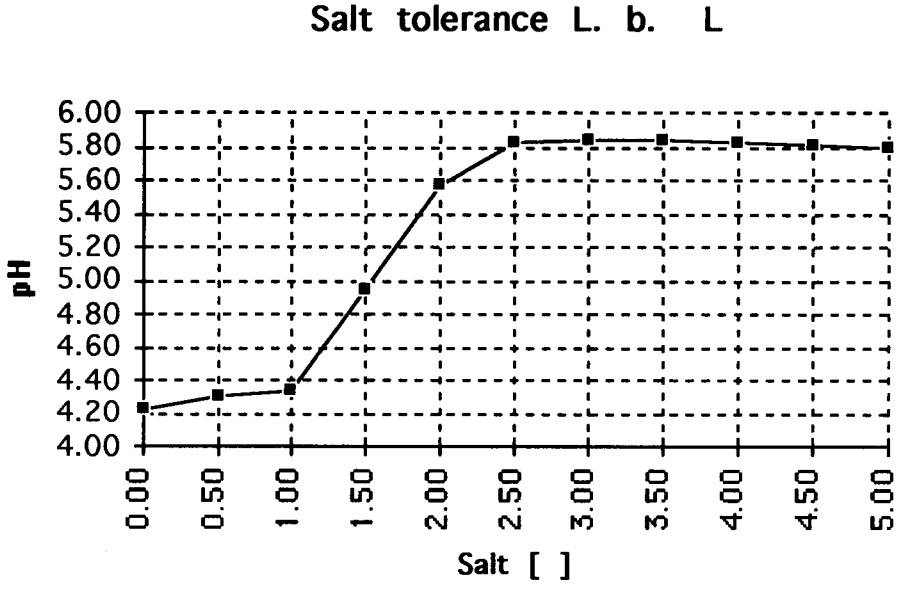


Table 31. Salt sensitivity of *L. bulgaricus* strain M grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.22	4.32	4.29	0.05	4.28
0.50%	4.73	4.76	4.71	0.03	4.73
1.00%	4.29	4.58	4.52	0.15	4.46
1.50%	4.94	4.82	4.93	0.07	4.90
2.00%	5.47	5.34	5.41	0.07	5.41
2.50%	5.69	5.72	5.75	0.03	5.72
3.00%	5.72	5.81	5.82	0.06	5.78
3.50%	5.73	5.84	5.83	0.06	5.80
4.00%	5.73	5.84	5.84	0.06	5.80
4.50%	5.71	5.82	5.81	0.06	5.78
5.00%	5.73	5.82	5.81	0.05	5.79

Figure 18. Salt sensitivity of *L. bulgaricus* strain M grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

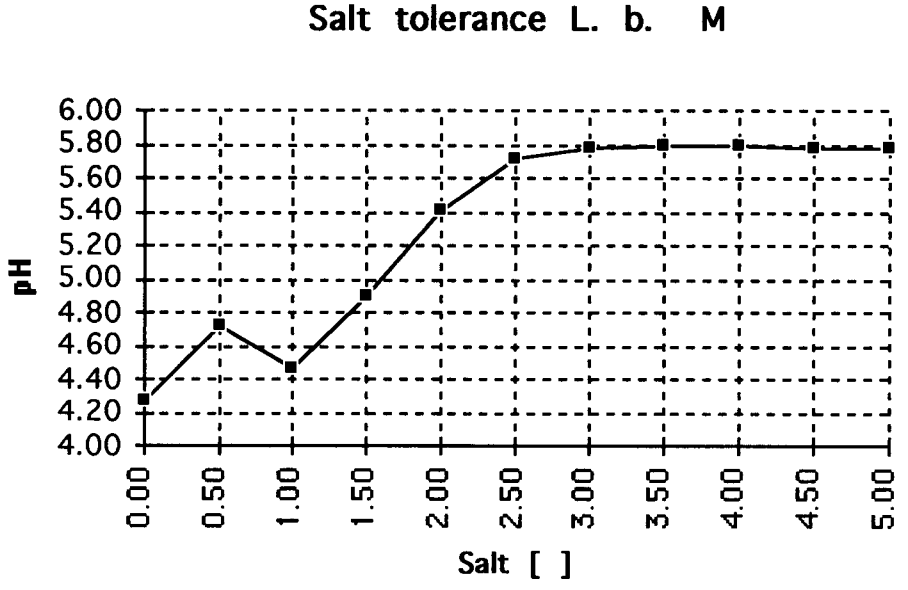


Table 32. Salt sensitivity of *L. bulgaricus* strain N grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.16	4.09	4.12	0.04	4.12
0.50%	3.72	4.01	3.98	0.16	3.90
1.00%	3.65	3.97	3.91	0.17	3.84
1.50%	3.93	4.31	4.19	0.19	4.14
2.00%	5.06	5.11	5.12	0.03	5.10
2.50%	5.33	5.59	5.54	0.14	5.49
3.00%	5.70	5.67	5.66	0.02	5.68
3.50%	5.72	5.68	5.70	0.02	5.70
4.00%	5.71	5.66	5.69	0.03	5.69
4.50%	5.71	5.65	5.69	0.03	5.68
5.00%	5.71	5.66	5.68	0.03	5.68

Figure 19. Salt sensitivity of *L. bulgaricus* strain N grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

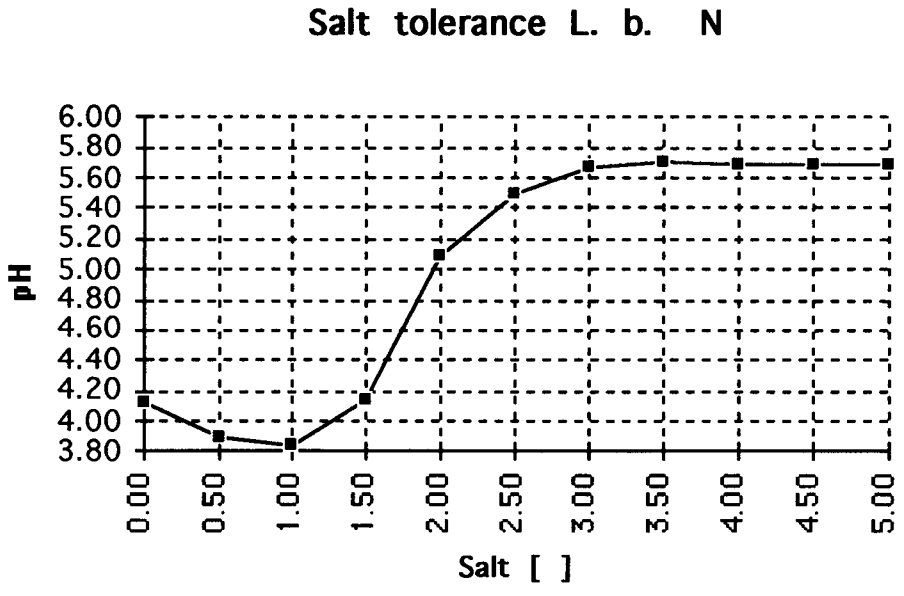


Table 33. Salt sensitivity of *L. bulgaricus* strain O grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.80	4.97	4.95	0.09	4.91
0.50%	5.54	5.22	5.41	0.16	5.39
1.00%	5.78	5.64	5.72	0.07	5.71
1.50%	5.78	5.69	5.77	0.05	5.75
2.00%	5.77	5.81	5.81	0.02	5.80
2.50%	5.73	5.80	5.83	0.05	5.79
3.00%	5.78	5.71	5.84	0.07	5.78
3.50%	5.75	5.85	5.83	0.05	5.81
4.00%	5.73	5.81	5.82	0.05	5.79
4.50%	5.73	5.83	5.81	0.05	5.79
5.00%	5.72	5.83	5.81	0.06	5.79

Figure 20. Salt sensitivity of *L. bulgaricus* strain O grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

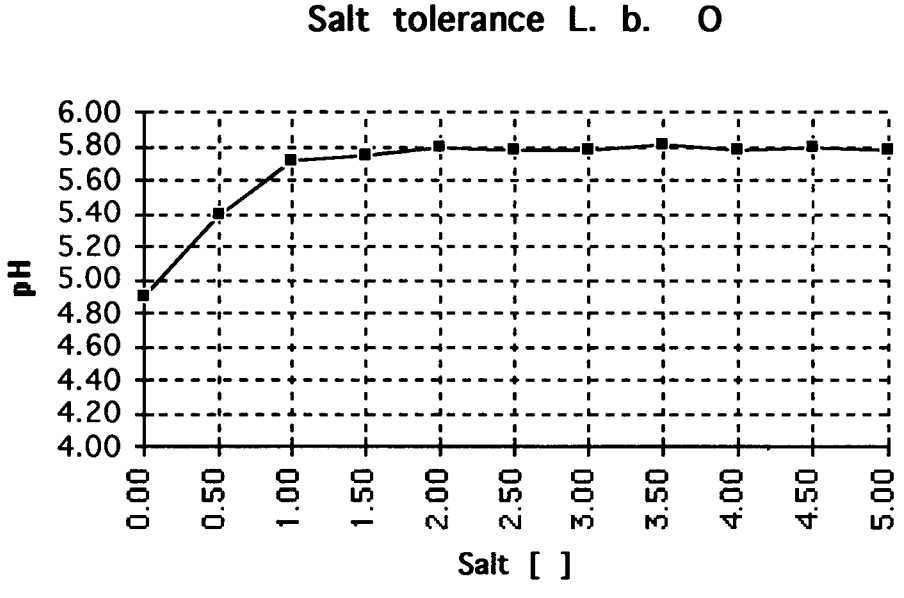


Table 34. Salt sensitivity of *L. bulgaricus* strain P grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.22	4.20	4.25	0.03	4.22
0.50%	4.02	4.18	4.17	0.09	4.12
1.00%	4.28	4.67	4.33	0.21	4.43
1.50%	4.68	5.05	4.87	0.19	4.87
2.00%	4.99	5.38	5.20	0.20	5.19
2.50%	5.33	5.63	5.51	0.15	5.49
3.00%	5.74	5.64	5.75	0.06	5.71
3.50%	5.81	5.64	5.76	0.09	5.74
4.00%	5.82	5.64	5.74	0.09	5.73
4.50%	5.81	5.60	5.74	0.11	5.72
5.00%	5.81	5.63	5.74	0.09	5.73

Figure 21. Salt sensitivity of *L. bulgaricus* strain P grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

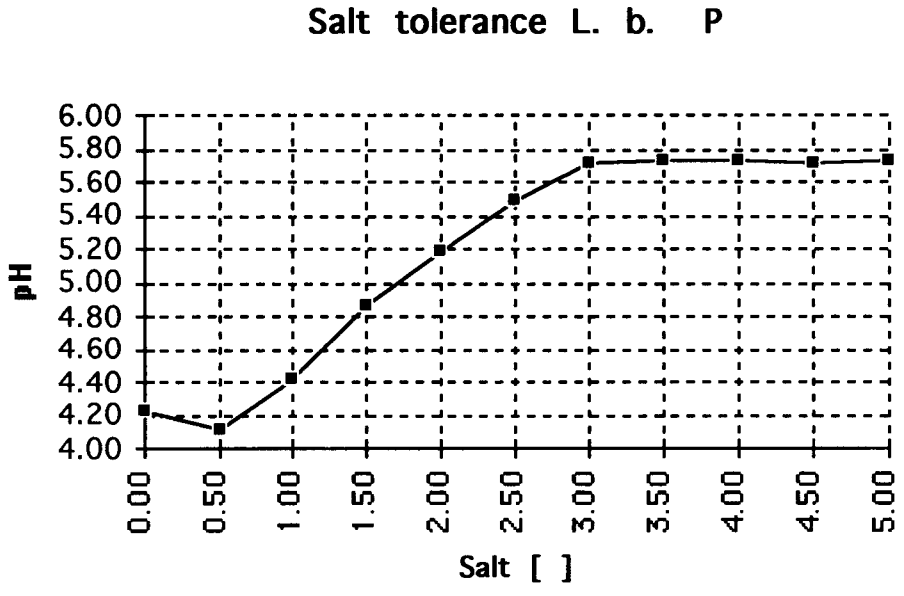


Table 35. Salt sensitivity of *L. bulgaricus* strain Q grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	3.74	3.83	3.79	0.05	3.79
0.50%	3.76	3.91	3.83	0.08	3.83
1.00%	3.70	3.86	3.91	0.11	3.82
1.50%	4.54	4.60	4.72	0.09	4.62
2.00%	4.81	4.95	4.96	0.08	4.91
2.50%	5.13	5.21	5.19	0.04	5.18
3.00%	5.60	5.63	5.62	0.02	5.62
3.50%	5.75	5.78	5.77	0.02	5.77
4.00%	5.74	5.81	5.82	0.04	5.79
4.50%	5.74	5.81	5.82	0.04	5.79
5.00%	5.75	5.82	5.83	0.04	5.80

Figure 22. Salt sensitivity of *L. bulgaricus* strain Q grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

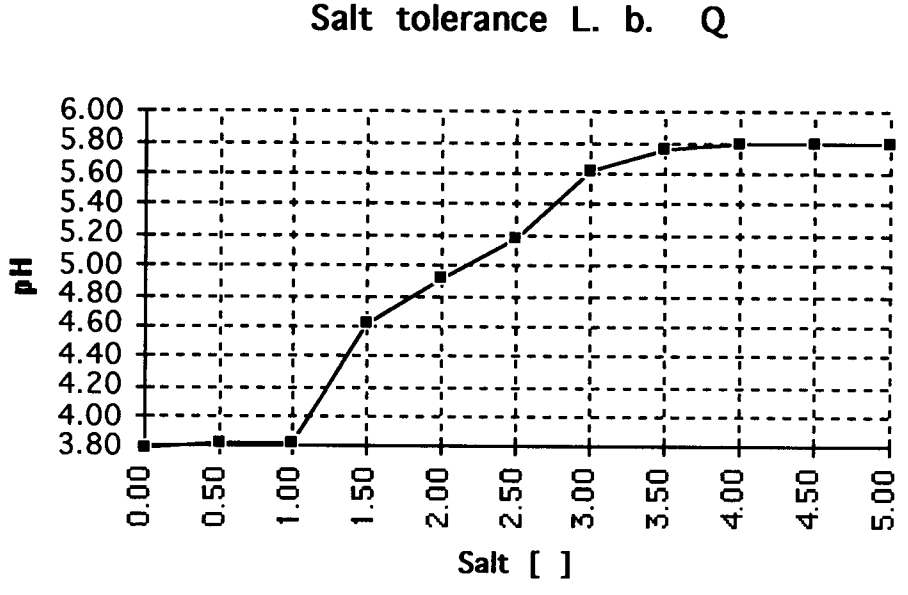


Table 36. Salt sensitivity of *L. bulgaricus* strain R grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.12	4.16	4.11	0.03	4.13
0.50%	4.13	4.20	4.14	0.04	4.16
1.00%	4.28	4.37	4.26	0.06	4.30
1.50%	4.29	4.40	4.37	0.06	4.35
2.00%	5.31	5.42	5.60	0.15	5.44
2.50%	5.62	5.59	5.74	0.08	5.65
3.00%	5.68	5.63	5.77	0.07	5.69
3.50%	5.73	5.73	5.80	0.04	5.75
4.00%	5.72	5.74	5.81	0.05	5.76
4.50%	5.71	5.71	5.81	0.06	5.74
5.00%	5.72	5.70	5.80	0.05	5.74

Figure 23. Salt sensitivity of *L. bulgaricus* strain R grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

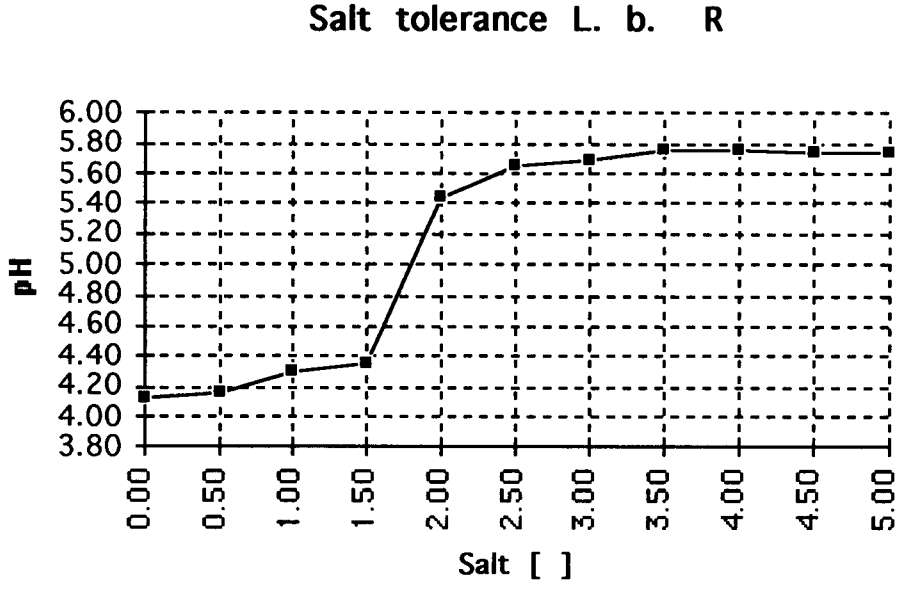


Table 37. Salt sensitivity of *L. bulgaricus* strain S grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.37	4.28	4.33	0.05	4.33
0.50%	4.44	4.61	4.62	0.10	4.56
1.00%	4.41	5.42	4.48	0.56	4.77
1.50%	4.91	5.33	5.18	0.21	5.14
2.00%	5.49	5.61	5.63	0.08	5.58
2.50%	5.77	5.81	5.81	0.02	5.80
3.00%	5.87	5.84	5.84	0.02	5.85
3.50%	5.84	5.85	5.87	0.02	5.85
4.00%	5.83	5.85	5.88	0.03	5.85
4.50%	5.83	5.85	5.88	0.03	5.85
5.00%	5.83	5.85	5.88	0.03	5.85

Figure 24. Salt sensitivity of *L. bulgaricus* strain S grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

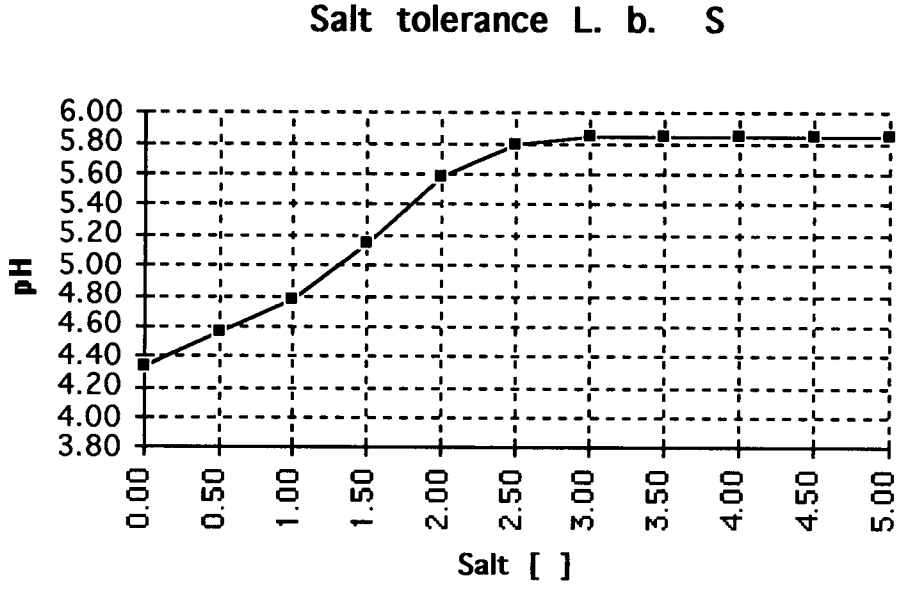


Table 38. Salt sensitivity of *L. helveticus* strain A grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.90	4.76	4.87	0.07	4.84
0.50%	5.05	4.86	4.99	0.10	4.97
1.00%	5.23	5.30	5.28	0.04	5.27
1.50%	5.43	5.46	5.45	0.02	5.45
2.00%	5.57	5.60	5.59	0.02	5.59
2.50%	5.64	5.78	5.70	0.07	5.71
3.00%	5.77	5.85	5.89	0.06	5.84
3.50%	5.83	5.86	5.88	0.03	5.86
4.00%	5.84	5.84	5.88	0.02	5.85
4.50%	5.84	5.85	5.87	0.02	5.85
5.00%	5.90	5.86	5.87	0.02	5.88

Figure 25. Salt sensitivity of *L. helveticus* strain A grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

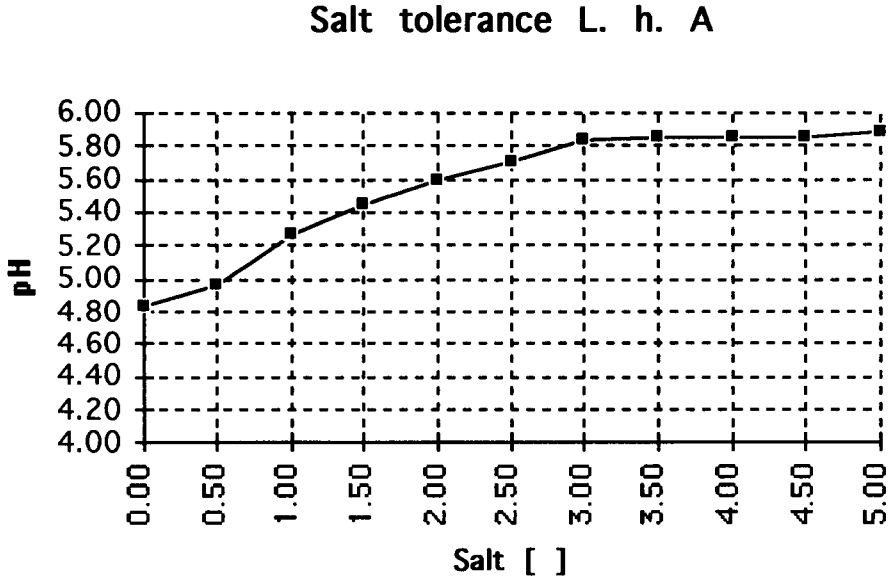


Table 39. Salt sensitivity of *L. helveticus* strain B grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.25	4.27	4.25	0.01	4.26
0.50%	4.17	4.19	4.18	0.01	4.18
1.00%	4.17	4.34	4.20	0.09	4.24
1.50%	5.14	5.38	5.25	0.12	5.26
2.00%	5.69	5.80	5.74	0.06	5.74
2.50%	5.86	5.86	5.85	0.01	5.86
3.00%	5.89	5.91	5.86	0.03	5.89
3.50%	5.98	5.92	5.86	0.06	5.92
4.00%	5.98	5.91	5.87	0.06	5.92
4.50%	5.99	5.91	5.87	0.06	5.92
5.00%	5.97	5.90	5.86	0.06	5.91

Figure 26. Salt sensitivity of *L. helveticus* strain B grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

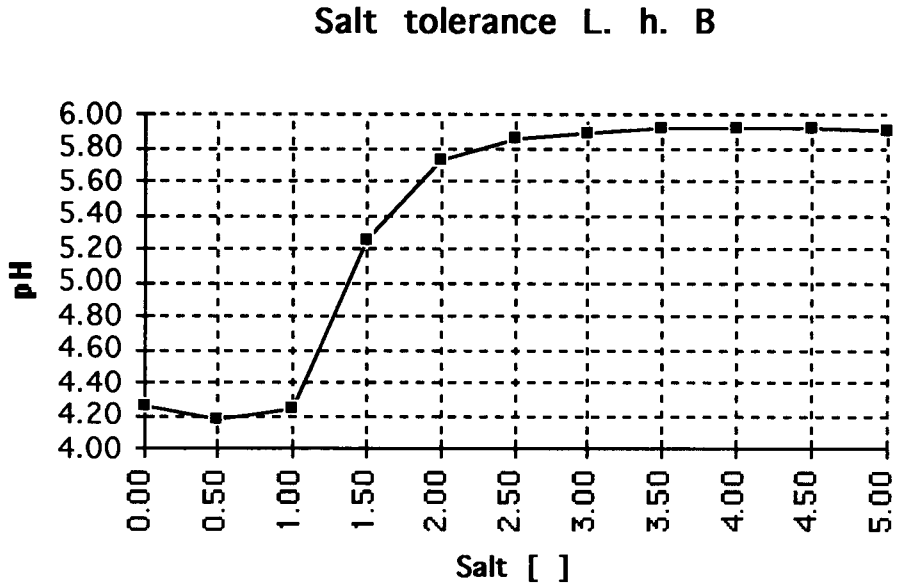


Table 40. Salt sensitivity of *L. helveticus* strain C grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.65	4.60	4.58	0.04	4.61
0.50%	4.83	4.95	4.81	0.08	4.86
1.00%	5.09	4.99	5.07	0.05	5.05
1.50%	5.39	5.24	5.25	0.08	5.29
2.00%	5.65	5.69	5.70	0.03	5.68
2.50%	5.89	5.77	5.80	0.06	5.82
3.00%	5.93	5.75	5.84	0.09	5.84
3.50%	5.94	5.83	5.90	0.06	5.89
4.00%	5.93	5.85	5.92	0.04	5.90
4.50%	5.93	5.87	5.92	0.03	5.91
5.00%	5.94	5.94	5.91	0.02	5.93

Figure 27. Salt sensitivity of *L. helveticus* strain C grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

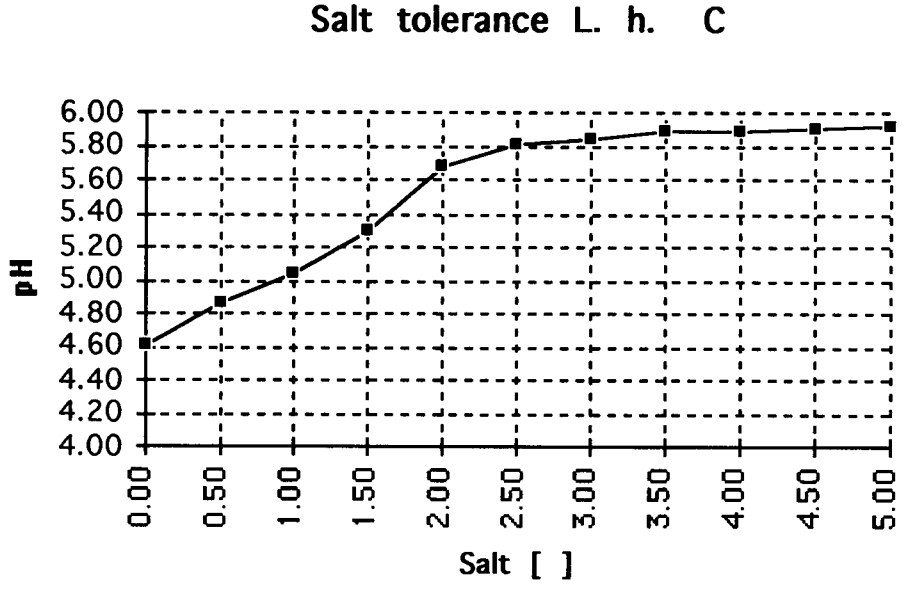


Table 41. Salt sensitivity of *L. helveticus* strain D grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	3.99	4.15	4.04	0.08	4.06
0.50%	4.45	4.54	4.41	0.07	4.47
1.00%	4.63	4.70	4.60	0.05	4.64
1.50%	4.95	5.02	4.90	0.06	4.96
2.00%	5.44	5.43	5.45	0.01	5.44
2.50%	5.66	5.62	5.70	0.04	5.66
3.00%	5.78	5.75	5.80	0.03	5.78
3.50%	5.91	5.88	5.89	0.02	5.89
4.00%	5.93	5.90	5.91	0.02	5.91
4.50%	5.94	5.91	5.95	0.02	5.93
5.00%	5.93	5.91	5.94	0.02	5.93

Figure 28. Salt sensitivity of *L. helveticus* strain D grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

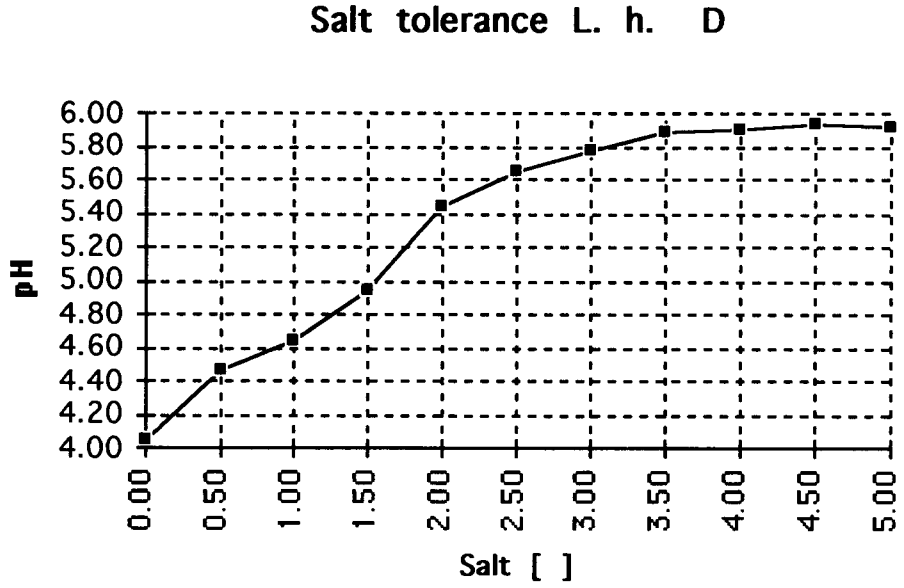


Table 42. Salt sensitivity of *L. helveticus* strain E grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.44	4.38	4.50	0.06	4.44
0.50%	4.43	4.41	4.52	0.06	4.45
1.00%	4.26	4.32	4.41	0.08	4.33
1.50%	4.49	4.45	4.60	0.08	4.51
2.00%	5.20	5.16	5.31	0.08	5.22
2.50%	5.39	5.41	5.42	0.02	5.41
3.00%	5.52	5.58	5.67	0.08	5.59
3.50%	5.63	5.71	5.75	0.06	5.70
4.00%	5.68	5.80	5.82	0.08	5.77
4.50%	5.71	5.83	5.85	0.08	5.80
5.00%	5.73	5.82	5.84	0.06	5.80

Figure 29. Salt sensitivity of *L. helveticus* strain E grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

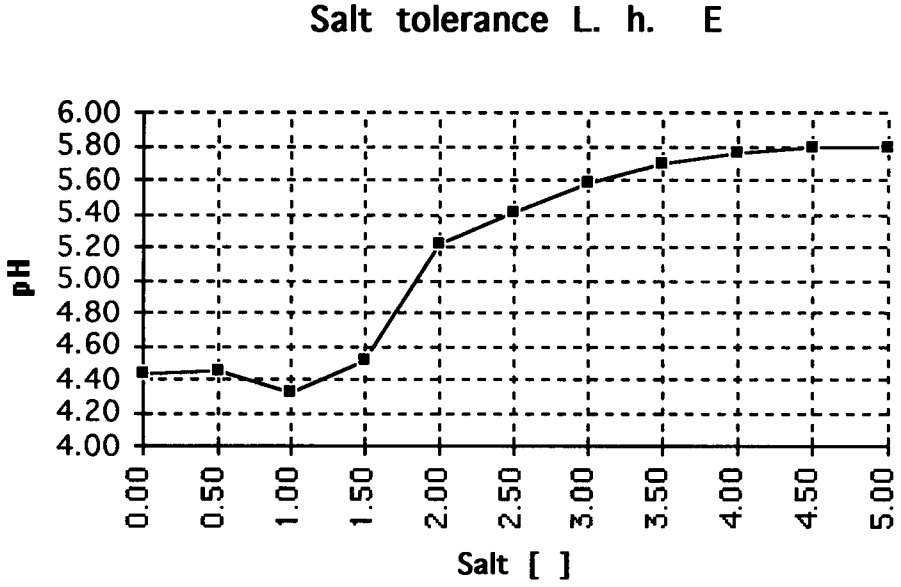


Table 43. Salt sensitivity of *L. helveticus* strain F grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.56	4.54	4.46	0.05	4.52
0.50%	4.49	4.50	4.46	0.02	4.48
1.00%	4.42	4.45	4.39	0.03	4.42
1.50%	4.84	4.90	4.76	0.07	4.83
2.00%	5.14	5.20	5.11	0.05	5.15
2.50%	5.31	5.34	5.29	0.03	5.31
3.00%	5.37	5.40	5.38	0.02	5.38
3.50%	5.51	5.60	5.58	0.05	5.56
4.00%	5.63	5.72	5.74	0.06	5.70
4.50%	5.64	5.72	5.74	0.05	5.70
5.00%	5.64	5.71	5.72	0.04	5.69

Figure 30. Salt sensitivity of *L. helveticus* strain F grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

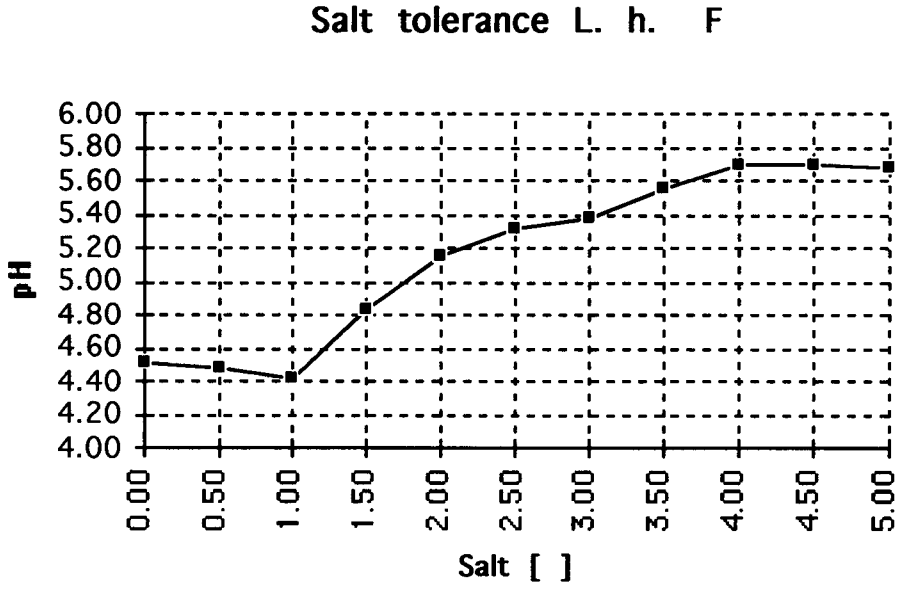


Table 44. Salt sensitivity of *L. helveticus* strain G grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.22	4.29	4.09	0.10	4.20
0.50%	4.12	4.27	4.04	0.12	4.14
1.00%	3.98	4.25	4.12	0.14	4.12
1.50%	4.38	4.50	4.40	0.06	4.43
2.00%	4.83	4.90	4.80	0.05	4.84
2.50%	5.29	5.34	5.25	0.05	5.29
3.00%	5.52	5.65	5.60	0.07	5.59
3.50%	5.66	5.70	5.74	0.04	5.70
4.00%	5.74	5.74	5.74	0.00	5.74
4.50%	5.73	5.75	5.75	0.01	5.74
5.00%	5.76	5.77	5.74	0.02	5.76

Figure 31. Salt sensitivity of *L. helveticus* strain G grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

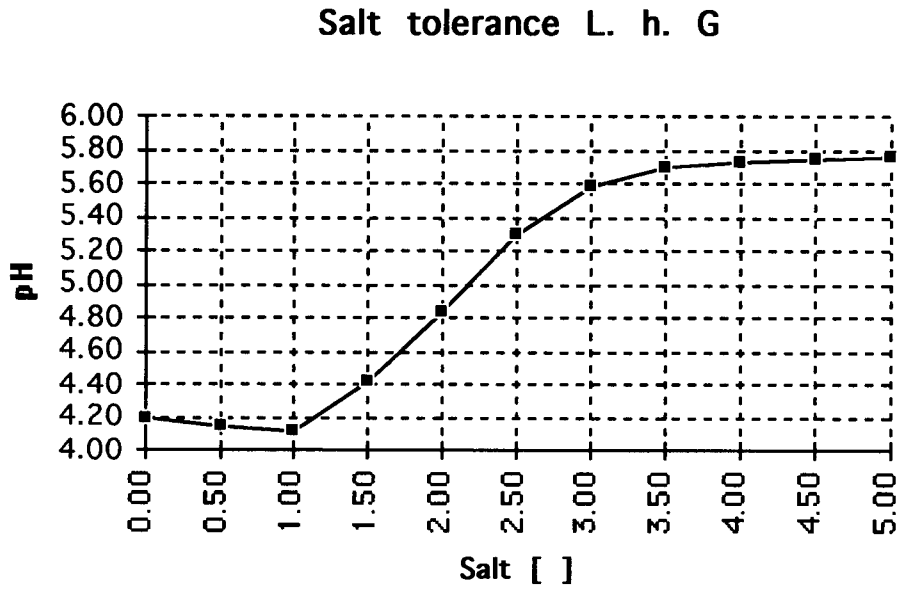


Table 45. Salt sensitivity of *L. helveticus* strain H grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.46	4.38	4.49	0.06	4.44
0.50%	4.61	4.66	4.67	0.03	4.65
1.00%	4.72	4.95	5.00	0.15	4.89
1.50%	5.20	5.22	5.27	0.04	5.23
2.00%	5.46	5.34	5.26	0.10	5.35
2.50%	5.62	5.81	5.61	0.11	5.68
3.00%	5.65	5.87	5.75	0.11	5.76
3.50%	5.75	5.88	5.80	0.07	5.81
4.00%	5.73	5.88	5.84	0.08	5.82
4.50%	5.72	5.87	5.85	0.08	5.81
5.00%	5.73	5.87	5.87	0.08	5.82

Figure 32. Salt sensitivity of *L. helveticus* strain H grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

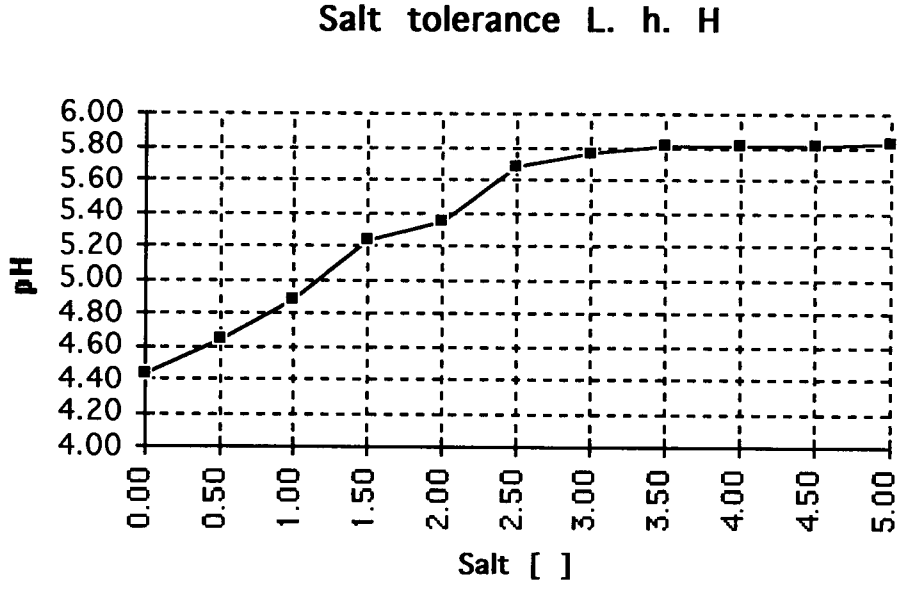


Table 46. Salt sensitivity of *L. helveticus* strain I grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.12	4.03	4.06	0.05	4.07
0.50%	4.64	4.56	4.60	0.04	4.60
1.00%	5.21	4.84	4.92	0.19	4.99
1.50%	5.29	5.09	5.13	0.11	5.17
2.00%	5.36	5.72	5.60	0.18	5.56
2.50%	5.64	5.80	5.75	0.08	5.73
3.00%	5.80	5.72	5.80	0.05	5.77
3.50%	5.80	5.81	5.81	0.01	5.81
4.00%	5.79	5.81	5.81	0.01	5.80
4.50%	5.77	5.80	5.80	0.02	5.79
5.00%	5.80	5.79	5.79	0.01	5.79

Figure 33. Salt sensitivity of *L. helveticus* strain I grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

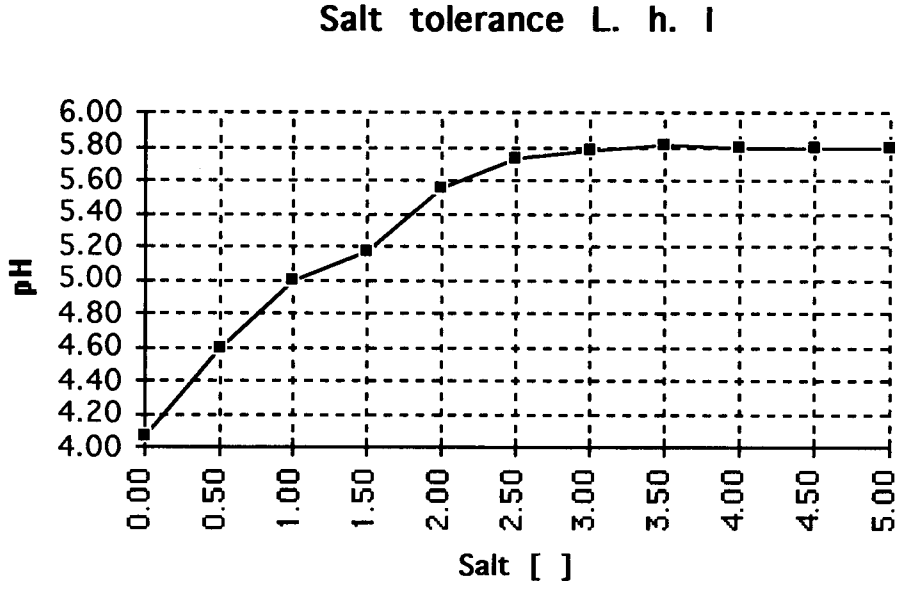


Table 47. Salt sensitivity of *L. helveticus* strain J grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	3.70	3.76	3.79	0.05	3.75
0.50%	4.17	4.37	4.21	0.11	4.25
1.00%	4.31	4.37	4.36	0.03	4.35
1.50%	5.08	5.16	5.21	0.07	5.15
2.00%	5.29	5.29	5.31	0.01	5.30
2.50%	5.58	5.39	5.48	0.10	5.48
3.00%	5.69	5.66	5.64	0.03	5.66
3.50%	5.76	5.78	5.77	0.01	5.77
4.00%	5.76	5.77	5.76	0.01	5.76
4.50%	5.71	5.76	5.76	0.03	5.74
5.00%	5.71	5.78	5.74	0.04	5.74

Figure 34. Salt sensitivity of *L. helveticus* strain J grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

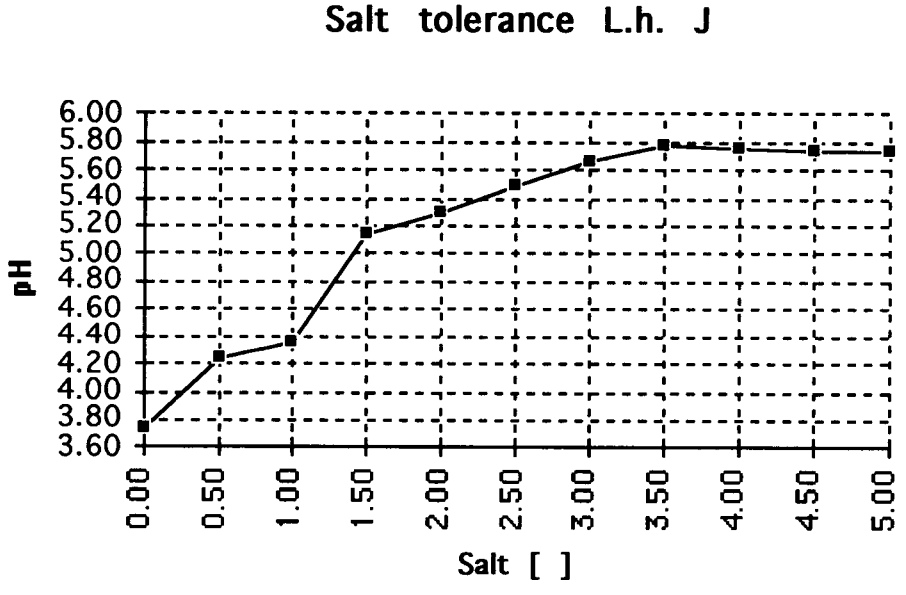


Table 48. Salt sensitivity of *L. lactis* strain A grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.66	4.70	4.77	0.06	4.71
0.50%	4.72	4.78	4.80	0.04	4.77
1.00%	4.64	4.74	4.70	0.05	4.69
1.50%	4.78	4.90	4.98	0.10	4.89
2.00%	5.02	5.03	5.07	0.03	5.04
2.50%	5.43	5.55	5.64	0.11	5.54
3.00%	5.63	5.70	5.77	0.07	5.70
3.50%	5.73	5.76	5.80	0.04	5.76
4.00%	5.76	5.78	5.81	0.03	5.78
4.50%	5.89	5.81	5.81	0.05	5.84
5.00%	5.90	5.85	5.83	0.04	5.86

Figure 35. Salt sensitivity of *L. lactis* strain A grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

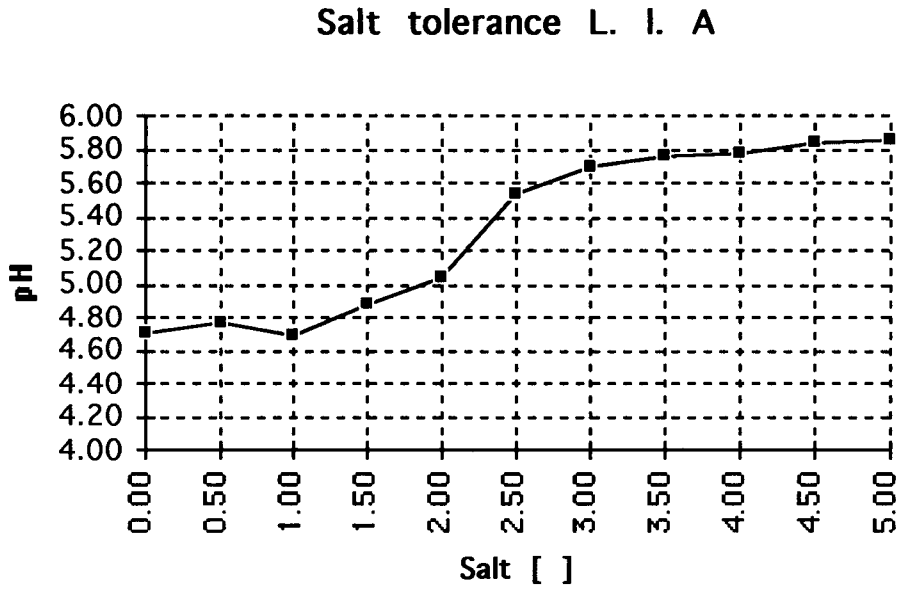


Table 49. Salt sensitivity of *L. lactis* strain B grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.67	4.56	4.74	0.09	4.66
0.50%	4.66	4.62	4.73	0.06	4.67
1.00%	5.04	4.72	5.05	0.19	4.94
1.50%	5.24	5.23	5.25	0.01	5.24
2.00%	5.63	5.50	5.66	0.09	5.60
2.50%	5.76	5.67	5.70	0.05	5.71
3.00%	5.78	5.69	5.75	0.05	5.74
3.50%	5.77	5.78	5.79	0.01	5.78
4.00%	5.75	5.79	5.79	0.02	5.78
4.50%	5.73	5.79	5.78	0.03	5.77
5.00%	5.76	5.81	5.77	0.03	5.78

Figure 36. Salt sensitivity of *L. lactis* strain B grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

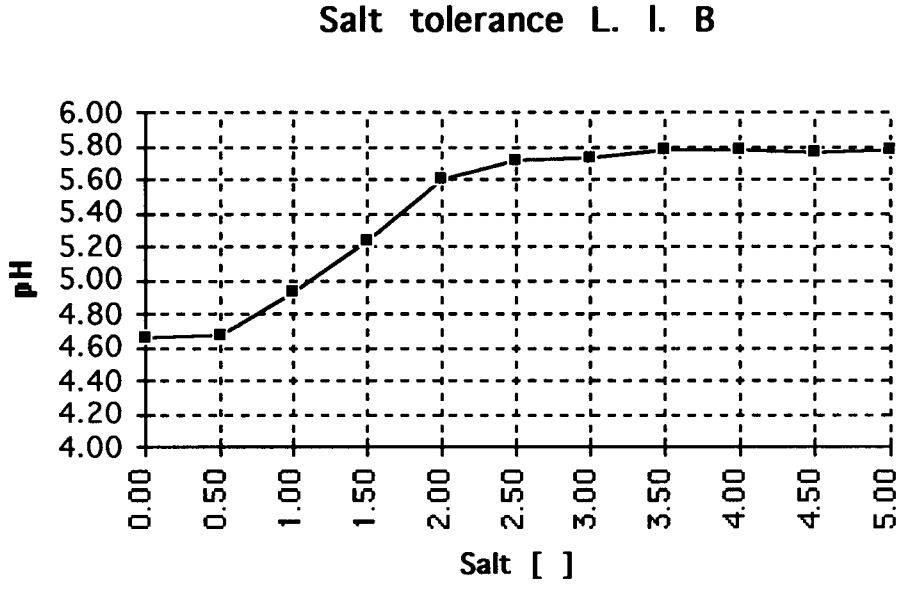


Table 50. Salt sensitivity of *L. lactis* strain C grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.48	4.42	4.37	0.06	4.42
0.50%	4.52	4.71	4.49	0.12	4.57
1.00%	4.67	4.77	4.58	0.10	4.67
1.50%	4.80	4.86	4.71	0.08	4.79
2.00%	5.21	5.11	5.13	0.05	5.15
2.50%	5.56	5.58	5.51	0.04	5.55
3.00%	5.56	5.64	5.52	0.06	5.57
3.50%	5.55	5.72	5.63	0.09	5.63
4.00%	5.65	5.72	5.75	0.05	5.71
4.50%	5.75	5.71	5.76	0.03	5.74
5.00%	5.75	5.71	5.74	0.02	5.73

Figure 37. Salt sensitivity of *L. lactis* strain C grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 44°C for 6 hours. Values are the average of three trials expressed graphically.

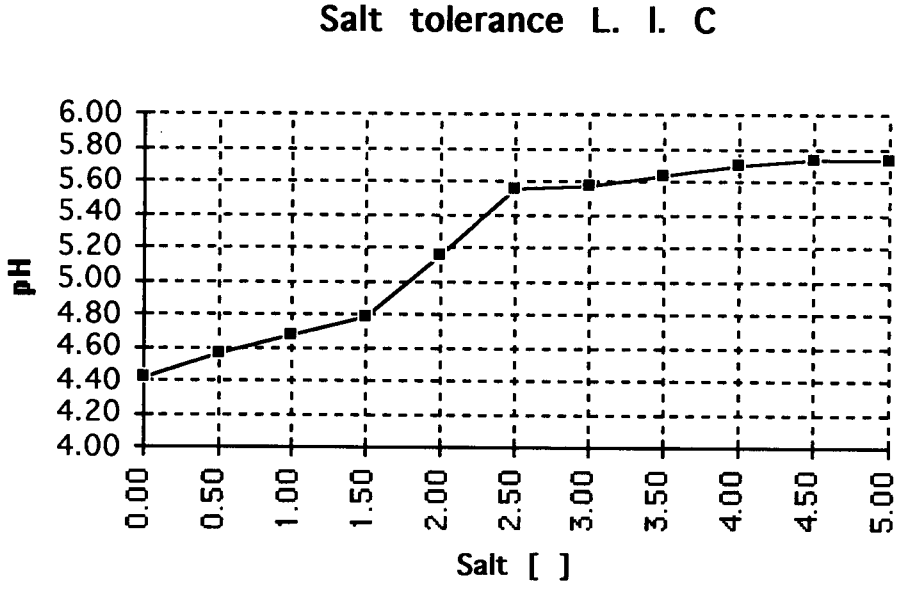


Table 51. Salt sensitivity of *S. thermophilus* strain 1 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	5.33	5.03	5.16	0.15	5.17
0.50%	5.50	5.53	5.55	0.03	5.53
1.00%	5.55	5.58	5.56	0.02	5.56
1.50%	5.76	5.70	5.72	0.03	5.73
2.00%	5.80	5.68	5.77	0.06	5.75
2.50%	5.82	5.74	5.81	0.04	5.79
3.00%	5.82	5.76	5.82	0.03	5.80
3.50%	5.83	5.85	5.82	0.02	5.83
4.00%	5.81	5.85	5.82	0.02	5.83
4.50%	5.80	5.82	5.81	0.01	5.81
5.00%	5.80	5.81	5.80	0.01	5.80

Figure 38. Salt sensitivity of *S. thermophilus* strain 1 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.

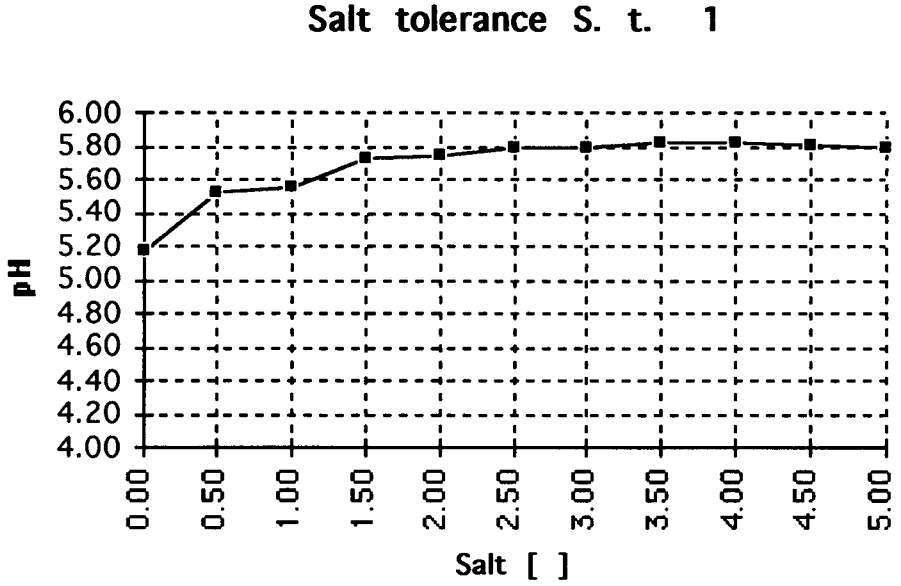


Table 52. Salt sensitivity of *S. thermophilus* strain 2 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	5.16	5.05	5.04	0.07	5.08
0.50%	5.45	5.38	5.39	0.04	5.41
1.00%	5.44	5.42	5.43	0.01	5.43
1.50%	5.79	5.70	5.72	0.05	5.74
2.00%	5.92	5.76	5.77	0.09	5.82
2.50%	5.93	5.76	5.78	0.09	5.82
3.00%	5.95	5.75	5.81	0.10	5.84
3.50%	6.01	5.81	5.82	0.11	5.88
4.00%	6.02	5.79	5.82	0.13	5.88
4.50%	6.02	5.79	5.81	0.13	5.87
5.00%	6.01	5.78	5.81	0.13	5.87

Figure 39. Salt sensitivity of *S. thermophilus* strain 2 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.

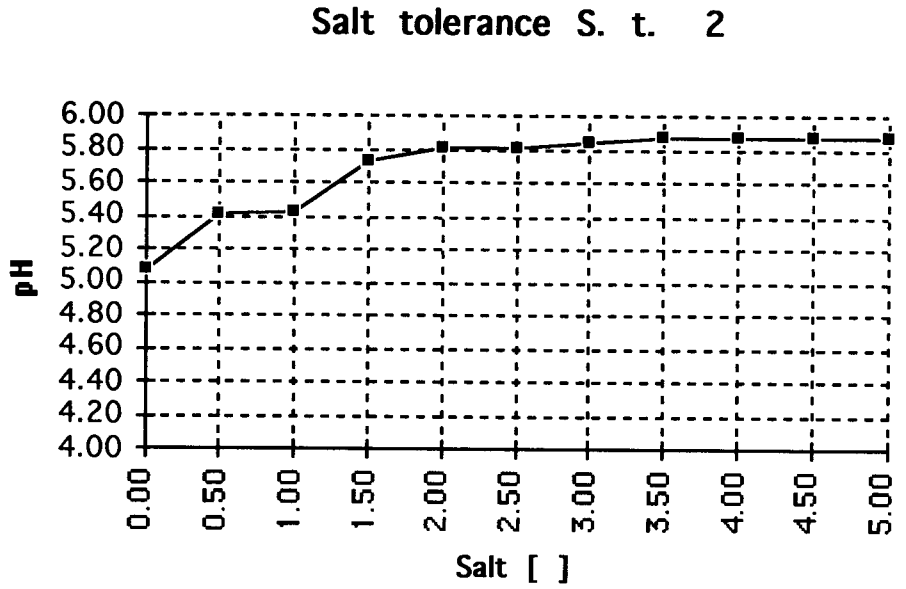


Table 53. Salt sensitivity of *S. thermophilus* strain 3 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	5.27	5.18	5.34	0.08	5.26
0.50%	5.43	5.33	5.49	0.08	5.42
1.00%	5.50	5.46	5.58	0.06	5.51
1.50%	5.59	5.53	5.62	0.05	5.58
2.00%	5.75	5.72	5.77	0.03	5.75
2.50%	5.85	5.84	5.86	0.01	5.85
3.00%	5.85	5.85	5.89	0.02	5.86
3.50%	5.91	5.85	5.91	0.03	5.89
4.00%	5.89	5.84	5.90	0.03	5.88
4.50%	5.89	5.84	5.89	0.03	5.87
5.00%	5.89	5.84	5.89	0.03	5.87

Figure 40. Salt sensitivity of *S. thermophilus* strain 3 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.

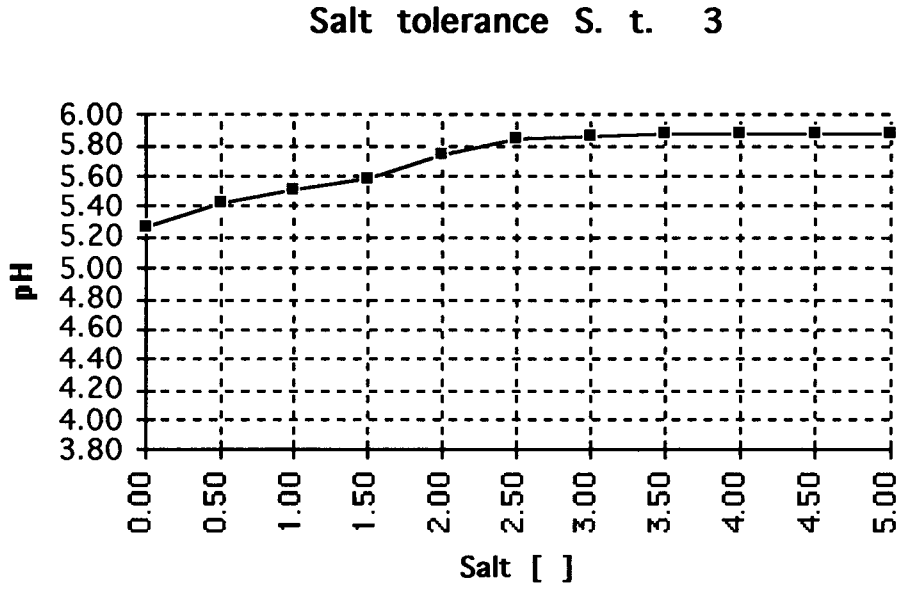


Table 54. Salt sensitivity of *S. thermophilus* strain 4 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	5.17	5.09	5.12	0.04	5.13
0.50%	5.39	5.23	5.25	0.09	5.29
1.00%	5.43	5.30	5.40	0.07	5.38
1.50%	5.67	5.67	5.65	0.01	5.66
2.00%	5.76	5.61	5.72	0.08	5.70
2.50%	5.82	5.79	5.77	0.03	5.79
3.00%	5.82	5.85	5.84	0.02	5.84
3.50%	5.84	5.85	5.85	0.01	5.85
4.00%	5.83	5.83	5.84	0.01	5.83
4.50%	5.82	5.82	5.82	0.00	5.82
5.00%	5.82	5.80	5.80	0.01	5.81

Figure 41. Salt sensitivity of *S. thermophilus* strain 4 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.

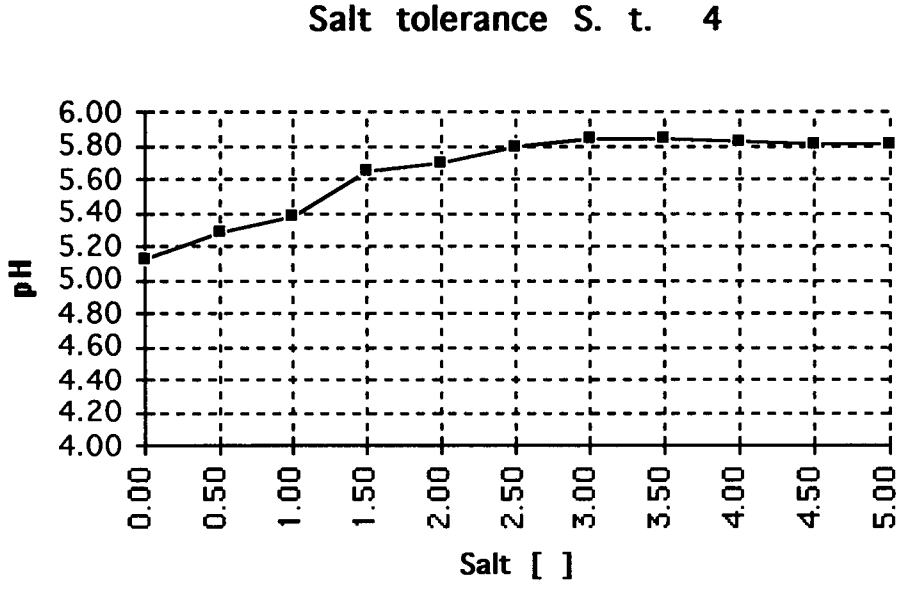


Table 55. Salt sensitivity of *S. thermophilus* strain 5 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.49	4.48	4.52	0.02	4.50
0.50%	4.45	4.21	4.31	0.12	4.32
1.00%	4.41	4.13	4.27	0.14	4.27
1.50%	4.77	4.35	4.65	0.22	4.59
2.00%	5.67	4.99	5.25	0.34	5.30
2.50%	5.77	5.55	5.70	0.11	5.67
3.00%	5.78	5.70	5.74	0.04	5.74
3.50%	5.80	5.71	5.80	0.05	5.77
4.00%	5.82	5.71	5.80	0.06	5.78
4.50%	5.82	5.72	5.79	0.05	5.78
5.00%	5.80	5.72	5.79	0.04	5.77

Figure 42. Salt sensitivity of *S. thermophilus* strain 5 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.

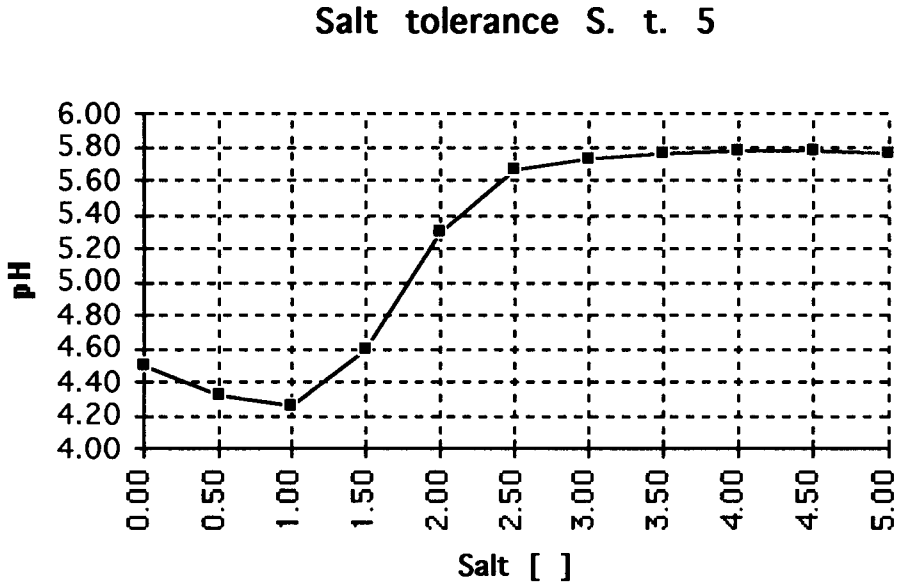


Table 56. Salt sensitivity of *S. thermophilus* strain 6 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	5.10	5.38	5.37	0.16	5.28
0.50%	5.02	5.30	5.25	0.15	5.19
1.00%	5.31	5.60	5.61	0.17	5.51
1.50%	5.58	5.85	5.83	0.15	5.75
2.00%	5.66	5.85	5.84	0.11	5.78
2.50%	5.74	5.88	5.86	0.08	5.83
3.00%	5.73	5.95	5.86	0.11	5.85
3.50%	5.71	5.96	5.85	0.13	5.84
4.00%	5.70	5.95	5.85	0.13	5.83
4.50%	5.69	5.95	5.85	0.13	5.83
5.00%	5.70	5.95	5.84	0.13	5.83

Figure 43. Salt sensitivity of *S. thermophilus* strain 6 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.

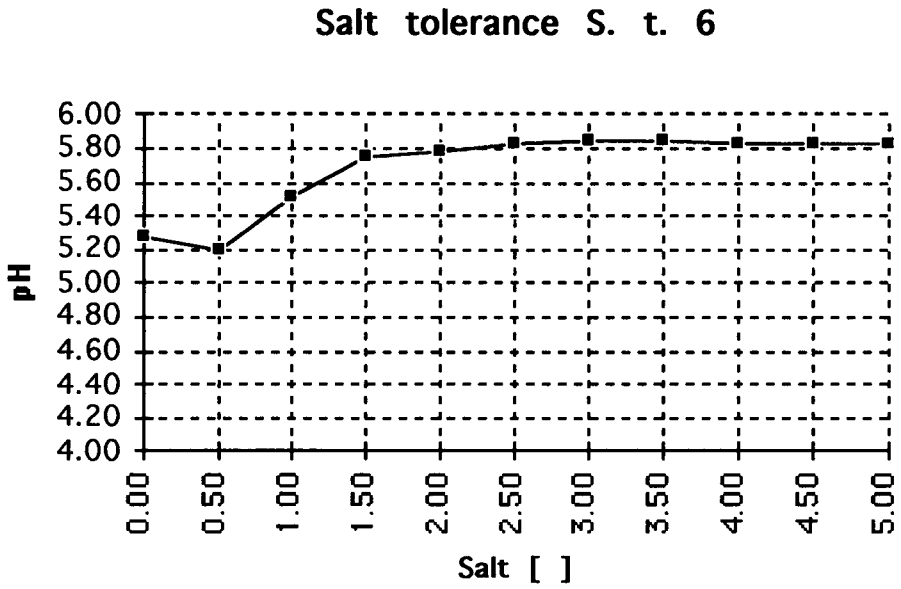


Table 57. Salt sensitivity of *S. thermophilus* strain 7 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.63	4.67	4.61	0.03	4.64
0.50%	4.37	4.52	4.25	0.14	4.38
1.00%	4.48	4.59	4.33	0.13	4.47
1.50%	4.70	4.77	4.64	0.07	4.70
2.00%	5.58	5.61	5.50	0.06	5.56
2.50%	5.67	5.70	5.61	0.05	5.66
3.00%	5.71	5.73	5.71	0.01	5.72
3.50%	5.71	5.73	5.70	0.02	5.71
4.00%	5.70	5.72	5.69	0.02	5.70
4.50%	5.70	5.72	5.66	0.03	5.69
5.00%	5.69	5.70	5.66	0.02	5.68

Figure 44. Salt sensitivity of *S. thermophilus* strain 7 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.

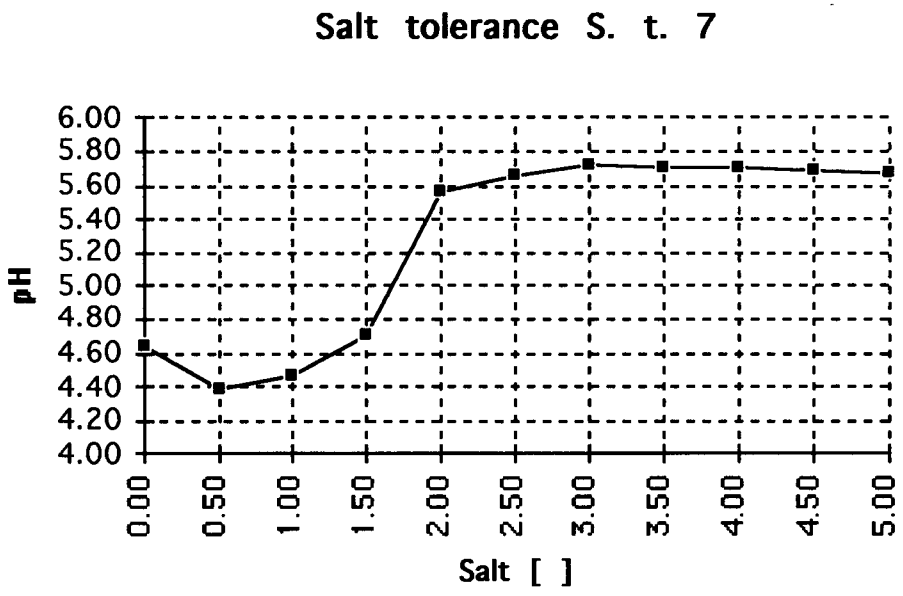


Table 58. Salt sensitivity of *S. thermophilus* strain 8 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.83	4.77	4.82	0.03	4.81
0.50%	4.80	4.76	4.80	0.02	4.79
1.00%	4.85	4.80	4.84	0.03	4.83
1.50%	5.13	5.02	5.12	0.06	5.09
2.00%	5.48	5.36	5.44	0.06	5.43
2.50%	5.60	5.55	5.58	0.03	5.58
3.00%	5.80	5.70	5.66	0.07	5.72
3.50%	5.80	5.71	5.80	0.05	5.77
4.00%	5.80	5.71	5.80	0.05	5.77
4.50%	5.80	5.72	5.79	0.04	5.77
5.00%	5.79	5.72	5.78	0.04	5.76

Figure 45. Salt sensitivity of *S. thermophilus* strain 8 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.

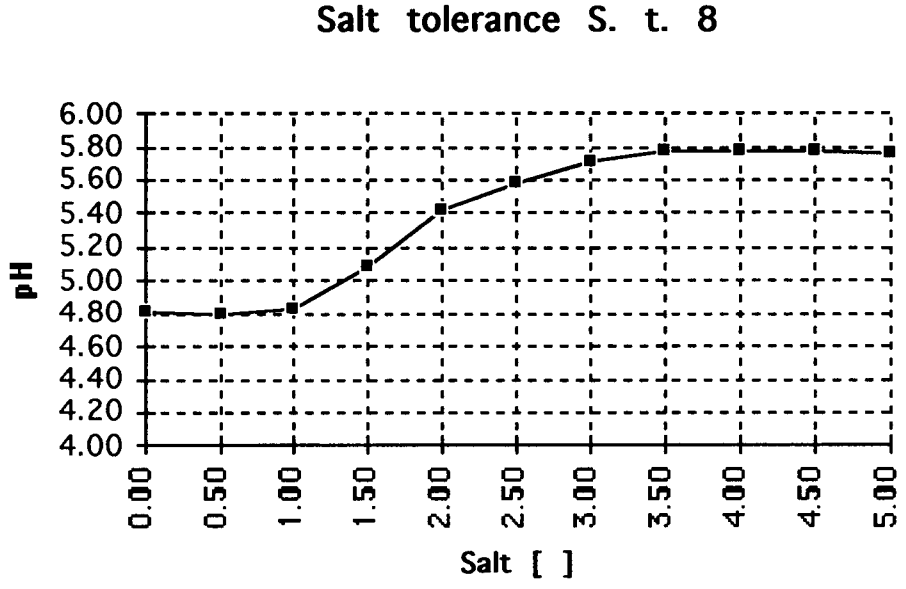


Table 59. Salt sensitivity of *S. thermophilus* strain 9 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	5.08	4.95	5.12	0.09	5.05
0.50%	5.41	5.26	5.39	0.08	5.35
1.00%	5.50	5.30	5.48	0.11	5.43
1.50%	5.77	5.65	5.70	0.06	5.71
2.00%	5.79	5.74	5.79	0.03	5.77
2.50%	5.80	5.77	5.82	0.03	5.80
3.00%	5.80	5.80	5.82	0.01	5.81
3.50%	5.80	5.79	5.80	0.01	5.80
4.00%	5.79	5.79	5.79	0.00	5.79
4.50%	5.79	5.78	5.78	0.01	5.78
5.00%	5.78	5.77	5.78	0.01	5.78

Figure 46. Salt sensitivity of *S. thermophilus* strain 9 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.

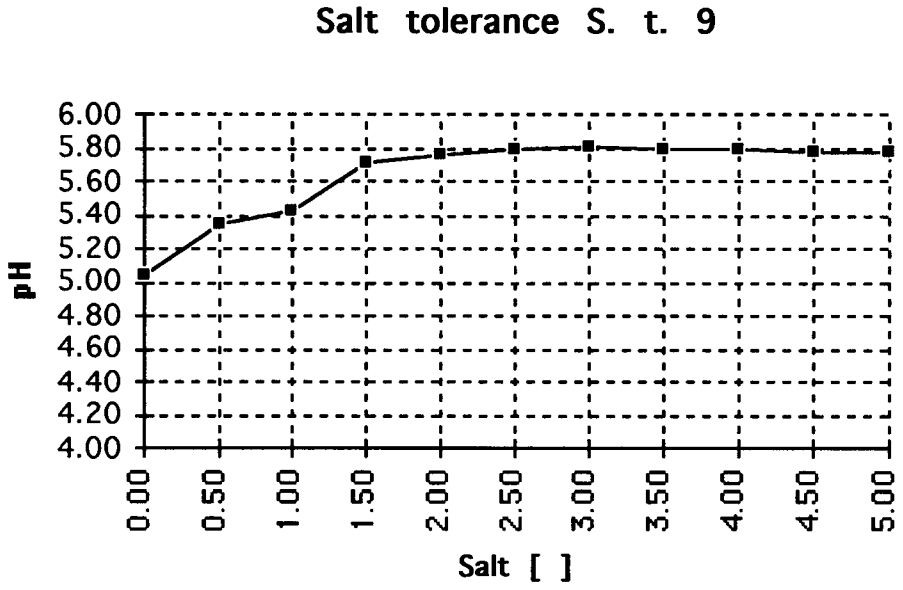


Table 60. Salt sensitivity of *S. thermophilus* strain 10 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.82	4.95	4.99	0.09	4.92
0.50%	5.16	5.20	5.21	0.03	5.19
1.00%	5.19	5.23	5.25	0.03	5.22
1.50%	5.35	5.40	5.45	0.05	5.40
2.00%	5.78	5.79	5.80	0.01	5.79
2.50%	5.80	5.83	5.80	0.02	5.81
3.00%	5.80	5.83	5.80	0.02	5.81
3.50%	5.80	5.83	5.80	0.02	5.81
4.00%	5.79	5.81	5.79	0.01	5.80
4.50%	5.79	5.80	5.78	0.01	5.79
5.00%	5.78	5.79	5.78	0.01	5.78

Figure 47. Salt sensitivity of *S. thermophilus* strain 10 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.

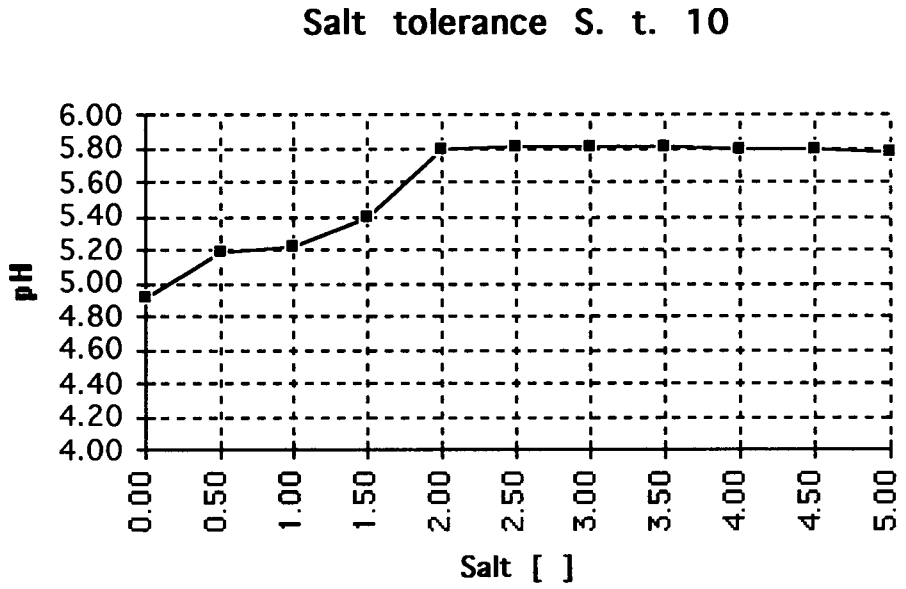


Table 61. Salt sensitivity of *S. thermophilus* strain 11 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	5.11	5.13	5.09	0.02	5.11
0.50%	5.44	5.42	5.40	0.02	5.42
1.00%	5.48	5.40	5.42	0.04	5.43
1.50%	5.70	5.79	5.69	0.06	5.73
2.00%	5.84	5.73	5.71	0.07	5.76
2.50%	5.97	5.80	5.79	0.10	5.85
3.00%	5.98	5.80	5.80	0.10	5.86
3.50%	5.97	5.82	5.81	0.09	5.87
4.00%	5.93	5.81	5.85	0.06	5.86
4.50%	5.92	5.79	5.85	0.07	5.85
5.00%	5.92	5.78	5.85	0.07	5.85

Figure 48. Salt sensitivity of *S. thermophilus* strain 11 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.

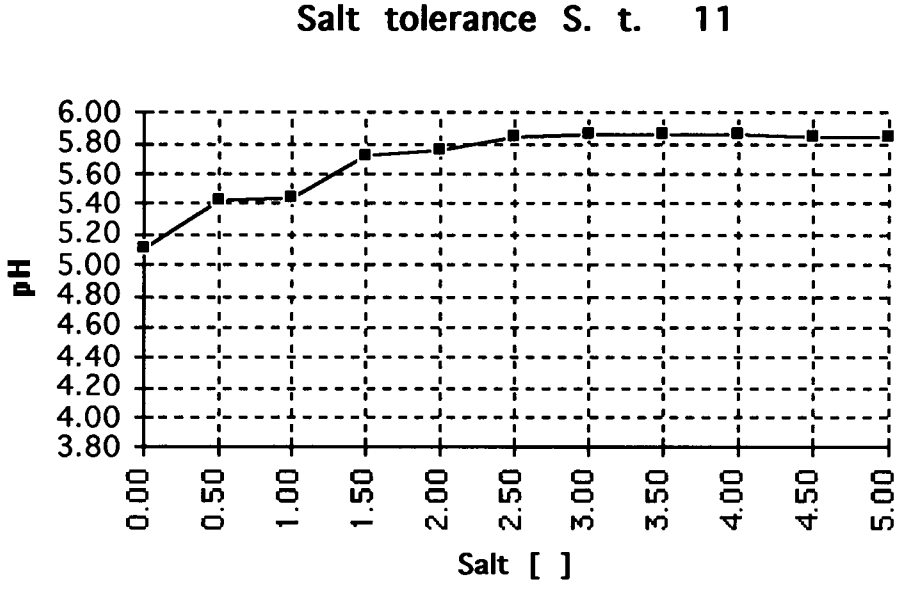


Table 62. Salt sensitivity of *S. thermophilus* strain 12 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.97	4.91	4.89	0.04	4.92
0.50%	5.30	5.28	5.25	0.03	5.28
1.00%	5.28	5.29	5.27	0.01	5.28
1.50%	5.81	5.77	5.71	0.05	5.76
2.00%	5.93	5.73	5.86	0.10	5.84
2.50%	5.93	5.80	5.89	0.07	5.87
3.00%	5.92	5.76	5.91	0.09	5.86
3.50%	5.89	5.78	5.92	0.07	5.86
4.00%	5.87	5.79	5.91	0.06	5.86
4.50%	5.85	5.77	5.90	0.06	5.84
5.00%	5.85	5.75	5.89	0.07	5.83

Figure 49. Salt sensitivity of *S. thermophilus* strain 12 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.

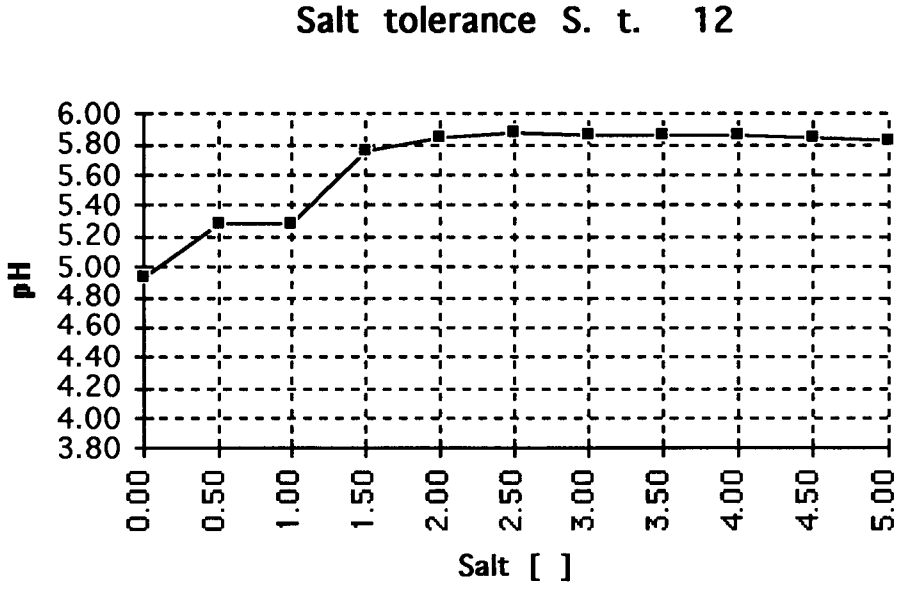


Table 63. Salt sensitivity of *S. thermophilus* strain 13 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.97	5.02	4.93	0.05	4.97
0.50%	5.09	5.12	5.05	0.04	5.09
1.00%	5.05	5.10	5.02	0.04	5.06
1.50%	5.21	5.31	5.18	0.07	5.23
2.00%	5.71	5.78	5.65	0.07	5.71
2.50%	5.89	5.80	5.79	0.06	5.83
3.00%	5.93	5.80	5.84	0.07	5.86
3.50%	5.89	5.87	5.88	0.01	5.88
4.00%	5.86	5.87	5.90	0.02	5.88
4.50%	5.85	5.84	5.89	0.03	5.86
5.00%	5.84	5.84	5.87	0.02	5.85

Figure 50. Salt sensitivity of *S. thermophilus* strain 13 grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 38°C for 6 hours. Values are the average of three trials expressed graphically.

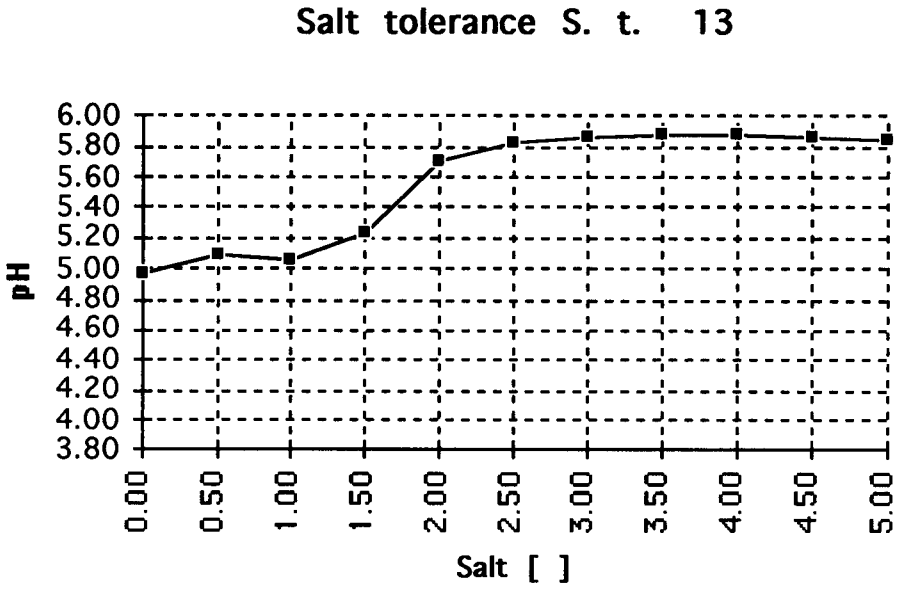


Table 64. Salt sensitivity of *L. bulgaricus* CR 14 and *S. thermophilus* 2 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	4.21	4.64	4.57	0.23	4.47
0.50%	4.58	4.46	4.54	0.06	4.53
1.00%	4.75	4.63	4.80	0.09	4.73
1.50%	5.10	5.04	5.18	0.07	5.11
2.00%	5.65	5.59	5.69	0.05	5.64
2.50%	5.66	5.61	5.71	0.05	5.66
3.00%	5.72	5.65	5.78	0.07	5.72
3.50%	5.75	5.70	5.85	0.08	5.77
4.00%	5.77	5.71	5.84	0.07	5.77
4.50%	5.80	5.72	5.85	0.07	5.79
5.00%	5.80	5.71	5.87	0.08	5.79

Figure 51. Salt sensitivity of *L. bulgaricus* CR 14 and *S. thermophilus* 2 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed graphically.

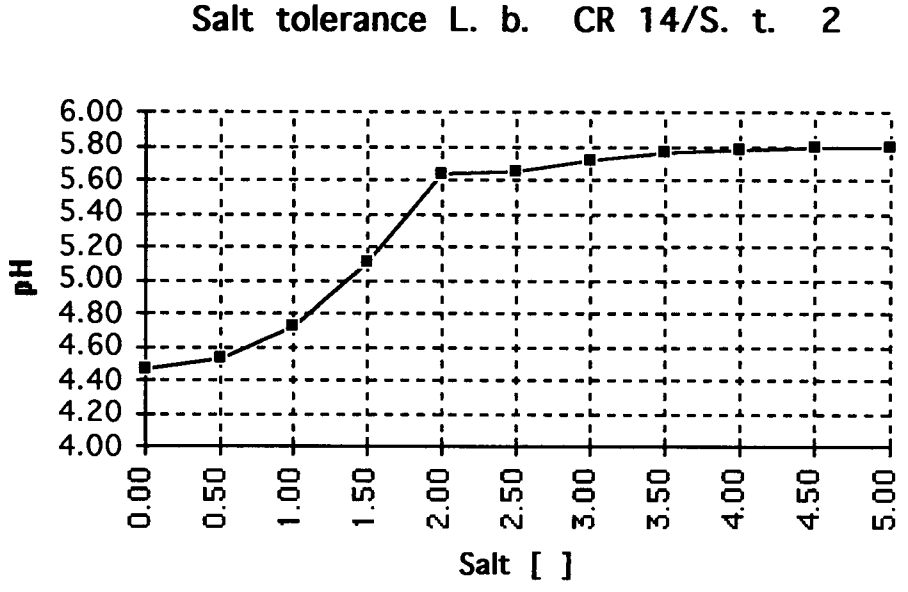


Table 65. Salt sensitivity of *L. bulgaricus* Q and *S. thermophilus* 2 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	3.70	3.69	3.67	0.02	3.69
0.50%	3.98	3.75	3.79	0.12	3.84
1.00%	3.94	3.80	3.76	0.09	3.83
1.50%	4.52	4.31	4.48	0.11	4.44
2.00%	5.64	5.64	5.64	0.00	5.64
2.50%	5.61	5.63	5.69	0.04	5.64
3.00%	5.73	5.72	5.80	0.04	5.75
3.50%	5.74	5.78	5.79	0.03	5.77
4.00%	5.74	5.78	5.82	0.04	5.78
4.50%	5.76	5.77	5.82	0.03	5.78
5.00%	5.74	5.77	5.84	0.05	5.78

Figure 52. Salt sensitivity of *L. bulgaricus* Q and *S. thermophilus* 2 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed graphically.

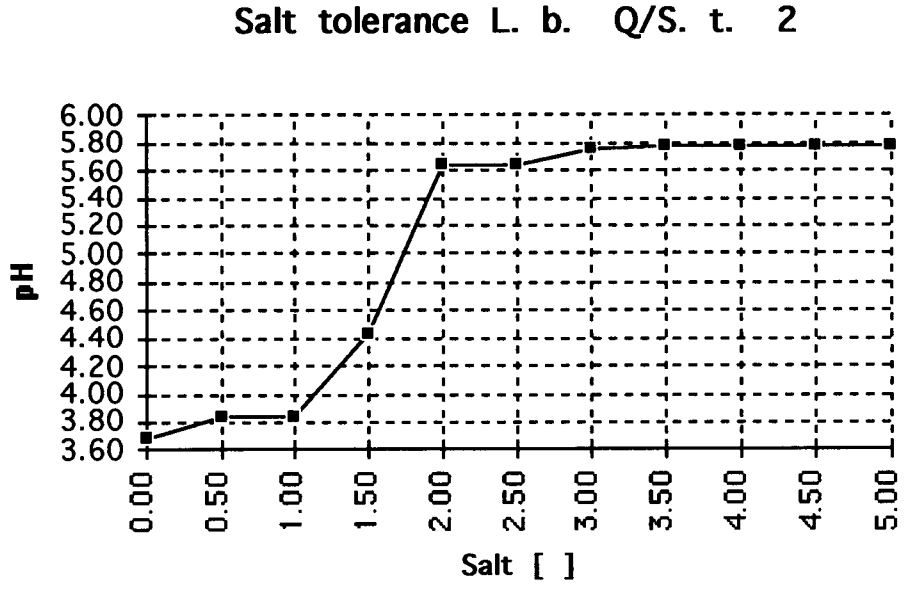


Table 66. Salt sensitivity of *L. bulgaricus* C and E, and *S. thermophilus* 7 and 12 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	3.64	3.62	3.62	0.01	3.63
0.50%	3.77	3.72	3.51	0.14	3.67
1.00%	3.83	3.71	3.65	0.09	3.73
1.50%	4.10	3.90	3.90	0.12	3.97
2.00%	5.62	5.56	5.59	0.03	5.59
2.50%	5.49	5.34	5.39	0.08	5.41
3.00%	5.79	5.73	5.73	0.03	5.75
3.50%	5.97	5.86	5.89	0.06	5.91
4.00%	5.99	5.87	5.88	0.07	5.91
4.50%	5.99	5.86	5.87	0.07	5.91
5.00%	5.99	5.86	5.87	0.07	5.91

Figure 53. Salt sensitivity of *L. bulgaricus* C and E, and *S. thermophilus* 7 and 12 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed graphically.

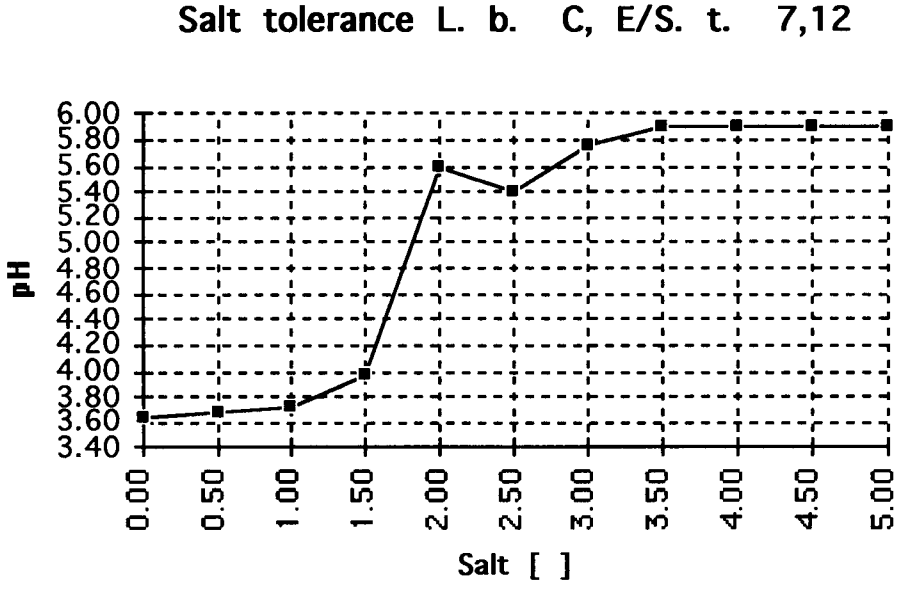
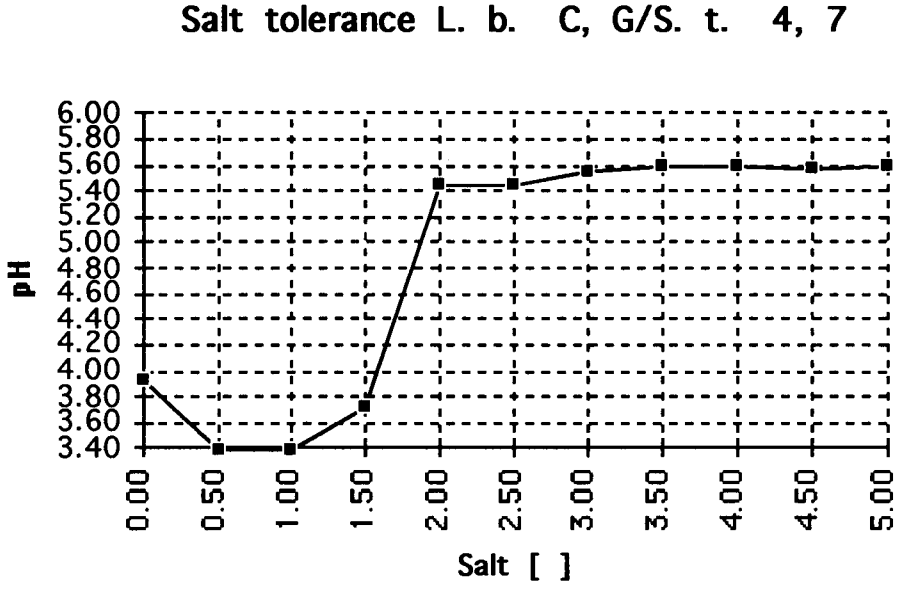


Table 67. Salt sensitivity of *L. bulgaricus* C and G, and *S. thermophilus* 4 and 7 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed as final pH.

Salt [ ]	Trial 1	Trial 2	Trial 3	STDEV	Mean
0.00%	3.92	3.90	3.97	0.04	3.93
0.50%	3.46	3.42	3.31	0.08	3.40
1.00%	3.48	3.37	3.31	0.09	3.39
1.50%	3.83	3.72	3.64	0.10	3.73
2.00%	5.43	5.56	5.37	0.10	5.45
2.50%	5.58	5.43	5.32	0.13	5.44
3.00%	5.63	5.56	5.44	0.10	5.54
3.50%	5.69	5.58	5.50	0.10	5.59
4.00%	5.67	5.59	5.51	0.08	5.59
4.50%	5.67	5.57	5.49	0.09	5.58
5.00%	5.68	5.57	5.50	0.09	5.58

Figure 54. Salt sensitivity of *L. bulgaricus* C and G, and *S. thermophilus* 4 and 7 strains grown from a 1% inoculum in 11% NFM supplemented with sodium chloride in concentrations from 0% to 5%, increasing by 0.5% increments, and incubated at 42°C for 6 hours. Values are the average of three trials expressed graphically.



Carbohydrate Fermentation

Analytab Products Rapid CH 50 identifies the carbohydrates expected to be fermented by *L. bulgaricus* as glucose, fructose, mannose, and lactose. Strains A, B, C, D, E, H, I, J, K, M, N, O, P, R, S, and CR 14 fermented these carbohydrates as expected. Strains F and G did not ferment fructose, but did ferment galactose. Strain L fermented saccharose in addition to the expected carbohydrates. Strain Q fermented saccharose, but did not ferment fructose. Bergey's Manual (1986) indicates *Lactobacillus delbrueckii* subsp. *bulgaricus* ferments glucose, fructose, and lactose.

Analytab Products identifies the carbohydrates expected to be fermented by *L. helveticus* are galactose, glucose, fructose, mannose, lactose, melibose, saccharose, and trehalose. Strains D, E, F, G, H, fermented these carbohydrates as expected. Strains A, B, C, I, and J did not ferment Melibose or Saccharose. Bergey's Manual (1986) indicates *Lactobacillus helveticus* ferments galactose, glucose, fructose, mannose, lactose, maltose, saccharose, and trehalose.

The carbohydrates expected to be fermented by *L. lactis* are glucose, fructose, mannose, N-acetyl glucosamine, lactose, melibose, saccharose, and trehalose. None of the three strains fermented melibose, and strain B also fermented galactose. Bergey's Manual (1986) indicates *Lactobacillus delbrueckii* subsp. *lactis* ferments glucose, fructose, mannose, lactose, amygdalin, esculin, saccharose, and trehalose.

The carbohydrates expected to be fermented by *S. thermophilus* are glucose, fructose, lactose, and saccharose. Strains 1, 3, 4, 5, 6, 8, 9, 11, and 13 fermented these carbohydrates as

expected. Strains 2, 7, 10, and 12 also fermented ribose, galactose, mannose, N-acetyl glucosamine, arbutin, esculin, salican, cellibose, maltose, trehalose, starch, and β gentibiose. This is similar to *Lactococcus lactis* subsp. *lactis.* Bergey's Manual (1986) indicates *Streptococcus salivarious* subsp. *thermophilus* ferments glucose, fructose, mannose, lactose, and saccharose.

CONCLUSIONS

Lactobacilli are well known to be more proteolytic than *S. thermophilus* cells. However, in milk(or starter media) incubated only six hours, differences between rods and cocci are not apparent. The range for cocci is 17 mM to 22 mM, excluding values of 28 and 46. The range for rods is 11 mM to 35 mM. From this it appears that longer incubation is necessary to show a difference where proteolysis is concerned. Fermentation runs were extended to 16 hours and strains *L. bulgaricus* J and *S. thermophilus* 3 were selected to test this theory. *L. bulgaricus* J liberated 719 mM free amino acids compared to 459 mM free amino acids by *S. thermophilus* 3. This experimental modification demonstrated that the difference in proteolysis between rods and cocci is significant; however, the difference between rods and cocci is insignificant in only six hours. In cheese undergoing aging, sufficient time lapses so that the effect on cheese breakdown by individual strains will be expressed according to the ability of each strain. Therefore strain selection based on difference in proteolysis as measured over 16 hours is significant.

Mixed rod and coccus strains, in three out of four cases, showed significant stimulation of proteolysis as a result of cooperative growth. The exception, L. b. CR 14/S. t. 2, was inhibited in proteolysis likely because one of the two strains produced a bactericin inhibiting the other.

Rods (lactobacilli) are not known to be formate producers and preliminary tests substantiated this fact. From the standard curve it

should be noted that recovery of formate from milk is incomplete, and that the slope of the curve is flat. From formate solutions in buffer the standard curve is more respectable. However, formate values reported in Tables 7 & 8 were determined from the equation provided in the Boehringer Mannheim assay kit for formic acid.

Considering formate production by cocci, Table 7 shows data for 13 strains ranging from 4 to 20.3 mg/L. Most of these values are lower than the published values of Perez et al. (1991); However, they incubated strains for 16 hours. When we did the same, with *S. thermophilus* 3, formate production values for this strain increased from 6.7 mg/L at six hours, to as high as 20 mg/L, or about 435 mM. These value agree with data of Perez et al. who reported values ranging from 400 to 750 mM.

Mixed rod and coccus strains, in three out of four cases, were stimulated in formate production by more than 200%. The exception was L. b. CR 14/S. t. 2, in which formate production decreased by 65% from *S. thermophilus*. 2 grown alone.

It is evident that *L. bulgaricus* needs carbon dioxide for optimal growth in milk (Driessen et. al., 1982). However, rods (lactobacilli) are not known to be carbon dioxide producers and our preliminary tests substantiated this fact. Only four of 32 rods produced more than 200 µl CO_2 in 6 hours at 44°C.

Cocci produced large quantities of carbon dioxide. Ten of 13 strains produced at least 798 µl carbon dioxide. Strains 1,8, and 9 were all well below 150 µl CO_2, an indication of weak urease activity.

Our mixed rod and coccus strains demonstrated weaker carbon dioxide production then expected. The two-strain cultures were both

below 100 μl CO_2, where the four-strain cultures were between the 200 to 400 μl CO_2 range. These values were well below the majority of *S. thermophilus* strains. These findings are contrary to those of Driessen et. al. (1982) who reported that production of carbon dioxide by *S. thermophilus* in a pure culture is not as rapid as in a mixed culture. The difference in carbon dioxide production between two and four strain cultures must be noted and may be attributed to synergism between the strains.

Bacteriophage contamination of starter cultures is one of the main causes of starter culture failure during cheese and cultured dairy product manufacture (Wright and Klaenhammer, 1984). It has been demonstrated that bacteriophage require divalent ions , in particular calcium, for adsorption and subsequent proliferation (Cherry and Watson, 1949; Collins et al., 1950; Babel, 1958). Studies by Hargrove et al., (1961), demonstrated that addition of phosphate to starter milk inhibits phage proliferation. The majority of previous phosphate sensitivity studies tested mesophilic strains using phosphate ion concentrations ranging from 0.5% to 2.0%. These concentrations proved to be too high for our thermophilic strains and concentrations of 0.1%, 0.3%, and 0.5% phosphate ion therefore were used.

Acid production by *L. bulgaricus*, in the presence 0.1% phosphate ion (0.19% total phosphate salt), showed slight stimulation in 9 of 19 strains. The presence of 0.3% phosphate ion (0.58% total phosphate salt), caused widely varied acid production according to strain. Trends were much the same for *L. helveticus*,; three of 10 strains showed slight stimulation in the presence 0.1% phosphate ion

while 0.3% phosphate ion caused widely varied acid production according to strain. *Lactobacillus lactis* demonstrated widely varied acid production according to strain in the presence 0.1% phosphate ion.

The *S. thermophilus* strains were inhibited to a lesser a degree by phosphate salts, findings which are supported by Thunnell, (1989). Two strains, 5 & 7, were not inhibited at 0.5% phosphate ion (0.97% total phosphate salt) also, the widely varied acid production did not occur, and inhibition occured at a more gradual rate, partly because of the overall slower rate of acid production by cocci as opposed to rods.

Our mixed rod and coccus strains demonstrated varied phosphate sensitivities. The two-strain culture acid production was not inhibited by 0.1% phosphate ion, but acid production inhibition did occur at higher concentrations. The four-strain cultures were stimulated by all three concentrations of phosphate tested.

While small amounts of NaCl have stimulatory effects on lactic cultures, larger proportion exhibit toxic properties (Meister and Ledford, 1979). The rods showed toxic effects between 2.5% and 3.0% NaCl, while only 7 of 32 strains showed stimulation at low concentrations. In general the cocci were more sensitive to NaCl with 6 of 13 strains showing sensitivity at 0.5% NaCl. Sensitivity was a more gradual effect in the cocci primarily to the overall slower rate of acid production.

Mixed cultures revealed more tolerance to NaCl with no inhibition occurring at concentrations of 1.0%. Culture L. b. C, G/S. t. 4, 7 was actually stimulated at concentrations through 1.5%. The

synergistic properties of the mixed strains appear to help increase NaCl tolerance.

From the data collected in this study certain recommendations can be made for growing rod-coccus cultures in starter media for subsequent use to inoculate cheese milk. Strains to be combined should be tested individually and together to insure they are compatible. This can be done simply by testing the mixture for acid production as compared to the single strains. Also for mixed culture growth, starter media should not contain more than 1.0% sodium chloride and not more than 0.3% phosphate ion. These are conserative values and take into consideration the high sensitivities of some strains. Also highly proteolytic rods, *L. bulgaricus* H, I, J and *L. helveticus* I, should be used with caution and evaluated in limited cheesemaking trials; they may cause soft bodied cheeses, bitterness, and reduced yields. Strains of *S. thermophilus* producing low amounts of formate, 6, 8 and12, should also be avoided since they likely will not provide enough formate to optimize C-1 metabolism by the rods.

A culture consisting of *L. bulgaricus* J, *L. helveticus* I, *S. thermophilus* 5 and 11 should be further examined as a starter culture for mozzarella cheese production.

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