published fermentation processes, showed an optimum aeration rate (table 4). At high aeration a yeast product with a relatively low lysine content, 13.2 per cent, was obtained. At low aeration, the highest lysine content, 16.5 per cent, was obtained, although the cell yield was reduced. At the lowest aeration used, both lysine content and cell growth were reduced.

SUMMARY

Conversion of 2-oxoadipic acid to lysine with *Saccharomyces cerevisiae* was accomplished in aerated and agitated fermentors under fermentation conditions resembling those used in baker's yeast process.

With this process, a yeast product containing about 16 per cent hot water-extractable lysine (calculated as hydrochloride) was obtained. The conversion efficiency of 2-oxoadipic acid to lysine was about 0.63 (weight basis) in a fermentation medium containing molasses as the sole carbon source. Most of the conversion took place during the growth period. Aeration, concentration of the added 2-oxoadipic acid, and time of precursor addition affected the lysine content and the conversion efficiency.

REFERENCES

BROQUIST, H. P. AND STIFFEY, A. V. 1959 Biosynthesis of lysine from adipic acid precursors by yeast. Federation Proc., **18**, 198.

COOPER, C. M., FERNSTROM, G. A., AND MILLER, S. A. 1944 Performance of agitated gas-liquid contactors. Ind. Eng. Chem., **36**, 504–509.

Difco Laboratories, Inc. 1953 *Difco manual of dehydrated culture media and reagents*, 9th ed., p. 233. Detroit, Michigan.

ESPOSITO, R. G., ALBRECHT, A. M., AND BULLOCK, M. W. 1959 The biosynthetic pathway of lysine in *Saccharomyces cerevisiae*. Bacteriol. Proc., **1959**, 121.

FRIEDEMANN, J. E. AND HAUGEN, G. E. 1943 Pyruvic acid. II. The determination of keto acids in blood and urine. J. Biol. Chem., **147**, 415–442.

STRASSMAN, M. AND WEINHOUSE, S. 1953 Biosynthetic pathways. III. The biosynthesis of lysine by *Torulopsis utilis*. J. Am. Chem. Soc., **75**, 1680–1684.

UNDERKOFLER, L. A. AND HICKEY, R. J. 1954 *Industrial fermentations*, vol. 1, pp. 273–306. Chemical Publishing Co., New York, New York.

Fecal Streptococci

I. Cultivation and Enumeration of Streptococci in Surface Waters

BERNARD A. KENNER, HAROLD F. CLARK, AND PAUL W. KABLER

Robert A. Taft Sanitary Engineering Center, Bureau of State Services, Public Health Service, U. S. Department of Health, Education, and Welfare, Cincinnati, Ohio

Received for publication April 13, 1960

The streptococci have been under consideration as indicators of fecal pollution for many years. Their poor acceptance as a measure of fecal pollution from human and warm-blooded animal excreta has been due in part to the relatively low recovery rates in comparison to coliform densities in polluted waters; the multiplicity of detection procedures; poor agreement between the various methods for their quantitative enumeration; and the lack of detailed and systematic studies on the sources, survival, and interpretation of streptococci in various types of waters. Furthermore, undue emphasis has been placed on the *Streptococcus faecalis* group (enterococci) with little or no regard for other streptococcal strains present in the gut of humans and warm-blooded animals or birds. The predominating species of streptococci may vary markedly in various animal excreta. Some detection methods yield excellent results with human fecal samples but give poor quantitative recovery of the streptococci present in pig or cow feces.

Due to limited space, it is not appropriate to review all the procedures that have been recommended for the detection of streptococci of sanitary significance. Commonly used media of this type are SF medium of Hajna and Perry (1943) or the subsequent modification by Hajna (1951) as BAGG broth, Winter and Sandholzer (1946) presumptive and confirmatory media and azide dextrose broth of Roth as recommended by Mallmann and Seligmann (1950) with confirmation of presumptive positive tubes in ethyl violet azide broth of Litsky, Mallmann, and Fifield (1953, 1955). A newer medium for the membrane filter technique with a considerable increase in productivity was described by Slanetz and Bartley (1957).

The original use of sodium azide in medium for the isolation of streptococci should probably be credited to Hartman (1936). Substances with wide acceptance in other countries as suppressive or inhibitive agents of nonstreptococcal bacterial growth are potassium tellurite used by Harold (1936) and Flemming (1932)

or thallium acetate described in the work of Cooper and Linton (1947). However, the type of media made considerable difference in both the number of streptococcal species isolated and the quantitative recovery when examining sewage from various sources and feces of different animal species.

In this report a medium for the growth of fecal streptococci is described, which with minor modifications can be used in a multiple tube (most probable number; MPN) method, with a membrane filter (MF) procedure, or by the agar pour plate technique. The growths of several fecal and nonfecal streptococci, as well as some nonstreptococcal species, on this media are compared to the growths of the same bacteria on other media.

MATERIALS AND METHODS

The streptococcal medium developed for this investigation and designated as KF streptococcal medium (KF) had the composition as shown in table 1. This medium as described may be used for the multiple tube method (MPN streptococcal test), using 10 ml of single strength broth with water samples of 1 ml or less per tube and 20-ml quantities of 1.5 strength broth for 10-ml quantities of water samples. It is adaptable for membrane filter technique by adding 1 ml of sterile 1 per cent triphenyltetrazolium chloride (or 1 ml of 1 per cent sterile tetrazolium red solution) solution to each 100 ml of the broth. For the agar plate count, add 2 per cent agar before sterilization of the medium and, when the KF streptococcal agar is melted for pouring plates, add 1 ml of 1 per cent sterile tetrazolium solution for each 100 ml of agar medium. The tetrazolium solution should be sterilized by Seitz filtration and such stored solutions may be boiled 5 min before using. An alternate procedure consists of boiling the tetrazolium solution for 5 min and using immediately. Sterilization of medium containing tetrazolium or the sterilization of tetrazolium solutions under steam pressure is not recommended.

TABLE 1

Composition of KF streptococcal medium

	g
Proteose peptone No. 3	10
Yeast extract (Bacto)*	10
Sodium chloride (analytical reagent)	5
Sodium glycerophosphate	10
Maltose, cp	20
Lactose, cp	1
Sodium azide†	0.4
Sodium carbonate (analytical reagent)	0.636
Brom cresol purple (water soluble)	0.015
	ml
Distilled water	1,000

Sterilize at 121 C (15 lb) for 10 min. The sterile medium should have a pH of between 7.2 to 7.3 and should not be used when the pH is less than 7.0.

* Difco Laboratories, Inc., Detroit, Michigan.

† Eastman Kodak Co., Rochester, New York.

Growth of a variety of streptococci, a species of *Pediococcus*, a species of *Leuconostoc*, and three species of *Lactobacillus* in dextrose azide presumptive broth and confirmation in ethyl violet azide (EVA) broth were compared with growths in KF streptococcal medium as (a) liquid broth in tubes, (b) with the membrane filter method, and (c) as a pour plate using solid medium.

Water samples from 25 sources, including streams, lakes, springs, and sewage, were examined by five streptococcal procedures and by a confirmed test for the coliform group. In the multiple tube procedures (MPN) five portions each in three usable decimal dilutions were examined. Appropriate quantities of sample were filtered through membrane filters to secure at least one membrane with between 20 and 60 streptococcal colonies. The MPN and membrane filter procedures were used with KF streptococcal medium; multiple tube tests with BAGG broth and with a dextrose azide (DA) presumptive test followed by confirmation of all positive tubes in EVA broth; and the membrane filter technique with M-enterococcus agar. Dehydrated media were used for all test procedures with the exception of KF medium and directions described by the authors of each reference test were followed. Tests using KF medium were incubated 48 $\pm$ 2 hr at 35 $\pm$ 1 C and all membrane filter tests incubated in an atmosphere saturated with water vapor. MPN tubes of KF streptococcus medium were considered positive when *turbid growth occurred with a bright yellow (acid) color* of the indicator and in the absence of marked foaming. Where foaming occurred, the streptococci were confirmed by a Gram stain. On membrane filters all red and pink colonies visible with 15 diameters magnification were counted as streptococcal colonies.

Approximately 700 colonies were picked by the random sample procedure from membrane filter tests for further verification as streptococci. Each colony was cultivated in brain heart infusion broth for approximately 24 hr at 35 C and examined for typical morphology of gram positive cocci in chains of two or more organisms, with or without pleomorphism. Organisms from positive tubes of liquid medium were examined for typical streptococcal morphology.

Ten samples of surface waters were compared by the membrane filter procedure and agar plate counting procedure, using KF streptococcal medium. All colonies on the plate with a red or pink color visible with 15$\times$ magnification were counted as streptococci.

RESULTS

Growths of several streptococcal species and a few nonstreptococcal microorganisms on KF media and in

dextrose azide-ethyl violet azide (DA-EVA) are compared in table 2.

Streptococcus mitis, *S. salivarius*, *S. bovis*, *S. equinus*, and the enterococcal group consisting of *S. faecalis*, *S. faecalis* var. *liquefaciens*, *S. faecalis* var. *zymogenes* with related *S. faecalis* biotypes gave growth in KF streptococcal broth, on KF streptococcal membrane filter tests, and on KF streptococcal agar. There was a trace of turbidity in KF broth and scanty growth of some minute colonies on KF solid media with *Pediococcus cerevisiae* and *Lactobacillus plantarum* but no acid was produced in the liquid medium and no pink or red color change noted in the colonies on KF solid media. No growth was demonstrated on the KF media (broth or solid media) with *S. cremoris*, *S. lactis*, *S. pyogenes*, *S. thermophilus*, *S. uberis*, *Leuconostoc mesenteroides*, *Lactobacillus lactis*, *L. acidophilus*, and *L. plantarum*.

Results of the examination of 25 water samples are shown in table 3.

In the comparison of the data in table 3, the density obtained by the DA-EVA procedure was used as the reference test. Ratios for each test were calculated by dividing the density obtained by the DA-EVA test into the density given by each of the other test procedures. Median ratios were 0.62 for BAGG MPN, 1.28 for M-enterococcus agar MF, 2.42 for KF streptococcal medium MF, and 5.64 for the same medium by the multiple tube method.

The confirmed coliform density by a 5-tube test in 3 decimal dilutions was included to show the level of

TABLE 2

Growth characteristics of selected bacterial species on KF streptococcal medium and in dextrose azide-ethyl violet azide (DA-EVA) streptococcal medium

Culture	Source of Culture	Growth in DA Broth		Growth in EVA Broth			Growth and Reaction in KF Streptococcal Media							
							Broth			Membrane filter, 48 hr			Agar pour plate, 48 hr	
		Hours		Hours		Sediment in tube	Hours		Indicator	Growth	Colony		Colony	
		24	48	24	48		24	48			Color	Size in mm	Growth	Color
Streptococcus:														
S. faecalis	ATCC 7080	v	+	+		Blue	+		Acid	+	Red	1–2	+	Red
S. faecalis var. *zymogenes*	ATCC 6054	v	+	v	+	Blue	v	+	Acid	+	Red	1–2	+	Red
S. faecalis var. *liquefaciens*	ATCC 4532	v	+	v	+	Blue	v	+	Acid	+	Red	1–2	+	Red
S. faecalis biotypes	Feces	v	+	v	v	v	v	+	Acid	+	Red	1–2	+	Red
S. durans	ATCC 9810	v	+	v	+	Blue	v	+	Acid	+	Pink	1–2	+	Pink
S. bovis	ATCC 9809	+		−	+	Blue	+		Acid	+	Pink	0.3–0.5	+	Pink
S. equinus	ATCC 9812	+		−	−		+		Acid	+	Pink	0.7–1.0	+	Pink
S. mitis	ATCC 9811	+		−	+	Blue	−	+	Weak acid	+	Pink	0.3–0.5	+	Pink
S. salivarius	ATCC 9756	+		+		White	+		Acid	+	Pink	0.3–0.5	+	Pink
S. cremoris	ATCC 9625	−	+	−	−		−	−	No change	−			−	
S. lactis	ATCC 7962	−	+	−	+	White	−	−	No change	−			−	
S. pyogenes	ATCC 10389	+		−	+	White	−	−	No change	−			−	
S. thermophilus	ATCC 7952	−	+	−	−		−	−	No change	−			−	
S. uberis	ATCC 9927	−	+	±	+	White	−	−	No change	−			−	
Pediococcus cerevisiae	ATCC 8081	−	+	−	+	Blue	−	±	No change	±	White	0.1–0.2	±	White
Leuconostoc mesenteroides	ATCC 9135	−	+	−	−		−	−	No change	−			−	
Lactobacillus lactis	ATCC 10697	−	+	−	−		−	−	No change	−			−	
Lactobacillus acidophilus	ATCC 9857	−	+	−	−		−	−	No change	−			−	
Lactobacillus plantarum	ATCC 10241	−	+	−	+	Blue	−	±	No change	±	Colorless	0.1–0.2	±	Colorless
Escherichia coli	Feces	−	−	−	−		−	−		−			−	
E. coli	River water	−	−	−	−		−	−		−			−	
Aerobacter aerogenes	Feces	−	−	−	−		−	−		−			−	
A. aerogenes	River water	−	−	−	−		−	−		−			−	

+, Growth; ±, trace of growth (slight turbidity in tube or minute colonies on solid surface); v, reaction variable; and −, no growth.

pollution of the surface water samples by the usual test procedure.

The relative efficiency of the membrane filter procedure using KF medium as a liquid and KF medium solidified with 2 per cent agar as a pour plate count were compared on a short series of 10 water samples. Data are presented in table 4. The 10 samples have an average ratio (MF:plate count) of 0.94 with a range in ratios from 0.83 to 1.03.

Using a random sampling procedure, 698 colonies picked from membranes or plates during the study were all shown to be species of the streptococci with gram positive cocci in chains of two or more cells and with or without pleomorphism. Gram stained preparations from MPN tubes always showed the presence of streptococci when acid was produced to give a yellow color. Some other bacteria were able to grow in liquid medium but did not produce acid in sufficient quantity to give a yellow color.

Results and Discussion

The various formulas of KF media (broth, membrane filter, or agar plate tests) gave characteristic growth, summarized in table 2, with *S. bovis*, *S. equinus*, *S. mitis*, *S. salivarius*, and the enterococci consisting of *S. durans* and *S. faecalis* with its varieties of *S. liquefaciens*, *S. zymogenes* and closely related biotypes. All the above streptococci produced turbid growth in the KF broth with an acid reaction demonstrated by the yellow color change produced in the bromocresol purple indicator. Characteristic reaction on the membrane filter or agar plate KF test was a red or pink colony with variations in diameter from 0.3 to 2 mm. All of these streptococci are interpreted to be in the fecal streptococcal group since *S. mitis* and *S. salivarius* have been frequently isolated from human feces and domestic fecal wastes, *S. bovis* and *S. equinus* from domestic animal feces and slaughter house wastes, and

TABLE 3

Comparison of streptococcal densities per 100 ml of sample by most probable number (MPN) and membrane filter (MF) tests on four different media

Sample	Confirmed Coliform, MPN	DA-EVA Test, MPN	BAGG Broth, MPN	KF Streptococcal Medium, MPN	M-Enterococcus Agar, MF	KF Streptococcal Medium, MF	Ratios by Various Tests			
							BAGG / DA-EVA	KF (MPN) / DA-EVA	M-Enterococcus agar / DA-EVA	KF (MF) / DA-EVA
Well	130	4	350	210	10	10	87.5	52.50	2.50	2.50
Lake A	33	8	33	920	6	40	4.23	118.00	0.77	5.13
Lake B	45	20	20	No test	30	4,000	1.00		1.50	200.00
Lagoon	1,700	68	230	790	130	100	3.38	11.60	1.91	1.47
River A	3,300	200	200	1,300	100	4,100	1.00	6.50	0.50	20.50
Pond	400	200	200	4,900	100	500	1.00	24.50	0.50	2.50
River B	1,400	200	200	2,300	260	710	1.00	11.50	1.30	3.55
Lake C	490	340	78	No test	430	570	0.23		1.27	1.68
River C	11,000	450	200	17,000	1,300	1,400	0.44	37.78	2.89	3.11
River D	13,000	680	200	4,900	950	1,300	0.29	7.21	1.40	1.91
River E	17,000	1,300	780	4,900	2,700	3,000	0.60	3.77	2.08	2.31
Creek A	13,000	3,300	1,300	2,300	4,400	8,000	0.39	0.70	1.33	2.42
River F	46,000	4,000	2,000	3,600	4,700	4,900	0.50	0.90	1.18	1.23
Creek B	1,100	4,300	4,300	No test	59,000	56,000	1.00		13.72	13.00
Creek C	92,000	4,600	4,900	22,000	13,000	27,000	1.07	4.78	2.83	5.87
Creek D	7,900	7,900	3,300	7,900	5,700	5,700	0.42	1.00	0.72	0.72
Creek E	22,000	7,900	4,900	7,000	8,800	28,000	0.62	0.89	1.11	3.54
Creek F	92,000	7,900	13,000	54,000	15,000	18,000	1.65	6.84	1.90	2.28
Creek G	33,000	11,000	4,500	79,000	12,000	9,400	0.41	7.18	1.09	0.86
Creek H	49,000	13,000	2,000	23,000	10,000	7,900	0.15	1.77	0.77	0.61
River G	49,000	23,000	4,000	33,000	9,000	32,000	0.17	1.44	0.39	1.39
Creek I	54,000	35,000	7,900	92,000	16,000	54,000	0.23	2.63	0.46	1.54
Creek J	350,000	35,000	200	54,000	24,000	56,000	0.01	1.54	0.69	1.60
Sewage A	1,700,000	700,000	490,000	2,200,000	880,000	1,900,000	0.70	3.14	1.26	2.71
Sewage B	17,000,000	700,000	450,000	4,900,000	990,000	1,700,000	0.64	7.00	1.41	2.43
Median							0.62	5.47	1.28	2.42

DA = dextrose-azide; EVA = ethyl violet azide; BAGG = buffered azide glucose glycerol broth.

enterococci from both the above sources. Also each of these species has been isolated from combined human and slaughter house sewage.

No growth was observed with *S. cremoris*, *S. lactis*, *S. pyogenes*, *S. thermophilus*, or *S. uberis*, nor with the nonstreptococcal bacteria such as *Leuconostoc mesenteroides*, *L. lactis*, and *L. acidophilus*.

A trace of turbidity occurred at the end of 48 hr in the broth procedure with *P. cerevisiae* and *L. plantarum* but these reactions were considered negative since there was insufficient acid produced to give the characteristic yellow color to the indicator. A few small colonies appeared on the solid KF media at the end of 48 hr with the two above species but these reactions were considered negative for the fecal streptococcal group because the characteristic red or pink color reaction in the colony was absent.

Comparison of the KF media with the DA-EVA test shows slightly better productivity of the DA-EVA with *S. mitis* examined, however, the DA-EVA yields false positive reactions with the strains tested of *P. cerevisiae* and *L. plantarum* (which are eliminated by the KF media) and gives variable reactions with some of the fecal streptococcal *S. faecalis* biotypes.

Differences in the numbers of streptococci recovered from various surface waters may be due to the greater number of streptococcal strains which grow on KF medium as compared to the other media tested. This possibility is suggested by the data summarized in table 2, and is strongly supported by observations in another current study which showed that streptococcal yields were essentially the same from human feces when cultured in KF medium and in DA-EVA, but that much larger numbers of streptococci were recovered from domestic animal feces when grown in KF medium than when examined by the DA-EVA method. This discrepancy in numbers is apparently due to the ability of some strains of *S. bovis*, *S. mitis*, *S. salivarius*, *S. equinus*, and some *S. faecalis* biotypes to grow on KF medium yet unable to grow on other media studied. It is recognized that because of the relatively small number of strains from each species examined, the suggestion of preferential growth is not statistically confirmed.

TABLE 4

Comparison of streptococcal densities by membrane filter (MF) method and pour plate agar count using KF streptococcal medium

Sample	MF Count per Ml	Plate Count per Ml	Ratio, MF Count:Plate Count
River no. 1	15,000	15,000	1.00
Creek no. 1	11,000	12,000	0.92
River no. 2	5,900	7,100	0.83
Creek no. 2	23,000	23,000	1.00
Sewage no. 1	620,000	700,000	0.89
Pond water no. 1	20	20	1.00
Creek no. 3	2,800	2,700	1.03
Pond water no. 2	33	40	0.83
Well no. 1	10	10	1.00
Well no. 2	42	45	0.93

Summary

A basic liquid medium (KF) for the enumeration of fecal streptococci was described for use with the multiple tube procedure (most probable number; MPN) and minor modifications were outlined for its adaption to a membrane filter method or a streptococcal agar plate count technique. The KF media were evaluated with various species of streptococci and some nonstreptococcal strains which have a few common growth characteristics with the streptococci. These data indicate typical strains of *Streptococcus bovis*, *S. mitis*, *S. salivarius*, *S. equinus*, and the enterococcal group, including closely related biotypes, produce growth on the KF media, whereas negative reactions resulted with *S. cremoris*, *S. lactis*, *S. pyogenes*, *S. thermophilus*, and *S. uberis* as well as with such microorganisms as *Pediococcus cerevisiae*, *Leuconostoc mesenteroides*, and 3 species of *Lactobacillus*. Fecal streptococci are considered by the authors to include *S. bovis*, *S. mitis*, *S. salivarius*, *S. equinus*, as well as the enterococci and their closely related biotypes. The KF medium as an MPN test procedure and as a membrane filter method, was compared with M-enterococcus agar (membrane filter; MF), BAGG broth (MPN), and dextrose azide (DA) presumptive broth with confirmation in ethyl violet azide (EVA) (MPN) test on 25 water samples of varying degree and type of pollution. The KF medium, used as an MPN test or as a membrane filter procedure yielded higher results in the recovery of streptococci from this series of polluted waters than were obtained by other comparative tests. The identification as streptococci of 698 colonies selected at random and the failure to find any nonstreptococcal bacteria during this study suggests the increased recovery of KF medium might be due to increased streptococcal growth. The possibilities that the difference in productivity in media was due to the number of species isolated and that a greater number of streptococcal species were able to grow on KF medium were suggested as a theory to account for differences in productivity. The membrane filter procedure using KF medium was the most convenient method where verification of streptococci was required or identification of the species by subsequent biochemical tests was desired. The membrane filter method or the agar plate counting procedures are recommended over the multiple tube procedure,

where they are applicable. Preliminary isolation on solid media is mandatory where a classification of species or groups is intended to eliminate possible overgrowths by certain streptococcal strains.

REFERENCES

COOPER, K. E. AND LINTON, A. H. 1947 The value of thallium acetate for the isolation of genococci and streptococci. Monthly Bull. Ministry Health Service, **6**, 204.

FLEMING, A. 1932 On the specific antibacterial properties of penicillin and potassium tellurite. J. Pathol. Bacteriol., **35**, 831.

HAJNA, A. A. 1951 A buffered azide glucose-glycerol broth for the presumptive and confirmative tests for fecal streptococci. Public Health Lab., **9**, 80–81.

HAJNA, A. A. AND PERRY, C. A. 1943 Comparative study of presumptive and confirmative media for bacteria of the coliform group and for fecal streptococci. Am. J. Public Health, **33**, 550–556.

HAROLD, C. H. 1936 *Annual report metropolitan water board London (31st)*. Her Majesty's Stationery Office, London.

HARTMAN, G. 1936 Ein Beitrag zur Reinzuchtung von Mastitisstreptokokken aus verunreinigtem Material. Milchwirtsch. Forsch., **18**, 116–122.

LITSKY, W., MALLMANN, W. L., AND FIFIELD, C. W. 1953 A new medium for the detection of enterococci in water. Am. J. Public Health, **43**, 873–879.

LITSKY, W., MALLMANN, W. L., AND FIFIELD, C. W. 1955 Comparison of the most probable numbers of *Escherichia coli* and enterococci in river waters. Am. J. Public Health, **45**, 1049–1053.

MALLMANN, W. L. AND SELIGMANN, E. B. 1950 A comparative study of media for the detection of streptococci in water and sewage. Am. J. Public Health, **40**, 286–289.

SLANETZ, L. W. AND BARTLEY, C. H. 1957 Numbers of enterococci in water, sewage, and feces determined by the membrane filter technique with an improved medium. J. Bacteriol., **74**, 591–595.

WINTER, C. E. AND SANDHOLZER, L. A. 1946 Recommended procedures for detecting the presence of enterococcus. Commercial Fisheries TL 2, U. S. Dept. of Interior, Fish and Wildlife Service. (Nov.)

Microflora within Healthy Tomatoes[1]

ZDENKA SAMISH, R. ETINGER-TULCZYNSKA, AND MIRIAM BICK

Department of Food Technology, Agricultural Research Station, Ministry of Agriculture, Rehovot, Israel

Received for publication April 13, 1960

The tissue of normal, healthy, undamaged fruit is generally considered to be sterile. Yet, literature proving this hypothesis is scarce (Fernback, 1888), whereas at times such statements appear without experimental evidence (Allen, 1950; Burcik, 1950). A number of investigators, however, have reported instances in which bacteria were found in various parts of healthy plants particularly in storage organs, (Hennig and Villforth, 1940; Szilvasi, 1942; Sanford, 1948; Tervet and Hollis, 1948; Hollis, 1951; Schanderl, 1953; Tonzig and Bracci-Orsenigo, 1955; Dawid, 1957; Starkey, 1958). Evidence has been accumulated and reported by us on the occurrence of bacteria within fresh, healthy cucumbers (Samish, Dimant, and Marani, 1957; Samish and Dimant, 1959) and this study has now been expanded to tomato fruits.

MATERIALS AND METHODS

In a series of preliminary experiments an efficient method of sterilizing the surface of fresh tomatoes was developed. Fresh, firm, healthy, and unblemished tomatoes were thoroughly washed, scrubbed, and rewashed with a detergent, immersed for 10 to 15 min in a detergent solution, and rinsed. They were then kept for 15 min in sodium hypochlorite containing 300 mg available chlorine, and for 5 min in a weak solution of sodium thiosulfate, again rinsed with sterile water, and finally immersed for 30 min in 80 per cent alcohol containing 0.02 per cent iodine. The tomatoes were then flamed and their pulp extracted by either of two methods:

1. A sterile wide-mouthed glass pipette was forced into the fruit and rotated so as to macerate the inner tissue of the tomato; aliquots of the pulpy juice were then pipetted and transferred directly upon nutrient media.

2. The fruit was cut open with a sterile knife, and pieces of the inner pulp dissected, transferred into a sterile jar containing pieces of broken glass, and dispersed by shaking.

Aliquots of the pulpy juice were transferred, with or without dilution with buffers, into test tubes containing nutrient broth or agar slants. Generally 1 ml of tomato pulp was used for each sample, 3 to 6 samples being prepared from each tomato. The concentration and composition of the buffers and nutrient substrates were modified in the course of the experiments. The samples were incubated at 29 to 30 C and observed

[1] Publication of the Agricultural Research Station, Rehovot, Israel, 1959, Series 295-E. This study was supported by the U. S. Department of Agriculture, Agricultural Research Service (Public Law 480), Project No. UR-A10-(30)-3.