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The enumeration of lactic streptococci

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THE ENUMERATION OF LACTIC STREPTOCOCCI

by

Robert William Baughman

A Dissertation Submitted to the

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Of Science and Technology
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INTRODUCTION

Many media and techniques now used for the enumeration of lactic streptococci are inadequate. This situation is surprising because controlled growth of lactic streptococci in starter cultures, cultured milk and cheese of all types is of paramount importance. Few studies, however, have been concerned with attempts to improve existing methodology for determining numbers of lactic streptococci.

Media commonly used for the enumeration of lactic streptococci are either general purpose media or were developed primarily for the isolation of lactobacilli. Many of these media will not support the growth of fastidious strains of lactic streptococci. Even with the less fastidious strains there are differences in recovery from medium to medium and from strain to strain in the same medium. Therefore, the selection of a suitable medium presents a serious problem to the investigator when the enumeration of lactic streptococci is required for a particular study.

With the above considerations in mind, a study was undertaken to determine certain nutritional factors affecting the enumeration of lactic streptococci. A comparative study also was made of media that had been suggested as being

suitable for the enumeration of lactic streptococci. Concurrently, studies were made of the effects of various environmental factors on the enumeration of lactic streptococci. Information gained in such a study should enable the investigator to obtain more reliable estimates of the population of lactic streptococci in dairy products.

REVIEW OF LITERATURE

There were three major areas of concern in this study. They encompassed the effects of nutrition and environment as related to media evaluation. All were directly involved in the problem of enumeration of lactic streptococci and are included in this review of literature.

Nutritional Requirements of Lactic Streptococci

Amino acid and vitamin requirements

One of the first workers to consider the nutritional requirements of lactic streptococci in a synthetic medium was Niven (41). As reported in his study, each of 21 strains of *Streptococcus lactis* required pantothenic acid, nicotinic acid and biotin. Eighteen of the 21 strains required thiamin, whereas only one-third required riboflavin. None of the strains required folic acid or pyridoxamine. The latter compound stimulated the growth of all of the strains studied. The amino acid requirements were complex; a minimum of 14 were necessary to effect prompt growth. Asparagine and glutamine were necessary for the initiation of growth of all strains. None of the strains required the addition of tryptophan. Niven (41) stated that *Streptococcus cremoris*

had similar requirements although no data were given.

Smith (48) studied the growth of two strains of S. lactis in a glucose-citrate-salts medium. Amino acids, vitamins, purines and pyrimidines were added, but the culture did not respond. When yeast extract was added, good growth was obtained.

Pollock and Lindner (43) found that asparagine and glutamine were not required in a synthetic medium by any of the six strains of S. lactis included in their studies.

Wright and Skeggs (63) found that asparagine and glutamine were effective, in a defined medium, in promoting the growth of several strains of S. lactis. In the absence of asparagine or glutamine a vitamin-free tryptic hydrolysate of casein was effective in promoting the growth of S. lactis. The activity of the casein digest was much greater than could be accounted for on the basis of asparagine or glutamine content. They concluded that a factor, or factors, more effective than asparagine or glutamine must be present.

Stokes (53), using a synthetic medium, found that six out of 17 strains grew as well or better when thymine was substituted for folic acid. Adenine, in addition to either thymine or folic acid, was necessary for maximum growth. Guanine or xanthine could be substituted for adenine but were

less effective.

Snell and Mitchell (49) also noted that adenine was essential for growth of S. lactis in a synthetic medium.

Anderson and Elliker (2) studied the nutrition of five strains of S. lactis and 30 strains of S. cremoris in a chemically defined medium. These strains, isolated from commercial cultures, required proline, isoleucine, valine, leucine, histidine, glutamine and methionine. All but one strain required arginine. Cystine, tryptophan and serine were not required by any of the strains studied. All of the other amino acids were required by one or more of the strains. Niacin, pantothenic acid and biotin were required by all strains studied. Pyridoxamine and thiamin were stimulatory. Most of the strains were stimulated by riboflavin and more than one-third required it for growth.

Hussain and McDonald (24) determined the amino acid requirements of three strains of S. lactis and six strains of S. cremoris in a synthetic medium. Their results agreed with the previous findings of Niven (41) and Anderson and Elliker (2).

Using a synthetic medium in which methionine, glycine or serine were omitted singly, Kizer et al. (28) found that

these amino acids were not essential to their strain of *S. lactis*. However, Anderson and Elliker (2) found that methionine was essential and glycine stimulatory for their strains of *S. lactis*. Folic acid and cyanocobalamin additions to the medium failed to have any effect on growth.

The effect of adding amino acids to milk was investigated by Anderson and Elliker (3). Slight stimulation of *S. lactis* and *S. cremoris* was obtained with histidine, methionine, glycine, lysine and tryptophan; leucine, isoleucine and serine depressed growth. Cystine, arginine, alanine, asparagine, glutamine, proline, tyrosine, valine, phenylalanine and threonine had no effect.

Reiter and Oram (45), using a synthetic medium, found that niacin, pantothenic acid and biotin were essential for six strains of *S. lactis* and 18 strains of *S. cremoris*. Pyridoxal stimulated, but was not essential for the strains studied. Folic acid, thiamin and cyanocobalamin were not required. Although riboflavin was required by all strains of *S. cremoris*, it was required by only one strain of *S. lactis*. Acetate was not essential, although it stimulated the growth of some strains. All strains of *S. lactis* and *S. cremoris* required glutamic acid, valine, methionine, leucine, iso-

leucine and histidine. Aspartic acid, citrulline and ornithine were not required by any of the strains studied. All strains of *S. cremoris* required proline and phenylalanine and most of them also required or were stimulated by tyrosine, lysine and alanine.

In an attempt to clarify the reported amino acid and vitamin requirements of lactic streptococci, a summary of data reported in the literature is given in Tables 1 and 2.

Mineral requirements

Hunter (23) considered the role that phosphate might play in increasing the yield of a plating medium. He suggested that the value of phosphate was due not only to its buffering effect, but also to a combination of the phosphate with some constituent of agar. This combination was thought to have a growth promoting action on the lactic streptococci.

DeMans and Galesloot (13) noted that the addition of manganese to milk stimulated the growth of betacocci but not that of lactic streptococci. The addition of manganese also stimulated the rate of citric acid fermentation.

Nurmikko and Karha (42) conducted experiments on the nutritional requirements of 124 strains of lactic acid bacteria isolated from Finnish dairy products. Some strains

Table 1. Amino acid requirements of lactic streptococci in synthetic media

Amino acid	Species	Number of strains and requirement[a]			Reference
		+	S	-	
Alanine	*S. lactis*		2	3	Anderson and Elliker (2)
	S. lactis		1	5	Reiter and Oram (45)
	S. cremoris	8	13	9	Anderson and Elliker (2)
	S. cremoris	2	12	4	Reiter and Oram (45)
Arginine	*S. lactis*	5			Anderson and Elliker (2)
	S. lactis			6	Reiter and Oram (45)
	S. cremoris	29			Anderson and Elliker (2)
	S. cremoris	3			Reiter and Oram (45)
Asparagine	*S. lactis*		1	4	Anderson and Elliker (2)
	S. lactis	3			Hussain and McDonald (24)
	S. lactis	21			Niven (41)
	S. lactis			6	Pollock and Lindner (43)
	S. lactis		3		Wright and Skeggs (63)
	S. cremoris		10	19	Anderson and Elliker (2)
Aspartic acid	*S. lactis*			5	Anderson and Elliker (2)
	S. cremoris		8	22	Anderson and Elliker (2)
Cystine	*S. lactis*			5	Anderson and Elliker (2)
	S. lactis			6	Reiter and Oram (45)
	S. cremoris			30	Anderson and Elliker (2)
	S. cremoris			18	Reiter and Oram (45)

[a] + = required, S = stimulatory, - = not required.

Table 1 (Continued)

Amino acid	Species	Number of strains and requirement[a] +	S	-	Reference
Glutamic acid	*S. lactis*	4			Anderson and Elliker (2)
	S. cremoris		27	3	Anderson and Elliker (2)
Glutamine	*S. lactis*	21			Niven (41)
	S. lactis	6			Reiter and Oram (45)
	S. lactis	5			Wright and Skeggs (63)
	S. cremoris	30			Anderson and Elliker (2)
	S. cremoris	18			Reiter and Oram (45)
Glycine	*S. lactis*		3	2	Anderson and Elliker (2)
	S. lactis			3	Kizer et al. (28)
	S. lactis			6	Reiter and Oram (45)
	S. cremoris	1	19	10	Anderson and Elliker (2)
	S. cremoris			18	Reiter and Oram (45)
Histidine	*S. lactis*	5			Anderson and Elliker (2)
	S. lactis	6			Reiter and Oram (45)
	S. cremoris	30			Anderson and Elliker (2)
	S. cremoris	18			Reiter and Oram (45)
Isoleucine	*S. lactis*	5			Anderson and Elliker (2)
	S. lactis	6			Reiter and Oram (45)
	S. cremoris	30			Anderson and Elliker (2)
	S. cremoris	18			Reiter and Oram (45)
Leucine	*S. lactis*	5			Anderson and Elliker (2)
	S. lactis	6			Reiter and Oram (45)
	S. cremoris	30			Anderson and Elliker (2)
	S. cremoris	18			Reiter and Oram (45)

Table 1 (Continued)

Amino acid	Species	Number of strains and requirement[a]			Reference
		+	S	-	
Lysine	*S. cremoris*	3	18	9	Anderson and Elliker (2)
	S. cremoris	5	8	5	Reiter and Oram (45)
Methionine	*S. lactis*	5			Anderson and Elliker (2)
	S. lactis			3	Kizer et al. (28)
	S. lactis	6			Reiter and Oram (45)
Phenylalanine	*S. lactis*			5	Anderson and Elliker (2)
	S. lactis	3		3	Reiter and Oram (45)
	S. cremoris	18		12	Anderson and Elliker (2)
	S. cremoris	18			Reiter and Oram (45)
Proline	*S. lactis*	3		2	Anderson and Elliker (2)
	S. lactis			6	Reiter and Oram (45)
	S. cremoris	29		1	Anderson and Elliker (2)
	S. cremoris	18			Reiter and Oram (45)
Serine	*S. lactis*			5	Anderson and Elliker (2)
	S. lactis			3	Kizer et al. (28)
	S. lactis			6	Reiter and Oram (45)
	S. cremoris		2	28	Anderson and Elliker (2)
	S. cremoris	5	10	3	Reiter and Oram (45)
Threonine	*S. lactis*			5	Anderson and Elliker (2)
	S. lactis			6	Reiter and Oram (45)
	S. cremoris	3	16	11	Anderson and Elliker (2)
Tyrosine	*S. lactis*			5	Anderson and Elliker (2)
	S. lactis			6	Reiter and Oram (45)
	S. cremoris	3	3	24	Anderson and Elliker (2)
	S. cremoris	8	5	5	Reiter and Oram (45)

Table 1 (Continued)

Amino acid	Species	Number of strains and requirement[a] +	S	-	Reference
Tryptophan	*S. lactis*			5	Anderson and Elliker (2)
	S. lactis			21	Niven (41)
	S. lactis			6	Reiter and Oram (45)
	S. cremoris		1	29	Anderson and Elliker (2)
	S. cremoris	2	2	14	Reiter and Oram (45)
Valine	*S. lactis*	5			Anderson and Elliker (2)
	S. lactis	6			Reiter and Oram (45)
	S. cremoris	30			Anderson and Elliker (2)
	S. cremoris	18			Reiter and Oram (45)

Table 2. Vitamin requirements of lactic streptococci in synthetic media

Vitamin	Species	Number of strains and requirement[a] +	S	-	Reference
Ascorbic acid	_S. lactis_		2	2	Anderson and Elliker (2)
	S. cremoris		4	26	Anderson and Elliker (2)
Biotin	_S. lactis_	5			Anderson and Elliker (2)
	S. lactis	21			Niven (41)
	S. lactis	6			Reiter and Oram (45)
	S. cremoris	30			Anderson and Elliker (2)
	S. cremoris	18			Reiter and Oram (45)
Cyanocobalamin	_S. lactis_			6	Reiter and Oram (45)
(B_{12})	_S. cremoris_			18	Reiter and Oram (45)
Folic acid	_S. lactis_		5		Anderson and Elliker (2)
	S. lactis			21	Niven (41)
	S. lactis			6	Reiter and Oram (45)
	S. lactis	6		11	Stokes (53)
	S. cremoris		29	1	Anderson and Elliker (2)
	S. cremoris			18	Reiter and Oram (45)
Niacin	_S. lactis_	5			Anderson and Elliker (2)
	S. lactis	21			Niven (41)
	S. lactis	6			Reiter and Oram (45)
	S. cremoris	30			Anderson and Elliker (2)
	S. cremoris	18			Reiter and Oram (45)

[a]+ = required, S = stimulatory, - = not required.

Table 2 (Continued)

Vitamin	Species	Number of strains and requirement[a] +	S	-	Reference
Pantothenic acid	*S. lactis*	5			Anderson and Elliker (2)
	S. lactis	21			Niven (41)
	S. lactis	6			Reiter and Oram (45)
	S. cremoris	30			Anderson and Elliker (2)
	S. cremoris	18			Reiter and Oram (45)
Pyridoxal	*S. lactis*			21	Niven (41)
Pyridoxamine	*S. lactis*		5		Anderson and Elliker (2)
	S. lactis		6		Reiter and Oram (45)
	S. cremoris		30		Anderson and Elliker (2)
	S. cremoris		18		Reiter and Oram (45)
Riboflavin	*S. lactis*	1		4	Anderson and Elliker (2)
	S. lactis	7		14	Niven (41)
	S. lactis	1		5	Reiter and Oram (45)
	S. cremoris	14		16	Anderson and Elliker (2)
	S. cremoris	18			Reiter and Oram (45)
Thiamin	*S. lactis*		5		Anderson and Elliker (2)
	S. lactis	18		3	Niven (41)
	S. lactis			6	Reiter and Oram (45)
	S. lactis	5		1	Stokes (53)
	S. cremoris		30		Anderson and Elliker (2)
	S. cremoris			18	Reiter and Oram (45)

had complex growth requirements and required unidentified growth factors. Some strains of _S_. _thermophilus_ required calcium for growth. They indicated that the presence of Ca^{++} counteracted the inhibition of growth in the presence of citrate and some other organic ions.

The inclusion of certain cations in synthetic media, although not indicative of specific growth requirements, was of interest. Concentrations used in three synthetic media are presented in Table 3.

Table 3. Concentrations of certain cations used in synthetic media

	Investigator		
	Niven (41)	Smith (48)	Reiter and Oram (45)
Cation	Cation concentration mg/liter		
Mg^{++}	8.0	20.0	24.0
Fe^{++}	0.8	2.0	1.0
Mn^{++}	0.5	2.5	0.0
Ca^{++}			18.0
Zn^{++}			1.0
Cu^{++}			0.6
Co^{++}			0.5

Miscellaneous nutritional requirements

Anderson and Elliker (3) found that a liver fraction, trypsinized skimmilk or peptonized milk, when added in a low concentration to reconstituted non-fat milk solids, stimulated growth of mixed strain starter cultures, as well as individual strains of S. lactis and S. cremoris. The liver fraction was effective in stimulating the initial growth of the less rapidly growing strains. On the other hand, the more rapidly growing strains were either inhibited or stimulated only slightly when this factor was added to milk.

Speck et al. (50) attempted to ascertain the cause of different growth responses of lactic streptococci in milk and in milk supplemented with certain stimulatory extracts. Aqueous extracts of pancreas tissue, when added to milk, were stimulatory for the lactic streptococci. Litmus milk bioautographs of paper chromatograms showed the presence of multiple stimulatory substances in the pancreas extract. Extracts of liver and yeast also contained various stimulatory substances. There was an inverse relationship between the number of components to which a culture responded and the rate at which a culture grew in milk.

Kennedy and Speck (26) added 1% corn steep liquor to milk. This resulted in stimulation of growth of both *S. lactis* and *S. cremoris*. *S. lactis* was stimulated by the addition of corn steep preparations to synthetic media which had been reported as complete for the optimum growth of this organism. The greatest response of *S. lactis* was obtained at concentrations below 0.1% in synthetic media.

Hunter (22) studied factors affecting the growth of two lactic cultures in a plating medium. Of six peptones that were used, culture ML grew rapidly only on Tryptone. Tryptone apparently supplied a certain factor or factors that were not present in the other peptones. The inclusion of lactose, as well as glucose in the medium, stimulated the growth of culture ML.

Garvie and Mabbitt (21) increased the rate of acid production by a slow variant of *S. cremoris* by adding various peptones to milk. The rate of acid production was raised to that of the parent strain. However, the stimulation took place without any apparent change in the rate of growth. They concluded that the change from a fast to a slow culture, which sometimes occurs on continued transfer, was due to the loss of ability to utilize nitrogenous compounds in milk.

Anderson et al. (4) analysed samples of milk from different cows and found considerable variation in the peptide content. Different cultures varied somewhat in their response to the peptide content of milk. The rate of acid production by lactic streptococci in most instances increased with an increase in the peptide content. Addition of 1 to 10% non-fat milk solids to fresh skimmilk did not affect the initial growth rate of lactic streptococci subsequently cultured in the milk. The addition of 0.1 to 1% of trypsin-hydrolysed skimmilk caused a marked increase in the initial growth rate of cultures.

Metcalf et al. (35) utilised Lactobacillus fermentum as the test organism in studying the growth factor or factors in certain vegetable juices. Tomato juice serum was used up to a level of 5%. This growth promoting substance was designated as the "T" factor. This "T" factor also was found in liver, string beans, carrots, beets, onions, cabbage, peppers, spinach and orange juice. In an attempt to find a substitute for this "T" factor many amino acids and vitamins were utilized. However, only thiamin was effective as a growth promoting factor.

Collins et al. (12) used a chemically defined medium

made by adding sodium acetate and sorbitan monooleate to the medium of Niven (41). The modified medium permitted the growth of all lactic streptococci strains tested that did not grow in the unsupplemented medium. The addition of sodium acetate and sorbitan monooleate was necessary for the growth of 22 strains of _S_. _cremoris_ and nine of 31 strains of _S_. _lactis_. A liver fraction could be substituted in somewhat smaller quantities for sodium acetate and sorbitan monooleate.

The acetate-citrate metabolism of the lactic streptococci was studied by Kizer and Speck (27). They found that strains of _S_. _cremoris_ responded significantly when a level of 10.0 mg per ml of acetate was used. No response was noted when citrate was added at the same concentration; with some strains this concentration of citrate proved to be inhibitory. _S_. _lactis_ responded significantly to this concentration of either acetate or citrate. In the absence of pantothenate neither _S_. _lactis_ or _S_. _cremoris_ was able to grow. This also was noted by Anderson and Elliker (2), Niven (41), and Reiter and Oram (45).

Koburger et al. (29) found that acid production by _S_. _lactis_ in milk was accelerated by the addition of pancreas extract. Three distinct components that were detected by

bioautography were isolated and identified. Identification of the purified fractions was by paper chromatography, ultraviolet and infra red spectra. The active compounds were found to be inosine, adenine and hypoxanthine.

Environmental Factors Affecting the Growth of Lactic Streptococci

Reiter and Oram (44) noted that growth initiation by *S. lactis* was dependent upon the presence of a small quantity of CO_2 in the medium. This requirement was low; however, growth was inhibited only when the medium was swept continuously with CO_2-free gas.

Whitehead et al. (62) found that the initial growth rate of *S. lactis* and *S. cremoris* in skimmilk depends upon the amount of CO_2 present in solution. If the skimmilk is swept continuously by CO_2-free gas, all strains show a prolonged lag period. For optimal initial growth lactic streptococci require the presence of CO_2 in solution in milk within a range of 0.2 to 2.3%. Yeast extract in an amount of about 0.5% can be substituted for CO_2 in skimmilk.

Factors Influencing Recovery of Injured Cells on Plating Media

Although not a direct factor in plating procedures as such, the treatment that a culture undergoes prior to plating might influence the productivity of any medium. Many investigators have assumed that media which are suitable for the development of bacteria grown under favorable conditions are equally suitable for bacteria which have been subjected to sub-lethal heat or toxic agents. By proper choice of medium, bacteria which have been exposed to sub-lethal temperatures or toxic agents may be non-viable on one medium and viable when another medium is used for plating.

Since there are indications that the plate incubation period is more critical for heat injured cells than those not subjected to heat treatment, the significance of the length of plate incubation upon the plate count will be briefly reviewed. Babel et al. (5) found that, with raw milk, incubation for 3 days at 32 or 35C gave no increase in count over 2 days of incubation. With pasteurized milk the additional day of incubation resulted in an increased count. It also was reported by Rowlands and Provan (46) that, with pasteurized milk, colony counts on plates incubated at 30 and

37C were increased by prolonging the incubation period from 48 to 72 hr. Nelson and Baker (40) observed that incubation periods shorter than 3 days at 25C and 4 days at 21C resulted in lower plate counts on certain samples of pasteurized milk. Kaufmann et al. (25) compared the growth of heated and unheated cultures and concluded that heated cells exhibited a longer lag phase than did unheated cells. Thomas (54) found that of the plate incubation temperatures of 35, 32, 28 and 21C, incubation at 28C for 4 days was the optimum for determining the maximum bacterial population of laboratory pasteurized milk.

The influence of plate incubation temperature on recovery of heat injured cells has been studied extensively and will only be briefly reviewed. Recent work by Thomas (54) showed that the average thermoduric colony count obtained at 35C for 2 days was only 31% of the average count obtained at 28C for 4 days. Even at an incubation temperature of 32C for 2 days, the average count was more than double that obtained at 35C for 2 days. Black (9) and Thomas et al. (56) found that the plate counts on raw and commercially pasteurized milk following incubation of plates at 37, 35 and 32C for 48 hr showed that counts were somewhat higher at 35C and still

higher at 32C. Thomas and Jenkins (55) concluded that incubation for 3 days at 30C, as compared with 3 days at 37C, gave an increase in count of approximately two times for raw and six times for pasteurized milk.

Peptones also have been investigated concerning their influence on the recovery of heat treated cells. Bowers and Hucker (10) compared Nutrient agar and an improved medium containing Tryptone, 0.5%; dextrose, 0.1%; skimmilk, 0.5%; and agar, 1.5%. They found the plate counts on the improved medium to be 30% higher on 134 samples of raw milk and 350% higher on 77 samples of pasteurized milk. The colony productivity of Nutrient agar and TGEM agar was compared by Nelson (38). He observed that only in rare instances were differences in plate counts of the unheated controls possibly attributable to differences in the media. The TGEM agar, however, was definitely superior to Nutrient agar for the development of heat treated bacteria. Bacteria which had been subjected to heat at sub-lethal levels were said to be more demanding in their requirements for growth than the unheated controls. In a later study Nelson (39) reported that variations in the Tryptone content of the plating medium and the time of addition of the peptone in the preparation of the medium influenced colony development by heat

treated bacteria. Black (8) compared the better peptones generally available for bacteriological use. He concluded that the better peptones gave comparable results. These peptones were not defined.

Carbohydrates also have received attention concerning their influence concerning recovery of heat treated bacteria. The earliest worker to point out the advantage of a carbohydrate containing medium for the enumeration of bacteria in pasteurized dairy products was Sherman (47). In a study of the effect of carbohydrates on thermal resistance of bacteria, Fay (18) found that the addition of carbohydrates, especially dextrose, to the plating medium increased considerably the count of surviving organisms in a number of cases.

The pH of the plating medium was found by Fay (18), Nelson (39) and Thomas (54) to influence the plate counts of thermoduric organisms. In general, the unheated cultures grew on solid media over a much wider pH range than did the heated cultures. Also a higher pH many times favored the heated cells.

There are many factors that affect the rate of mortality in a bacterial population and it would not be possible to review them all here. Watkins and Wilson (59) studied factors

that affected the mortality in a cell population. These factors included: cell age, concentration of the inoculum, intensity of toxic agents and temperature. In a study by White (61) cultures of *S. faecalis* were found to be more susceptible to heat at both a high and low pH as compared to a neutral pH. Mattick and Nichols (33) studied the effect of pH on the heat resistance of microorganisms in milk. As the pH decreased, the number of bacteria surviving also was found to decrease.

From this very brief review it was apparent that there are many factors concerning a plating medium that will influence cell recovery from heat treated cultures. Although most of this information deals with pasteurization-resistant bacteria, it is quite possible that many of these same factors will apply if lactic cultures that have undergone undue stress are to be plated.

Media Development

Hunter (23) was the first investigator to attempt to develop a satisfactory plating medium for the enumeration of lactic streptococci. Of eight different peptones that were studied only one (Bacto-peptone, Difco) was not found to be

satisfactory. He used only one culture in this study.

Mull (36) obtained erratic plate counts of S. lactis when Tomato juice agar or Tryptone-glucose-beef extract milk agar were used. Uniform counts were obtained with a modified Trypticase-soy agar.

Elliker et al. (16) studied various media that had been suggested as suitable for the enumeration of certain types of lactic acid bacteria. A medium was developed and termed Lactic agar. This was a modification of McLaughlin's (34) Triple sugar agar. They found that the size and number of lactic streptococci colonies were greater when sodium chloride, sodium acetate and glycine were included in the medium. It was noted, in some instances, that a combination of lactose, dextrose and sucrose gave better growth than when only one sugar or a combination of two sugars was used.

Galesloot et al. (20) developed a medium for the enumeration of lactic streptococci and other lactic acid bacteria. It was designated TGV agar. This medium was used in conjunction with their study of media for the isolation of aroma bacteria in starter cultures.

The following media, although developed primarily for the enumeration of lactobacilli, have been used by many

investigators for the enumeration of lactic streptococci.

Kulp and White (31) modified the original Tomato juice agar of Kulp (30) by adding 1% peptonized milk to the original formula. The modified medium was more satisfactory because *Lactobacillus acidophilus* developed larger colonies than in the original medium. Quantitative counts were higher with the new medium than with the original. Extensive tests indicated that this new medium was as good or better than any of the more complicated digest media previously used.

McLaughlin (34), using plating methods for determining numbers of lactobacilli, found that Whey or Tomato juice agar failed to give consistent results. A medium was developed which was called Triple sugar agar. This medium proved to be better than the commercially available Whey or Tomato juice agars. The addition of soybean peptone caused the colonies to be larger, but the average plate count was lower compared to that obtained with the regular Triple sugar agar. The addition of stimulatory materials, such as liver extract, yeast extract and cystine, in some instances, increased the size of colonies. The number of colonies was not significantly increased.

Fabian et al. (17) modified Tryptone-glucose agar and

Tryptone-glucose-beef extract agar by adding different amounts of filtered or unfiltered V-8 cocktail vegetable juice[1]. The most productive combination was then compared against ten other media commonly used for the enumeration of lactobacilli. The V-8 medium gave as high or higher counts than any of the other media. In addition, this medium inhibited *Sarcina lutea*, *Bacillus subtilis* and *Staphylococcus aureus*. None of the other media showed this inhibition.

Tittsler[2] later modified the medium of Fabian et al. (17) by the addition of Tryptose and yeast extract. This was designated an improved V-8 medium, because it yielded somewhat higher counts than did the original version.

Briggs (11) studied several media that had been recommended for the enumeration of lactobacilli. None supported good growth of all species. Experiments were described in which the optimum concentration and combinations of various ingredients were determined. A medium was developed that was reportedly satisfactory for the growth of all lactobacilli.

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[1]Campbell Soup Co., Camden, N. J.

[2]Tittsler, R. P. U.S.D.A., Washington, D. C. Information concerning an improved V-8 medium. Private communication. 1956.

Bacteriological investigations of citrus concentrates gave rise to another medium for the enumeration of lactobacilli. Murdock et al. (37) evaluated 18 media used for the enumeration of lactobacilli and leuconostocs in citrus concentrates. Orange serum agar, as developed by Troy and Beisel (57), was found to be most suitable of all the media studied. A dehydrated Orange serum agar was developed later by Stevens (52).

Other media, although not developed for the enumeration of lactic streptococci, have been used by many investigators for this purpose.

Penassay agar, Standard methods agar, and Whey agar, as manufactured by Difco Laboratories, Inc. (14), and APT agar, Eugonagar, L agar and Trypticase soy agar, as manufactured by Baltimore Biological Laboratory, Inc. (6), have been used for the enumeration of lactic streptococci. A brief description and the original source of each of these, with the exception of APT agar[1], can be found in the Difco Manual (14)

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[1] Baltimore Biological Laboratory, Inc., Baltimore 18, Maryland. Data from brochure on APT agar. Private communication. 1960.

or in *Products for the Microbiological Laboratory* (6), respectively.

Formulae for the previously mentioned media are found in Appendix A.

EXPERIMENTAL METHODS

Sources of Lactic Streptococci

Forty-five lyophilized lactic cultures were obtained from seven commercial sources in the United States. Both single and multiple-strain cultures were included. These cultures were representative of those currently being used for the manufacture of various cultured dairy products.

Propagation of Cultures

Skimmilk containing 2% milk fat was dispensed into 6 oz, screw-capped medicinal ovals, steamed for 1 hr and then cooled to 21C. A 1% inoculum was added. The cultures were incubated at 21C for 16 hr. These cultures were either used for experimental study or were cooled to about 4C and held at this temperature until the next transfer.

Lyophilized lactic cultures employed in this study were transferred at least three times prior to being used in any experiment. For any one experiment or experiments, cultures were transferred five times a week.

General Laboratory Procedure

Plating methods employed were those outlined in Standard Methods for the Examination of Dairy Products (1). Any modifications are specifically noted where applicable. All plating was done in duplicate.

Following incubation the colonies were counted with the aid of a Quebec colony counter. Plate counts were computed by arithmetically averaging the colony counts on duplicate plates and multiplying this average by the appropriate dilution factor (1) to obtain the numbers of bacteria/g.

Plate Counts Obtained at Selected Temperatures After Different Periods of Time

A single series of dilutions was prepared for each of seven lactic cultures. Duplicate plates were prepared from the appropriate dilution. Plates were poured with Eugonagar. Duplicate plates were incubated at 21, 25, 28 and 32C. Colonies that appeared at 2 days were marked with colored ink. Different colored inks were used to identify colonies appearing after incubation for 4, 7 and 10 days. This experiment was replicated three times. In the latter stage of this

investigation an incubation temperature of 18C was employed for comparative purposes.

Comparison of Plate Counts Obtained With Different Media

Commercial dehydrated media

Ten commercial dehydrated media that had been suggested as being suitable or were known to have been used for the enumeration of lactic streptococci were used. These media were: Whey agar, Difco; Penassay agar, Difco; Standard methods agar, Difco; Tomato juice agar, Difco; Brain heart infusion agar, BBL; Trypticase soy agar, BBL; Eugonagar, BBL; APT agar and L agar, BBL; Orange serum agar[1].

Each medium was prepared according to directions given by the manufacturer. Seventeen lactic cultures were plated in duplicate on each medium. Colonies were counted after incubation of the plates at 21C for 4 days. This experiment was replicated three times.

Laboratory media

Seven media that had been suggested as being satisfactory for the enumeration of lactic streptococci were examined.

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[1]Sunkist Growers, Products Dept., Ontario, Calif.

These media were: Lactic agar of Elliker et al. (16); Special Trypticase soy agar of Mull (36); Modified V-8 agar of Tittsler; Tomato juice agar of Briggs (11); V-8 agar of Fabian et al. (17); Tomato juice agar of Kulp and White (31) and TGV agar of Galesloot et al. (20). These media were prepared according to directions given in the references cited. Fourteen lactic cultures were plated in duplicate on each medium. Colonies were counted after incubation of the plates at 21C for 4 days. This experiment was replicated three times.

Plate Counts Obtained With Eugonagar and With Eugonagar Plus Filtered V-8 Cocktail Vegetable Juice

V-8 cocktail vegetable juice was first filtered through cotton and through Whatman No. 12 filter paper to obtain a clear serum. Eugonagar was modified by the addition of V-8 cocktail vegetable juice serum at three concentrations: 10, 20 and 30%. A single series of dilutions was prepared for each of 12 lactic cultures. Four sets of duplicate plates were prepared from the appropriate dilution. One set was poured with Eugonagar and the other sets with the modified Eugonagar. Colonies were counted after incubation at 21C for 4 days. This experiment was replicated three times.

The Effect of Different Levels of Trypticase in the Plating Medium on the Plate Count of Lactic Streptococci

A basal medium conforming to the following formula was used in this experiment and in subsequent experiments concerned with the addition or substitution of various compounds in the plating medium: Trypticase, 15 g; dextrose, 5 g; sodium chloride, 4 g; agar, 15 g and distilled water, 1 liter. The pH was 6.8. In this experiment the concentration of Trypticase was varied as follows (per liter) 5, 10, 15, 20, 25 and 30 g.

Six lactic cultures were plated in duplicate on each of the six media. Colonies were counted after incubation at 21C for 4 days. This experiment was replicated three times.

Effect of Selected Bacteriological Peptones on the Plate Count of Lactic Streptococci

The medium described on page 34 was modified by substituting 15 g/liter of Peptonized milk, Difco; Tryptone, Difco; Casamino acids, Difco or Phytone, BBL for the Trypticase, BBL.

Six lactic cultures were plated in duplicate on each of the above media and on the unmodified medium. Colonies were counted after incubation at 21C for 4 days. This experiment

was replicated three times.

Effect of Supplementing the Plating Medium With Beef Extract, Yeast Extract, Gelatin, Stimilac[1], Liver Fraction, Sodium Acetate or Tween 80 on the Plate Count of Lactic Streptococci

The medium described on page 34 was modified by adding singly each of the above compounds. Beef extract and yeast extract were used at a concentration of 5 g/liter; liver fraction, 1 g/liter; Stimilac, 2 g/liter; gelatin, 2 g/liter; sodium acetate, 1.5 g/liter; and Tween 80, 1 g/liter.

Six lactic cultures were plated in duplicate on each of the media. The unmodified medium served as a control. Colonies were counted after incubation at 21C for 4 days. This experiment was replicated three times.

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[1]Dried pancreas extract obtained from Marschall Dairy Laboratories, Madison, Wis.

Effect of Adding Glutamine, Asparagine or Both to the Plating Medium on the Plate Count of Lactic Streptococci

The medium described on page 34 was modified by adding singly, and in combination, 100 mg/liter of glutamine and/or asparagine. This level was used in the present study because Niven (41) reported that optimum growth was obtained in a synthetic medium when glutamine and/or asparagine were present at this concentration.

Four lactic cultures were plated in duplicate on each of the three media and on the unmodified medium. Colonies were counted after incubation at 21C for 4 days. This experiment was replicated three times.

Effect on Inosine on the Plate Count of Lactic Streptococci

An experimental medium designated as BW agar[1] was modified by the addition of inosine at concentrations of 0.1 and 1 g/liter. These concentrations had been stated by Koburger et al. (29) to stimulate acid production by lactic strepto-

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[1]Experimental medium no. 8 on page 125 of Appendix A.

cocci in milk.

Three lactic cultures were plated in duplicate on the modified medium. The unmodified medium served as a control. Colonies were counted after incubation at 21C for 4 days. This experiment was replicated three times.

In another experiment in the latter stages of this investigation three lactic cultures were held at 32C for 24 hr prior to plating. This treatment was employed after the culture had been incubated under normal conditions of temperature and time (21C for 16 hr).

A single series of dilutions was made for each of three lactic cultures. Twelve sets of duplicate plates were prepared from the appropriate dilution of each culture. Four sets were poured with BW agar containing 0.1 g of inosine/liter; four sets with BW agar containing 1 g of inosine/liter and four sets with the unmodified medium. Three sets of each series were incubated at 21, 25, 28 and 32C, respectively. Colonies were counted after incubation at 21, 25 and 28C for 4 days and after 32C for 3 days. This experiment was replicated three times.

Effect of $NaCO_3$, $NaHCO_3$ and KH_2PO_4 on the Plate Count of Lactic Streptococci

BW agar was modified by addition of carbonate and phosphate. The modifications were: (1) $NaCO_3$ was added, as a 4.0% solution, at the rate of 3.3 and 6.6 ml/100 ml. Cysteine hydrochloride, as a 5.0% solution, also was added at the rate of 0.5 and 1 ml, respectively. The higher concentration had been used by Maki and Foster (32) in their study on types of bacteria in the rumen. (2) $NaHCO_3$ was added, as a 10% solution, at concentrations of 0.1 and 0.2 g/ 100 ml. In conjunction with $NaHCO_3$, $Na_2S \cdot 9H_2O$ also was added at concentrations of 0.08 and 0.16 g/100 ml, respectively. Solutions of Na_2CO_3, $NaHCO_3$ and cysteine HCl were sterilized by Seitz filtration and added to the previously sterilized medium. (3) KH_2PO_4 was used at concentrations of 0.1 and 0.2 g/100 ml.

Three lactic cultures were plated in duplicate on the six modifications of BW agar. The unmodified medium served as a control. Colonies were counted after incubation at 21C for 4 days. This experiment was replicated three times.

Effect of Mg^{++}, Mn^{++}, Zn^{++}, Cu^{++} and Co^{++} on the Plate Count of Lactic Streptococci

The medium described on page 34 was modified by adding Mg^{++} (as $MgSO_4 \cdot 7H_2O$), 20 mg/liter; Mn^{++} (as $MnCl_2 \cdot 4H_2O$), 2 mg/liter; Zn^{++} (as $ZnSO_4 \cdot 7H_2O$), 1 mg/liter; Cu^{++} (as $CuSO_4 \cdot 5H_2O$), 0.8 mg/liter and Co^{++} (as $CoCl_2 \cdot 6H_2O$), 0.5 mg/liter. Another medium was prepared using Mg^{++} (as $MgSO_4 \cdot 7H_2O$) and Mn^{++} (as $MnCl_2 \cdot 4H_2O$) in combination, at concentrations of 30 and 3 mg/liter, respectively. Unmodified medium was used as the control.

Five lactic cultures were plated in duplicate on the three media listed above. Colonies were counted after incubation at 21C for 4 days. This experiment was replicated three times.

The Effect of Succinate, Malate, Fumarate or Citrate on the Plate Count of Lactic Streptococci

The medium described on page 34 was modified by adding singly each of the above compounds. Sodium citrate was used at concentrations of 1 and 2 g/liter. Succinate, fumarate and malate were used at concentrations of 0.1 g and 0.2 g/liter.

Four lactic cultures were plated in duplicate on the eight media listed above and on unmodified medium. Colonies

were counted after incubation at 21C for 4 days. This experiment was replicated three times.

Development and Evaluation of Experimental Media

Using information previously gained, various experimental media were prepared and evaluated. Four lactic cultures were plated in duplicate on each medium as well as Standard methods agar. Colonies were counted after incubation at 21C for 4 days. This experiment was replicated three times.

The formulae for the experimental media are found in Appendix A.

Plate Counts Obtained With Lactic Agar, TGV Agar and BW Agar

It was deemed desirable to compare the plate counts obtained with the two best laboratory media, previously described and evaluated, and those obtained with an experimental medium that was found to be satisfactory.

Three lactic cultures were plated in duplicate on each of these three media. Colonies were counted after incubation at 21C for 4 days. This experiment was replicated four times.

Effect of Holding Lactic Cultures at 4C for Two, Four and Seven Days on the Plate Count

At the end of incubation under normal conditions of temperature and time (21C for 16 hr) lactic cultures were stored at 4C. This storage treatment was selected to establish conditions in the culture that would make recovery of cells on a plating medium more difficult than from a fresh culture. Samples were removed at 2, 4 and 7 days for plating, in addition to a control sample secured for the initial count.

A single series of dilutions was made for each of eight lactic cultures. Six sets of duplicate plates were prepared from the appropriate dilution of each culture. Two sets were poured with Lactic agar, two with Eugonagar and the last two with BW agar. Three sets of duplicate plates were incubated at 21C; the other three sets at 18C. Colonies were counted after incubation at 21C for 4 days and after incubation at 18C for 6 days. This experiment was replicated four times.

Effect of Holding Lactic Cultures at 32C for 24 Hours on the Plate Count of Lactic Streptococci

To more adequately test the colony productivity of several media, lactic cultures were held at 32C for 24 hr prior to plating. This treatment was employed after the culture had been incubated under normal conditions of temperature and time (21C for 16 hr). Three separate experiments were performed.

In the first experiment, three lactic cultures were plated after being held at 32C for 24 hr. A single series of dilutions was made for each culture. Twelve sets of duplicate plates were prepared from the appropriate dilution of each culture. Four sets were poured with BW agar, four with Lactic agar and four with Eugonagar. One set of each medium was incubated at 18, 21, 25 and 28C, respectively. Colonies were counted after incubation at 21, 25 and 28C for 4 days and at 18C after 6 days. This experiment was replicated twice.

The second experiment was similar to the first except that control counts also were obtained. This experiment was replicated three times.

In the third experiment, a single series of dilutions

was made for each of eight lactic cultures. Six sets of duplicate plates were prepared from the appropriate dilution of each culture. Two sets were poured with BW agar, two with Lactic agar and two with Eugonagar. One set of each media was incubated at 18, the rest at 21C. This was repeated after the cultures had been held at 32C for 24 hr. Colonies were counted after incubation at 21C for 4 days and after incubation at 18C for 6 days. This experiment was replicated four times.

Plate Counts Obtained From Cottage Cheese With Lactic Agar, Eugonagar and BW Agar

Samples were secured from three different lots of Cottage cheese. Samples were taken immediately after the make operation was complete, just before salting and creaming. Part of a fresh sample was plated and the remainder was stored at 4C. Subsequent platings were made at 2, 4 and 7 days.

The initial dilution of cheese was made in sterile, phosphate-buffered, distilled water containing 2% sodium citrate. This dilution was blended for 2 min at high speed in a Waring Blendor. Subsequent dilutions were in sterile,

phosphate-buffered distilled water. Six sets of duplicate plates were prepared from the appropriate dilution. Two sets were poured with BW agar, two with Lactic agar and the last two with Eugonagar. Three sets of duplicate plates were incubated at 21C; the other three sets at 18C. Colonies were counted after incubation at 21C for 5 days and after incubation at 18C for 7 days.

To be sure that the colonies appearing on the media were not contaminants of a psychrophilic nature 30 colonies from plates of both fresh and stored Cottage cheese were picked into sterile, litmus milk. After incubation at 21C for about 20 hr the reaction was observed and smears made and examined.

Plate Counts Obtained From Cheddar Cheese With Lactic Agar, Eugonagar and BW Agar

Three lots of milk destined for Cheddar cheese were sampled at the end of the ripening period. Samples of curd were secured at intervals during the rest of the make operation and after the block of curd was taken from the press. Samples were secured at the following stages of process: sample of starter; end of ripening; after cooking; at time of

packing; at milling before salting; after the curd had been in the press overnight and at 3, 6 and 9 days after being removed from the press.

The initial dilution of cheese was made in sterile, phosphate-buffered distilled water containing 2% sodium citrate. This dilution was blended for 2 min at high speed in a Waring Blendor. Subsequent dilutions were in sterile phosphate-buffered distilled water. Six sets of duplicate plates were prepared from the appropriate dilution of starter, milk or curd. Two sets were poured with BW agar, two with Lactic agar and the last two with Eugonagar. Three sets of duplicate plates were incubated at 21C; the other three sets at 18C. Colonies were counted after incubation at 21C for 4 days and after incubation at 18C for 6 days.

Effect of an Agar Overlay on the Plate Counts of Lactic Streptococci

A single series of dilutions was prepared for each of ten lactic cultures. Six sets of duplicate plates were prepared from the appropriate dilution of each culture. Three sets were poured with Eugonagar and three sets with Eugonagar plus 10% filtered V-8 cocktail vegetable juice. After the

media had hardened, about 25 ml of sterile Eugonagar was added as an overlay to one set of plates containing Eugonagar, to another set containing Eugonagar plus 10% filtered V-8 cocktail vegetable juice about 25 ml of sterile Eugonagar plus 10% filtered V-8 cocktail vegetable juice was added as an overlay. One set poured with each medium received about 25 ml of a sterile 1.5% agar solution as an overlay. The remaining plates of each medium served as controls. Colonies were counted after incubation of the plates at 21C for 4 days. This experiment was replicated three times.

Effect of CO_2 on the Plate Counts of Lactic Streptococci

A single series of dilutions was prepared for each of ten lactic cultures. Four sets of duplicate plates were prepared from the appropriate dilution of each culture. Two sets were poured with Eugonagar and the remaining two sets with Eugonagar plus 10% filtered V-8 cocktail vegetable juice. One set of each medium was incubated at 21C for 4 days under normal atmospheric conditions. The other set of each medium was incubated at 21C for 4 days in an atmosphere containing

6% CO_2[1]. The experiment was repeated with levels of 10 and 14% CO_2. This experiment was replicated three times.

Effect of Plant Growth Hormones on the Growth of Lactic Streptococci

The following 15 plant growth hormones were used: o-chlorophenoxy acetic acid, p-chlorophenoxy acetic acid, 2,4 dichlorophenoxy acetic acid, ethyl-3-indole acetate, 2-furyl acrylic acid, gibberelic acid, indole-3 acetic acid, 3-indole butyric acid, 3-indole propionic acid, maleic acid hydrazide, napthalene acetic acid, a-naphthalene acetic acid, a-naphthalene acetimide, beta naphthoxy acetic acid, 2-4-5 trichlorophenoxy propionic acid and 2-4-5 trichlorophenoxy acetic acid. Aqueous solutions of each of the hormones were prepared using concentrations of 50, 100 and 200 ppm.

A single series of dilutions was prepared for each of eight lactic cultures. Triplicate plates were prepared from the appropriate dilution of each culture and poured with

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[1]Model 3560, CO_2 Incubator, National Appliance Co., Portland, Ore.

Eugonagar. A single, 1/2 in. filter paper disc[1] was saturated with a 50 ppm solution of a plant growth hormone. The disc was then placed on a plate of a lactic culture. This was repeated for the other two concentrations of this same plant growth hormone using two additional plates of the same lactic culture. This sequence was repeated for the other plant growth hormones and lactic cultures. The plates were incubated at 21C and examined at 2 days and 4 days for evidence of stimulation or inhibition of growth. This experiment was replicated twice.

Effect of Various Carbohydrates on the Growth of Lactic Streptococci

The effects of 12 carbohydrates on the growth of lactic streptococci were studied. Carbohydrates examined were: arabinose, inulin, levulose, mannose, melebiose, melezitose, raffinose, rhamnose, salicin, sorbose, starch and xylose.

A single series of dilutions was prepared for each of 12 lactic cultures. Duplicate plates were prepared from the appropriate dilution of each culture and poured with

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[1]Schleicher and Schuell No. 740-D, 12.7 mm in diameter.

Eugonagar. Redi Discs[1] were used to ascertain the effect of these carbohydrates on the growth of lactic streptococci. These were filter paper discs, 1/2 in. in diameter, impregnated with a filter-sterilized solution of a carbohydrate and then dried. A single Redi-Disc of a specific carbohydrate was placed on each of the duplicate plates of a lactic culture, and 0.1 ml of sterile, distilled water was added to each disc to dissolve the carbohydrate. This procedure was repeated for the entire series. The plates were examined at 2 and 4 days for any evidence of inhibition or stimulation of growth. This experiment was replicated three times.

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[1]Pennsylvania Biological Lab. Inc., Philadelphia, Pa.

RESULTS

Many problems are encountered when one attempts to present, in concise form data obtained during a study of this nature. The effects of different media on colony productivity are so diverse, and the counts so extreme, that it is difficult to evaluate the results without statistical interpretation.

Each experiment was analysed statistically using the multiple range test of Duncan (15). Any difference that was significant at $P < 0.05$ was considered to be real. To present the data in a brief, easily understandable form, the mean plate counts of all cultures or products used in any one experiment will be reported in this section. Complete data appear in Appendix B in which each count presented is an arithmetic average of the plate counts obtained.

In tables in this section, where it is feasible to do so, any two means which are not underscored by the same line, either horizontal or vertical are significantly different.

Plate Counts Obtained at Selected Temperatures After Different Periods of Time

The mean plate counts of seven lactic cultures obtained at four different temperatures are given in Table 4. These data indicate that there was no significant difference between plate counts obtained at 21 or 25C. Plate counts obtained at 21 or 25C were significantly higher than the counts obtained at 28 or 32C. There were no significant differences in plate counts when plates were incubated for 4, 7 or 10 days. There was a significant difference in plate counts obtained at 2 and 4 days of incubation at 21, 25 and 28C, but not at 32C.

Table 4. Mean plate counts of all cultures obtained at selected temperatures after different periods of time[a]

Incubation time, days	Plating medium Eugonagar — Incubation temperature C: 21	25	28	32	Avg.
	Mean plate count in millions/g[b]				
2	780	830	720	600	730
4	850	850	740	600	760
7	850	860	750	610	770
10	860	870	750	610	770
Avg.	840	850	740	600	

[a]Complete data in Table 30 of Appendix B.

[b]Average of three replications; cultures: FL-1, M-9, 6, 32, 41, 42 and W-2-6.

Plate Counts Obtained With Different Media

Commercial dehydrated media

The mean plate counts of 17 lactic cultures plated on ten media that have been used for the enumeration of lactic streptococci are given in Table 5. Examination of these data reveals that there were great differences in colony productivity by lactic streptococci on these media. Eugonagar and Standard Methods agar were significantly better than any

Table 5. Mean plate counts of all cultures obtained with commercial dehydrated media[a]

Plating medium									
Eugon-agar	Standard methods agar	APT agar	Penassay agar	Trypticase soy agar	L agar	Brain heart infusion agar	Tomato juice	Whey agar	Orange serum agar
Plate count in millions/g[b]									
820	690	530	540	400	400	330	68	67	54

[a]Complete data in Table 31 of Appendix B.

[b]Average of three replications; culture: FL-1, FL-3, K-11, Xi, Alpha, FC, 8, 15, 18, 32, 41, 42, M-9, M-16, 5-K, FD and W-2-6.

of the other media. No real difference existed between the plate counts obtained on APT agar or Penassay agar, Trypticase soy agar or L agar of the three media (Tomato juice agar, Whey agar and Orange serum agar) that were lowest in colony productivity. Also noted in this table is the fact that Standard methods agar gave plate counts that were ten times greater than those obtained on either Tomato juice agar or Whey agar. The difference in plate counts on Eugonagar, a medium not well known in this work, and Standard methods agar is about 17% in favor of the former medium. This difference, however, was not statistically significant.

Laboratory media

The mean plate counts of 14 lactic cultures on seven media are shown in Table 6. The variation in colony productivity on these media was not as great as on commercial dehydrated media. These data revealed that the Lactic agar of Elliker et al. (16) and TGV agar of Galesloot et al. (20) yielded plate counts that were not significantly different. These two media yielded counts that were significantly higher than the counts obtained on the other media used. The Tomato juice agar of Briggs (11), V-8 agar of Tittsler and V-8 agar of Fabian et al. (17) yielded plate counts that did

Table 6. Mean plate counts of all cultures obtained with laboratory media[a]

Plating medium						
Lactic agar Elliker et al. (16)	TGV agar Galesloot et al. (20)	Tomato juice agar Briggs (11)	Modified V-8 agar Tittsler	V-8 agar Fabian et al. (17)	Tomato juice agar Kulp and White (31)	Special Trypticase soy agar Mull (36)
Plate count in millions/g[b]						
760	710	570	550	540	500	470

[a]Complete data in Table 32 of Appendix B.

[b]Average of three replications; cultures: FL-1, FL-3, Xi, Alpha, 8, 15, 22, 23, 33, 40, M-9, FD, W-2-5 and W-2-6.

not differ significantly from each other. Another group of media among which there were no significant differences included the Tomato juice agar of Kulp and White (31), V-8 agar of Fabian et al. (17) and V-8 agar of Tittsler. Finally, a third group of media, among which no real difference in counts was apparent, included the V-8 agar of Fabian et al. (17), Tomato juice agar of Kulp and White (31) and Special Trypticase soy agar of Mull (36).

Plate Counts Obtained With Eugonagar and Eugonagar Plus Filtered V-8 Cocktail Vegetable Juice

The mean plate counts of all cultures plated on Eugonagar and Eugonagar plus filtered V-8 cocktail vegetable juice are shown in Table 7. These data clearly show the stimulatory effect of filtered V-8 cocktail vegetable juice on lactic streptococci. Further appraisal of these data shows that there was an increase of approximately 14% in the average count when either 10 or 20% filtered V-8 cocktail vegetable juice was added to Eugonagar. Counts obtained in the medium containing 30% filtered V-8 cocktail juice were significantly lower than those of the other two concentrations of filtered V-8 cocktail vegetable juice.

Table 7. Mean plate counts of all cultures obtained with Eugonagar and Eugonagar plus filtered V-8 cocktail vegetable juice[a]

Plating medium			
Eugonagar	Eugonagar plus 10% filtered V-8 cocktail vegetable juice	Eugonagar plus 20% filtered V-8 cocktail vegetable juice	Eugonagar plus 30% filtered V-8 cocktail vegetable juice
Plate count in millions/g[b]			
790	900	900	870

[a]Complete data in Table 33 of Appendix B.

[b]Average of three replications; cultures: 6, 15, 22, 23, 33, 41, FD, FL-3, M-9, Xi, W-2-5 and W-2-6.

Effect of Selected Bacteriological Peptones on the Plate Count of Lactic Streptococci

As shown in Table 8, there was no significant difference among Trypticase, Tryptone or Phytone when these peptones were used as a nitrogen source in a basal medium. These peptones gave significantly higher counts than either Peptonized milk or Casamino acids.

Table 8. Mean plate counts obtained with media containing various bacteriological peptones[a]

Bacteriological peptone				
Trypticase	Tryptone	Phytone	Casamino acids	Peptonized milk
Plate count in millions/g[b]				
600	600	580	540	520

[a]Complete data in Table 34 of Appendix B.

[b]Average of three replications; cultures: FL, M-9, 6, 33, Xi and W-2-6.

Effect of Different Levels of Trypticase in the Plating Medium on the Plate Count of Lactic Streptococci

As noted in Table 9 the highest plate counts were obtained at a concentration of 20 g/liter of Trypticase in a basal medium. A slight decrease in yield was obtained at a concentration of 25 g/liter of Trypticase and a sharp drop in counts occurred when the concentration of Trypticase was increased to 30 g/liter.

Table 9. Mean plate counts of all cultures obtained with a basal medium containing different concentrations of Trypticase[a]

Concentration of Trypticase in g/liter					
5	10	15	20	25	30
Plate count in millions/g[b]					
530	600	690	750	710	540

[a]Complete data in Table 35 of Appendix B.

[b]Average of three replications; cultures: FL-1, M-9, 6, Xi, W-2-5 and W-2-6.

Effect of Supplementing the Plating Medium With Beef Extract, Yeast Extract, Gelatin, Stimilac, Liver Fraction, Sodium Acetate or Tween 80 on the Plate Count of Lactic Streptococci

The mean plate counts reported in Table 10 show that only yeast extract and beef extract yielded counts that were significantly higher than any of the other compounds used to supplement a basal medium. Yeast extract was significantly better than beef extract at the levels tested. The addition of liver fraction, gelatin, Stimilac, sodium acetate or Tween 80 resulted in little change in count from unsupple-

mented basal medium. In addition, the medium containing liver fraction was dark brown in color, and the enumeration of colonies on this medium was difficult.

Table 10. Mean plate counts of all cultures obtained when a basal medium was supplemented with compounds that have been reported as stimulatory for lactic streptococci[a]

Supplement added/liter							
Yeast extract 5 g	Beef extract 5 g	Liver fraction 1 g	None	Gelatin 2 g	Stimilac 2 g	Sodium acetate 1.5 g	Tween 80 1 g
Plate count in millions/g[b]							
740	690	620	610	610	610	600	600

[a]Complete data in Table 36 of Appendix B.

[b]Average of three replications; cultures: FL-1, M-9, 6, Xi, W-2-5 and W-2-6.

Effect of Adding Glutamine, Asparagine or Both to the Plating Medium on the Plate Count of Lactic Streptococci

The mean plate counts that are given in Table 11 were not significantly different. Thus, glutamine and/or asparagine did not have any effect on the plate count at the level tested.

Table 11. Mean plate counts of all cultures obtained with a medium supplemented with glutamine and/or asparagine[a]

None	Supplement added/liter Glutamine 100 mg	Asparagine 100 mg	Glutamine and asparagine Each 100 mg
	Plate count in millions/g[b]		
650	630	660	620

[a]Complete data in Table 37 of Appendix B.

[b]Average of three replications; cultures: FL-1, M-9, 6 and W-2-6.

Effect of Inosine on the Plate Count of Lactic Streptococci

As shown in Table 12, the addition of inosine to BW agar did not increase significantly the plate count of fresh cultures above that obtained with unsupplemented BW agar. In a like manner, inosine exerted no stimulatory effect on cultures held at 32C for 24 hr prior to plating, regardless of the incubation temperature used.

Table 12. Mean plate counts of all cultures when BW agar was supplemented with inosine[a]

Inosine added/liter: None	0.1 g	1 g
Plate count in millions/g[b]		
740	760	750

[a]Complete data in Table 38 of Appendix B.

[b]Average of three replications; cultures: FL-1, 6 and W-2-6.

Table 13. Mean plate counts obtained of all cultures after they were held at 32C for 24 hours prior to plating on BW agar supplemented with inosine[a]

Incubation temp. C	Inosine added/liter: None	0.1 g	1 g
	Plate count in millions/g[b]		
21	19	18	18
25	15	14	14
28	10	10	9.3
32	5.5	5.1	5.0

[a]Complete data in Table 39 of Appendix B.

[b]Average of three replications; cultures: FL-1, 6 and W-2-6.

Effect of $NaCO_3$, $NaHCO_3$ and KH_2PO_4 on the Plate Count of Lactic Streptococci

The mean plate counts shown in Table 14 were not significantly different, whether BW agar was unsupplemented or was supplemented with the indicated concentrations of Na_2CO_3 and cysteine HCl, $NaHCO_3$ and Na_2S or KH_2PO_4.

Effect of Mg^{++}, Mn^{++}, Zn^{++}, Cu^{++} and Co^{++} on the Plate Count of Lactic Streptococci

As shown in Table 15, no significant difference was obtained with the combinations and levels of cations added to a basal medium.

Table 14. Mean plate counts obtained when BW agar was modified by the addition of carbonate and phosphate[a]

Supplement added/liter						
None	Na_2CO_3, 1.32 g; Cysteine HCl, 0.025 g	Na_2CO_3, 2.64 g; Cysteine HCl, 0.05 g	$NaHCO_3$, 1 g; $Na_2S \cdot 9H_2O$, 0.8 g	$NaHCO_3$, 2 g; $Na_2S \cdot 9H_2O$, 1.6 g	KH_2PO_4, 1 g	KH_2PO_4, 2 g
Plate count in millions/g[b]						
750	710	700	730	770	730	710

[a]Complete data in Table 40 of Appendix B.

[b]Average of three replications; cultures: FL-1, 6 and W-2-6.

Table 15. Mean plate counts of all cultures obtained when a basal medium was supplemented with selected cations[a]

None	Mg^{++}, 30 mg Mn^{++}, 3 mg	Mg^{++}, 20 mg; Mn^{++}, 2 mg; Zn^{++}, 1 mg; Cu^{++}, 0.8 mg; and Co^{++}, 0.5 mg
	Concentration of cations added/liter	
	Plate count in millions/g[b]	
630	610	620

[a]Complete in Table 41 of Appendix B.

[b]Average of three replications; cultures: FL-1, Xi, M-9, 6 and W-2-6.

Effect of Succinate, Malate, Fumarate and Citrate on the Plate Count of Lactic Streptococci

As shown in Table 16, no significant difference was obtained when selected organic acids were added individually, at the concentrations indicated to a basal medium.

Table 16. Mean plate counts obtained when a basal medium was supplemented with selected organic acids[a]

None	Citrate		Fumarate		Malate		Succinate	
	1 g	2 g	0.1 g	0.2 g	0.1 g	0.2 g	0.1 g	0.2 g
	Organic acid added/liter; Plate count in millions/g[b]							
620	610	580	610	610	570	580	620	610

[a]Complete data in Table 42 of Appendix B.

[b]Average of three replications.

Development and Evaluation of Experimental Media[1]

As shown in Table 17, significantly higher counts were obtained with media no. 1, 4, 5, 6, 7, 8, 10, 11 and 12 when compared with media 2, 3, 9 and Standard methods agar. Medium no. 8 was used in subsequent studies in which an experimental medium was used. It was designated as BW agar.

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[1]Composition of these media is given in Appendix A.

Table 17. Mean plate counts of all cultures obtained on experimental media and Standard methods agar[a]

Plating medium												
11	8	10	7	5	12	6	4	1	2	9	Standard methods agar	3
Plate count in millions/g[b]												
910	900	890	890	850	840	840	830	820	730	690	690	590

[a]Complete data in Table 43 of Appendix B.

[b]Average of three replications; cultures: FL-1, M-9, 6 and W-2-6.

Plate Counts Obtained With Lactic Agar, TGV Agar and BW Agar

No significant difference in plate counts was obtained among the three media as is shown in Table 18. More time and care are necessary, however, in counting Lactic agar or TGV agar plates than BW agar plates because of the brown, hazy condition of the first two media. It is easy to overlook small colonies on these media unless great care is exercised.

Table 18. Mean plate counts of all cultures obtained on Lactic agar, TGV agar and BW agar[a]

Plating medium		
Lactic agar	TGV agar	BW agar
Plate count in millions/g[b]		
640	620	660

[a]Complete data in Table 44 of Appendix B.

[b]Average of three replications; cultures: FL-1, M-9 and W-2-6.

Effect of Holding Lactic Streptococci at 32C for 24 Hours on the Plate Counts Obtained

If lactic cultures are injured by holding them at 32C for 24 hr, great differences in colony productivity are noted between Eugonagar and both BW agar and Lactic agar. These data are presented in Tables 19, 20 and 21. In Table 19, only counts after injury are presented, and in Table 20, counts of both fresh and injured cells are given for three cultures. In Tables 19 and 20 the differences between Eugonagar and both BW agar and Lactic agar are somewhat magnified because two out of three of the test cultures (6 and W-2-6) were particularly susceptible to this type of treatment. In

Table 21, the data obtained using eight cultures are presented. Although the average counts of the injured cultures were not as low as before, the difference is great between Eugonagar and both BW agar and Lactic agars. The average count obtained on Eugonagar was about 3.5 times as great as with BW agar and about six times as great as with Lactic agar.

Figure 1 illustrates the size and number of colonies produced on Eugonagar, BW agar and Lactic agar by fresh culture P-1. It is apparent that the colonies are larger as well as more numerous on Eugonagar and BW agar than on Lactic agar. Another factor of considerable interest is the clarity of Eugonagar and BW agar, compared with Lactic agar. This clarity of medium greatly facilitates the counting of colonies, especially the small ones. Eugonagar is somewhat better in this respect than BW agar.

Table 19. Mean plate counts obtained after holding cultures at 32C for 24 hours[a]

Incubation temp. C	Eugonagar	BW agar	Lactic agar
	Plating medium		
	Plate count in millions/g[b]		
18	84	7.0	4.3
21	76	5.9	4.1
25	52	3.5	2.9
28	43	2.3	2.1

[a]Complete data in Table 45 of Appendix B.

[b]Average of two replications; cultures: FL-1, 6 and W-2-6.

Table 20. Mean plate counts obtained from fresh cultures and after holding at 32C for 24 hours[a]

	Plating medium					
	Eugonagar		BW agar		Lactic agar	
	Treatment of cultures					
Incubation temp. C	Fresh[b]	Injured[c]	Fresh	Injured	Fresh	Injured
	Plate count in millions/g[d]					
18	650	66	670	5.4	650	4.0
21	700	55	760	4.2	660	3.6
25	650	32	600	2.8	590	2.6
28	590	17	490	2.0	440	1.5

[a]Complete data in Table 46 of Appendix B.

[b]Fresh counts not significantly different.

[c]Injured counts obtained with BW agar and Lactic agar were not significantly different but counts from both were significantly lower than counts on Eugonagar.

[d]Average of three replications; cultures: FL-1, 6 and W-2-6.

Table 21. Mean plate counts obtained from fresh cultures and after holding at 32C for 24 hours[a]

Plating medium					
Eugonagar		BW agar		Lactic agar	
Fresh[b]	Injured[c]	Fresh	Injured	Fresh	Injured
Plate count in millions/g[d]					
910	130	930	35	780	22

[a]Complete data in Table 47 of Appendix B.

[b]Fresh counts obtained on Eugonagar and BW agar were not significantly different but counts obtained on both Eugonagar and BW agar were significantly higher than on Lactic agar.

[c]Injured counts significantly different on all media.

[d]Average of four replications at 18 and 21C; culture: FL-1, 6, W-2-6, C-11, C-26, H-21, P-1 and P-1-A.

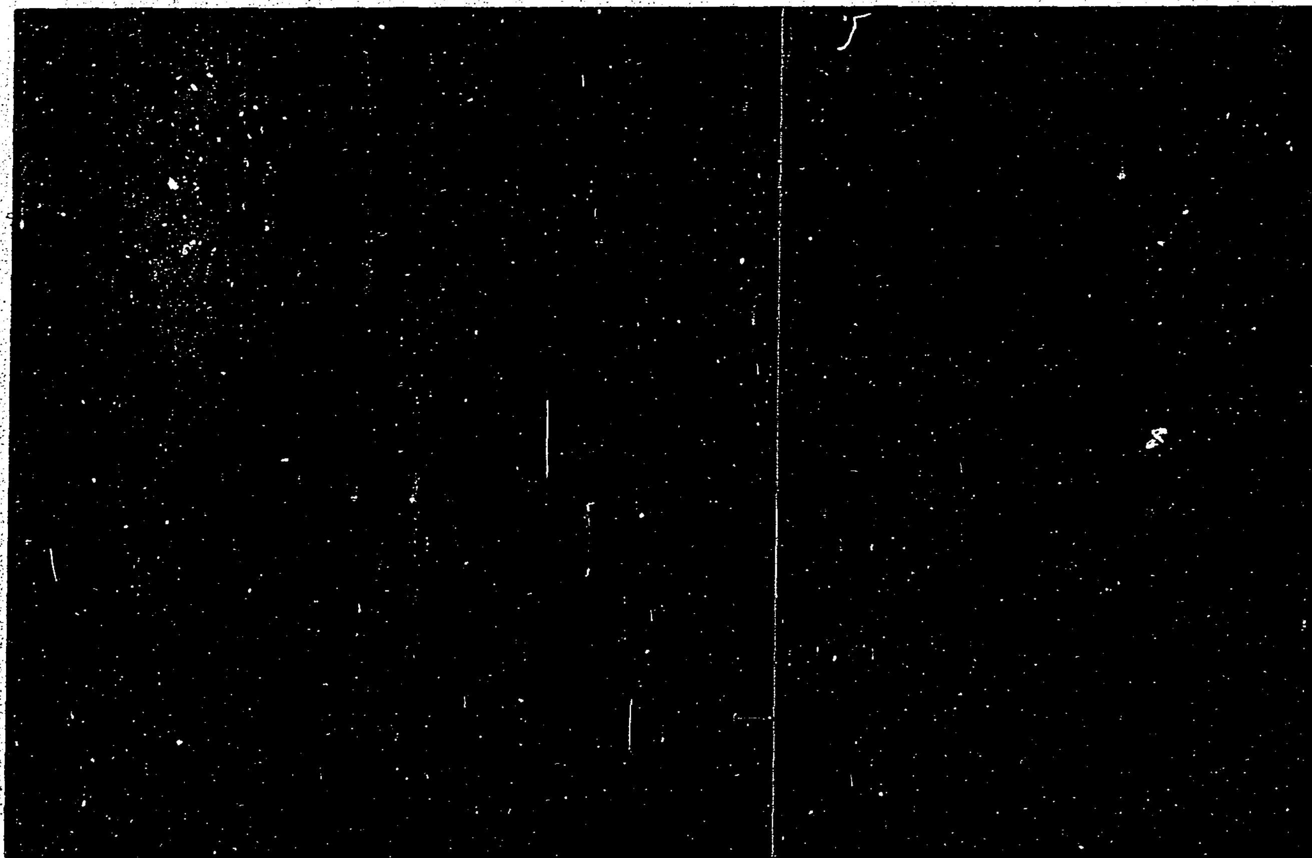

Figure 1. Comparison of the number and size of colonies of fresh culture P-1 when plated on Lactic agar (upper plate), Eugonagar (plate at lower right) and BW agar (plate at lower left)

Effect of Holding Lactic Cultures at 4C for Two, Four and Seven Days on the Plate Count Obtained

The mean plate counts given in Table 22 show that there is a significant difference between bacterial recovery on Lactic agar and either Eugonagar or BW agar regardless of the age of the test culture. No significant difference was found between Eugonagar and BW agar. Counts obtained on some cultures, however, were virtually the same when fresh samples were used as when the culture had been held for 2 days before plating (see Table 48 of Appendix B). This was true of all media. Evidently, some cultures could tolerate storage in an acid medium more than other cultures or perhaps these cultures produced less acid.

Table 22. Mean plate counts obtained after holding lactic cultures at 4C for two, four and seven days[a]

Time of sampling	Eugonagar	BW agar	Lactic agar
	Plating medium		
	Plate count in millions/g[b]		
Fresh	970	990	880
2 days	860	870	760
4 days	670	610	560
7 days	540	480	380

[a]Complete data in Table 48 of Appendix B.

[b]Average of three replications at 18 and 21C; cultures: FL-1, 6, W-2-6, C-11, C-26, H-21, P-1 and P-1-A.

Plate Counts Obtained From Cottage Cheese With Lactic Agar, Eugonagar and BW Agar

The data presented in Table 23 show that the mean plate counts obtained on either Eugonagar or BW agar were twice as great as those obtained on Lactic agar. This was true of the fresh curd, as well as curd held at 4C and sampled after 2, 4 and 7 days of storage.

The colonies that were picked into litmus milk were tentatively identified as lactic streptococci by the reaction

Table 23. Mean plate counts obtained from Cottage cheese[a]

Eugonagar	Plating medium BW agar Plate counts/g[b]	Lactic agar
3600	3400	1600

[a]Complete data in Table 49 of Appendix B.

[b]Average of three replications of fresh, 2-, 4- and 7-day old curd at plate incubation temperature of 18 and 21C.

in litmus milk and microscopic examination of the smears.

Figure 2 illustrates the comparative number and size of colonies obtained when Cottage cheese is plated on Lactic agar, Eugonagar and BW agar. All three plates in Figure 2 were made from the same dilution of the same sample. As can be noted in the figure, productivity on the latter two media is greater than on the former medium. Furthermore, the colonies on Lactic agar are small and difficult to discern against the hazy, brown background of this medium. On Eugonagar and BW agar, the colonies are larger and even the small colonies are readily observed against the clear background of these media.

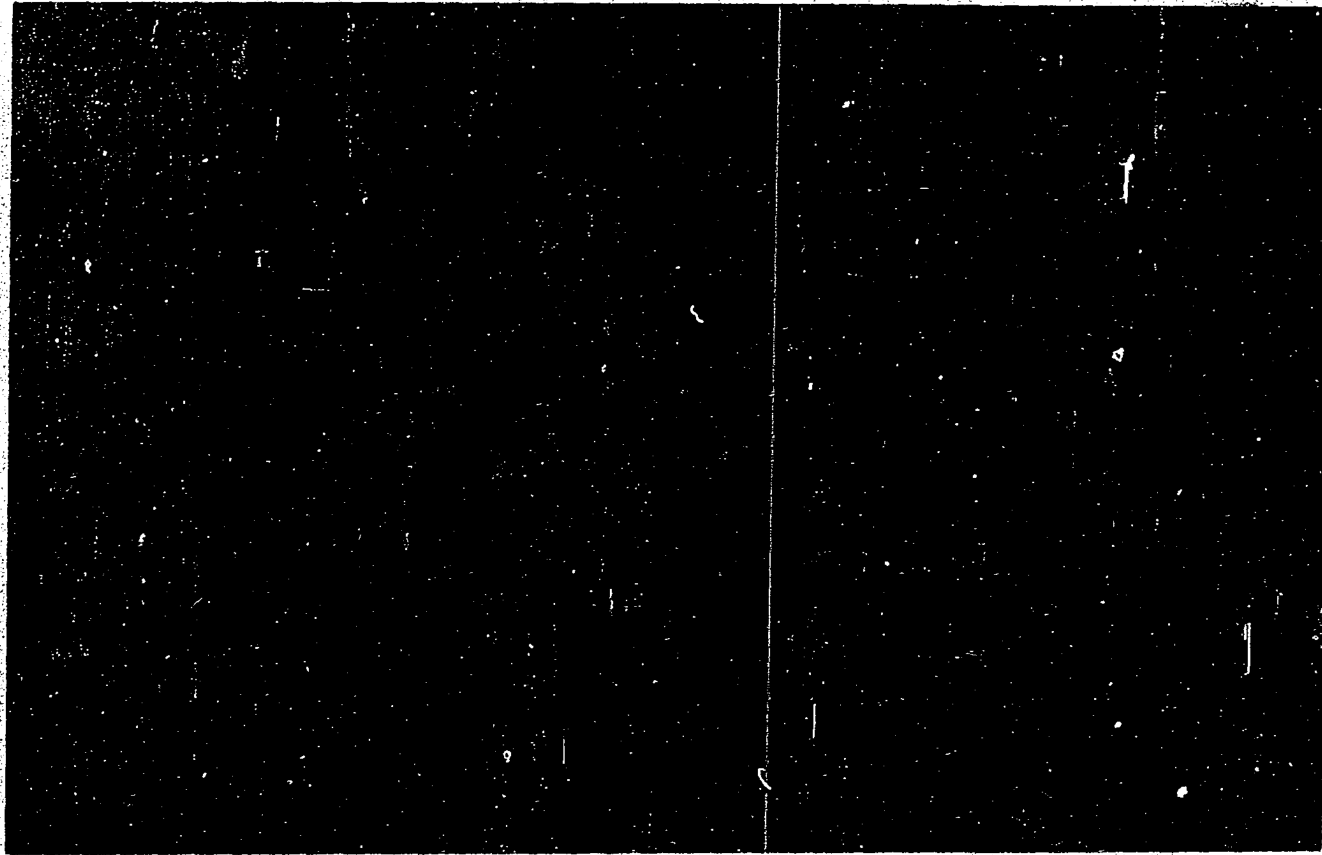

Figure 2. Comparison of the number and size of colonies of Cottage cheese when plated on Lactic agar (upper plate), Eugonagar (plate at lower right) and BW agar (plate at lower left)

Plate Counts Obtained From Cheddar Cheese With Lactic Agar, Eugonagar and BW Agar

Plate counts obtained at different stages of processing Cheddar cheese are presented in Table 24. Statistical evaluation of these data revealed that the plate counts obtained with Eugonagar and BW agar were significantly higher than those from Lactic agar. Plate counts obtained on Eugonagar and BW agar, however, were not significantly different.

Although not directly a part of this investigation, an interesting observation was made concerning the effect of cooking temperature on subsequent plate counts. Cooking temperatures of vats one, two and three were 39.2, 39.7 and 40C, respectively. The plate counts obtained from the curd in vat one at milling time were about twice those from vat two and about 1.5 times as great as those from vat three. These counts are presented in Tables 25, 26 and 27. After the Cheddar curd had been in the press overnight, the counts obtained with the curd from vat one were about three times those from vat two and about twice those from vat three. Final counts obtained with Cheddar curd after 9 days were higher from vat one than from either vat two or three.

Figure 3 shows the colony productivity of Lactic agar, Eugonagar and BW agar when used in plating Cheddar curd at milling time. Although the differences in counts are not great, the ease of identifying the colonies on either Eugonagar or BW agar make these media more desirable for routine use.

Table 24. Mean plate counts obtained from starter, milk and Cheddar curd[a]

Stage of process	Plating medium Eugonagar	 BW agar	 Lactic agar
	Plate count in millions/g[b]		
Starter	1100	1100	930
End of ripening	57	47	44
After cooking	720	680	560
Curd packed	480	470	400
Milling	510	490	440
Press (overnight)	86	79	76
3 days after press	57	52	41
6 days after press	38	34	28
9 days after press	32	29	24

[a]Complete data in Tables 25, 26 and 27.

[b]Average of three replications at plating temperatures of 18 and 21C.

Table 25. Plate counts obtained from starter, milk and Cheddar curd, vat one

Stage of process	Incubation temp. C	Eugonagar	BW agar	Lactic agar
		Plating medium		
		Plate count in millions/g[a]		
Starter	18	1200	1200	1000
	21	1200	1200	890
End of ripening	18	56	38	38
	21	64	38	38
After cooking (temp. 39.2C)	18	900	760	700
	21	840	790	640
Curd packed	18	540	560	430
	21	500	450	420
Milling	18	710	780	600
	21	710	690	640
Press (over-night)	18	160	140	140
	21	170	150	150
3 days after press	18	94	88	75
	21	100	97	70
6 days after press	18	89	86	64
	21	96	81	64
9 days after press	18	50	58	49
	21	61	51	49

[a]Average of duplicate plates.

Table 26. Plate counts obtained from starter, milk and Cheddar curd, vat two

Stage of process	Incubation temp. C	Eugonagar	BW agar	Lactic agar
		Plate count in millions/g[a]		
Starter	18	1100	1100	960
	21	1300	1100	960
End of ripening	18	60	60	56
	21	71	70	54
After cooking (temp. 39.7C)	18	660	680	520
	21	710	700	480
Curd packed	18	430	440	360
	21	440	420	340
Milling	18	340	370	350
	21	400	350	300
Press (overnight)	18	60	56	58
	21	54	45	50
3 days after press	18	38	31	24
	21	34	32	22
6 days after press	18	16	17	13
	21	14	16	13
9 days after press	18	15	12	10
	21	17	15	11

[a]Average of duplicate plates.

Table 27. Plate counts obtained from starter, milk and Cheddar curd, vat three

Stage of process	Incubation temp. C	Eugonagar	BW agar	Lactic agar
		Plate count in millions/g[a]		
Starter	18	940	900	930
	21	1100	920	840
End of ripening	18	48	40	44
	21	46	42	42
After cooking (temp. 40C)	18	620	580	480
	21	640	570	540
Curd packed	18	480	500	420
	21	520	480	440
Milling	18	460	420	400
	21	550	460	460
Press (over night)	18	69	70	54
	21	66	62	62
3 days after press	18	50	45	44
	21	57	53	40
6 days after press	18	38	47	26
	21	42	39	27
9 days after press	18	39	34	28
	21	40	34	25

[a]Average of duplicate plates.

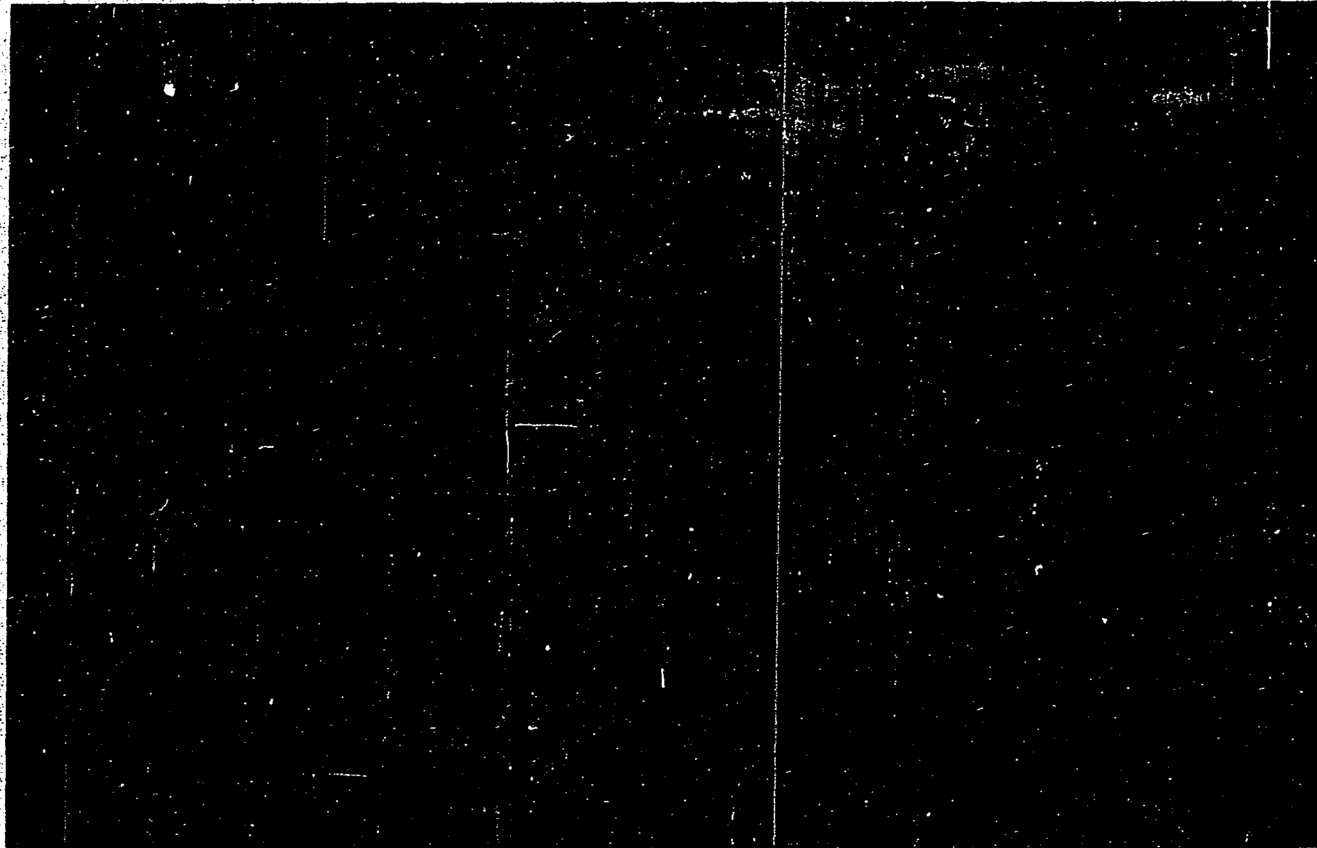

Figure 3. Comparison of the number and size of colonies of Cheddar curd at milling when plated on Lactic agar (upper plate), Eugonagar (plate at lower right) and BW agar (plate at lower left)

Effect of an Agar Overlay on the Plate Counts of Lactic Streptococci

The mean plate counts presented in Table 28 were not significantly different. Thus an agar overlay, used in combination with Eugonagar and Eugonagar plus 10% filtered V-8 cocktail vegetable juice did not affect the plate counts obtained. As has been shown before, there were significant differences between counts obtained on unsupplemented Eugonagar and counts obtained on Eugonagar plus 10% filtered V-8 cocktail vegetable juice.

Table 28. Mean plate counts of all cultures obtained with and without an agar overlay[a]

Plating medium					
Eugonagar			Eugonagar plus 10% filtered V-8 cocktail vegetable juice		
without overlay	with overlay of Eugonagar	with overlay of Plain agar	without overlay	with overlay of Eugonagar plus V-8 juice	with overlay of Plain agar
Plate count in millions/g[b]					
780	770	760	880	870	880

[a]Complete data in Table 50 of Appendix B.

[b]Average of three replications; cultures: FL-1, Alpha, Xi, M-9, 6, 32, 41, FD, W-2-5 and W-2-6.

Effect of CO_2 on the Plate Counts of Lactic Streptococci

As shown in Table 29, no significant increase in plate count was obtained by incubation of the plates in an atmosphere containing 6, 10 or 14% added CO_2. As would be expected from results reported previously, there were significant differences between counts obtained on unsupplemented Eugonagar and counts obtained on Eugonagar plus 10% filtered V-8 cocktail vegetable juice.

Table 29. Mean plate counts of all cultures obtained with and without added CO_2[a]

Plating medium							
Eugonagar				Eugonagar plus 10% filtered V-8 cocktail vegetable juice			
Normal atmosphere	with 6% CO_2	with 10% CO_2	with 14% CO_2	Normal atmosphere	with 6% CO_2	with 10% CO_2	with 14% CO_2
Plate count in millions/g[b]							
790	780	800	770	870	850	880	840

[a]Complete data in Tables 51, 52 and 53 of Appendix B.

[b]Average of three replications at each CO_2 concentration; cultures: FL-1, Alpha, Xi, M-9, M-16, 6, 23, 33, W-2-5 and W-2-6.

Effect of Plant Growth Hormones on Lactic Streptococci

None of the 15 plant growth hormones gave any indication of being either stimulatory or inhibitory for any of the 12 lactic cultures tested under the conditions of this investigation.

Effect of Various Carbohydrates on the Growth of Lactic Streptococci

Under the conditions of this investigation, none of the 12 carbohydrates tested gave any indication of being either stimulatory or inhibitory for the 12 lactic cultures used.

DISCUSSION

Many different media have been used for the isolation and enumeration of lactic streptococci. These media have been devised for various purposes. Some media, for example, were developed for the examination of dairy products, others for the microbiology of silage and still others for determining the nutritional requirements of the lactic streptococci. Frequently, media devised for one purpose have been used for other purposes. Thus, many different media have been used, at one time or the other, for the isolation and enumeration of lactic streptococci in dairy products. There is, however, lack of agreement as to which of the media now available is the better for this purpose. In addition, it would appear that there is still room for the development of even better media.

A plating medium used for the enumeration of lactic streptococci in starters or cultured dairy products should have as its primary objective high colony productivity. A second objective of this medium should be the production of easily discernible colonies. A possible source of error is that minute or "pin point" colonies might be overlooked, leading to erroneously low colony counts. Finally a plating

medium should, if at all possible, be simple, easy to prepare and use and yet yield consistently high recoveries of the lactic streptococci. A medium made of many separate ingredients is usually both time consuming and expensive to prepare.

In a well known dairy microbiology text, Foster et al. state that:

> It (S. lactis) is easily isolated from sour milk by plating appropriate dilutions on Trypticase soy agar, Orange serum agar, or Yeast extract agar containing a suitable carbohydrate. Tomato juice agar or Whey agar may be used. After incubation at 25 to 30C small, gray, circular, convex, glistening colonies may be transferred to litmus milk for further study (19, p. 13).

Do these statements mean that the choice of a medium or temperature of incubation for plate counts of lactic streptococci is of no importance? The implication is certainly there, although it might be argued, and rightly so, that isolation and enumeration have little relationship. The results obtained in this investigation indicate that the choice of a medium and incubation temperature are of paramount importance if maximum plate counts are to be obtained.

The results of this investigation indicate that a plate incubation temperature exceeding 25C will invariably result in lower plate counts. This is in contrast to an optimum growth temperature of 30C, as stated by Foster et al. (19).

Growth comparisons between a solid medium and milk are at the best difficult. It is fully appreciated that lactic cultures are normally grown at 21C and then transferred, if Cheddar cheese is being made, to a vat of milk at 32C. At this temperature the lactic streptococci grow rapidly. If plate counts were made of this same culture and the plates incubated at 32C there undoubtedly would be less colony productivity as compared to an incubation temperature of 21C. This certainly cannot be attributed to any form of metabolic shock, but must rather be attributed to the environment under which the lactic streptococci are expected to grow. This environment must then be a total of all the interrelated factors that are inherent in a plating medium.

Probably the most commonly used medium for the enumeration of lactic streptococci is Standard methods agar. The popularity of this medium can be attributed to its availability in many laboratories, rather than its being a superior medium. The results of the present investigation indicate that colony productivity of most lactic streptococci on Standard methods agar was low. These observations are in accord with some recent work of Johns[1]. He reported that some

[1]Johns, C. K. Canada Dept. of Agr., Ottumwa, Canada. Information concerning enumeration of lactic streptococci. Private communication. 1962.

strains of lactic streptococci failed to grow on Standard methods agar.

Eugonagar, at least according to published reports, has not been widely used for the enumeration of lactic streptococci. It was found to be the better of the commercial dehydrated media. Eugonagar certainly is not unique as far as medium components go. It contains, as the nitrogen source, a combination of Trypticase, 15 g/liter, and Phytone, 5 g/liter, making a total of 20 g of peptone/liter. The results of this investigation indicated that the optimum concentration for Trypticase was 20 g/liter. This amount of peptone is in accord with the work of Barkworth and Davis (7). They reported that colony productivity was low and colonies were very small when the plating medium contained less than 0.2% peptone. Other commercial dehydrated media also contain 20 g of peptone/liter. Tomato juice agar, Difco, contains a combination of Peptone, 10 g/liter and Peptonized milk, 10 g/liter. Peptone was shown by Hunter (22) to be unsuited for the growth of lactic streptococci. In this study, Peptonized milk gave the poorest colony productivity of the several peptones tested. L agar contains 20 g of Lactalysate/liter. This medium was devised by Vera

(58) for the express purpose of cultivation of Lactobacillus spp. She ascribes the growth promoting characteristics of this medium for Lactobacillus spp. due to the incorporation of Lactalysate, a hydrolysate of lactalbumin prepared by pancreatic digestion.

Cystine in Eugonagar should not have any influence on colony productivity. This amino acid has been shown not to be essential for the growth of lactic streptococci by Anderson and Elliker (2) and Reiter and Oram (45).

It appears that the peptone or peptones used in a medium greatly influence the colony productivity of that particular medium for the lactic streptococci.

The other commercial dehydrated media examined (Penassay agar, APT agar, L agar, Trypticase soy agar, Brain heart infusion agar, Tomato juice agar, Whey agar and Orange serum agar) can be classed as being unsuitable for the enumeration of lactic streptococci, if maximum plate counts are desired.

Of the laboratory media examined, the Lactic agar of Elliker et al. (16) and TGV agar of Galesloot et al. (20) gave similar results although differing considerably in composition. Both of these media were developed in an effort to afford better enumeration of the lactic streptococci. Although these two media gave good colony productivity, the

colonies were not easy to discern. This was true because of the hazy, brown appearance of the medium. One must be careful in counting colonies on these two media because small colonies are easily overlooked.

The four media that incorporate either filtered tomato juice or V-8 cocktail vegetable juice in their formulae were developed expressly for the enumeration of lactobacilli. As this investigation showed, plate counts obtained on these media were significantly lower than those obtained with Lactic agar of Elliker et al. (16) or TGV agar of Galesloot et al. (20). Their use for the enumeration of lactic streptococci cannot be recommended.

Citrate, which is incorporated in the Special trypticase soy agar of Mull (36), might prove inhibitory for strains of <u>S</u>. <u>cremoris</u>. Kizer and Speck (27) found that a concentration as low as 1 g/liter of citrate in their medium, a synthetic one, was definitely inhibitory for several strains of <u>S</u>. <u>cremoris</u>. Perhaps this medium has some selective action.

Stimulation is defined by Webster as:

> 1. Act of stimulating, or state of being stimulated; quickened activity. 2. Physiol. The irritating action of various stimuli on muscles, nerves, etc. by which activity is caused (60).

A bacteriologist might define stimulation as an increase in

metabolic activity brought about by the addition of some compound to a medium. Stimulation also might be defined as an increase in spore germination. There are many other examples that might be given.

Many compounds have been reported as being stimulatory for the lactic streptococci. Most of these compounds have been judged on their ability to either increase the rate of lactic acid production or the total amount of lactic acid produced. The test medium has usually been chemically defined, occasionally milk was used. To transpose these findings to a plating medium and hope for the same reaction is, perhaps, wishful thinking.

The addition of asparagine or glutamine to a test medium was found to have no effect on colony productivity. This is not surprising as the 15 g/liter of Trypticase in this medium would supply about 75 mg of aspartic acid/100 ml and about 250 mg of glutamic acid/100 ml. Niven (41) stated that 100 mg/100 ml of asparagine and/or glutamine were required in a synthetic medium. Pollock and Lindner (43) indicated that aspartic and glutamic acids could replace the amide forms. This indicates that the lactic streptococci can utilize these compounds interchangeably. It is evident that nothing is to

be gained by adding asparagine or glutamine to a medium already containing adequate amounts of aspartic and glutamic acids.

Tween 80, a compound reported to be stimulatory for the lactic streptococci had no effect when included in the basal medium. Reiter and Oram (45) reported that if possible stimulatory effects of Tween 80 were to be ascertained, biotin must not be present even in minute amounts. This was true as Tween 80 or biotin could be used interchangeably in a synthetic medium. The 15 g/liter of Trypticase used in the present basal medium supplied about 1.2 mcg/liter of biotin. Perhaps this amount of biotin is sufficient to supply the requirement for the lactic streptococci. If this was so, then the addition of Tween 80 would have little effect upon the lactic streptococci. Also many lactic streptococci have been shown by Anderson and Elliker (2) to be unaffected by Tween 80, even in a synthetic medium.

Stimilac, a dried extract of pancreas tissue, and inosine, extracted and purified from pancreas tissue, have both been shown to increase acid production by lactic streptococci when these materials are added to milk. This increased acid production might be strictly an increase in enzyme activity. This would have no effect on the colony productivity of a medium. Neither Stimilac or inosine gave any evidence of

increasing the colony productivity of a medium.

Liver fraction was used by Anderson and Elliker (2) to determine the maximum growth of lactic streptococci, measured as titratable acidity, that could be obtained in less than 24 hr. They indicated that the stimulatory effects of liver fraction suggests that peptides or peptide-like substances play an important part in the nutrition of lactic streptococci. Anderson and Elliker (2) indicated that the stimulatory factor in liver extract might be due to Sprince and Woolley's (51) strepogenin. Sprince and Woolley (51) have shown that some factor in casein stimulates the growth of _Lactobacillus casii_; and this factor is probably a peptide. This substance was called strepogenin. As Trypticase is a pancreatic digest of casein, it appears probable that this stimulatory factor might already be present in a medium containing Trypticase. Thus, it appears that liver fraction, if added to a medium containing Trypticase, offers little chance of being stimulatory.

The addition of gelatin, for its high glycine content, to a plating medium was reported by Elliker et al. (16) to favor the development of larger colonies. In this investigation, the addition of gelatin did not increase colony size or productivity of a basal medium. As this investigation progressed, it was apparent that colony size was just as large

on Eugonagar, which does not contain gelatin, as on BW agar, which contains gelatin. Both of these media yielded colonies that averaged somewhat larger than those obtained on Lactic agar, which contains gelatin. The comparisons in size were purely visual as no attempt was made to measure colony size. Actually, the amount of glycine supplied by Trypticase in the basal medium, 15 g/liter, is greater than the amount used by Reiter and Oram (45) in a synthetic medium. It is quite likely that there was an ample supply of glycine in the media tested, so that the addition of gelatin was unnecessary.

The stimulatory effects of yeast extract and beef extract, when added to plating media, are well known. Peptones contain small amounts of the vitamins generally considered to be essential for the lactic streptococci. Yeast extract will supply biotin, niacin, riboflavin and pantothenic acid, all of which have been reported (2, 41, 45) as essential for the lactic streptococci. In this study definite stimulation occurred when either yeast extract or beef extract was added to a basal medium containing 15 g of Trypticase/liter. In the many comparisons made between Eugonagar, which does not contain yeast extract and BW agar, which contained yeast extract, no significant differences were noted in the plate counts obtained. It appears that the amounts of

peptones, 20 g/liter, used in these two media supplied sufficient amounts of the necessary vitamins so that the yeast extract in BW agar had no influence on colony productivity.

Filtered tomato juice or V-8 cocktail vegetable juice have been used by many investigators (11, 17, 20, 31) in an effort to increase colony productivity of various media. Greater colony productivity, in this study, was obtained when filtered V-8 cocktail vegetable juice was added to Eugonagar. V-8 cocktail vegetable juice could, from lot to lot or year to year, conceivably vary in growth promoting factors. This variation might be due to variety of vegetable, area of the country in which grown, effects of processing and pH of the finished product. How great the possible influence these potential variables might have on the growth promoting ability of vegetable juices is not known. Due to these possible variations, it might be well not to depend on V-8 cocktail vegetable juice for its stimulatory action.

No specific requirements or possible stimulatory action has been reported for Mg^{++}, Mn^{++}, Zn^{++}, Cu^{++} or Co^{++}. Three investigators (41, 45, 48) specified certain amounts of these cations in their synthetic media. The amounts used varied as follows: Mg^{++} 8 to 24 mg/liter; Mn^{++} 0.5 to 2.5 mg/

liter and Fe^{++} 0.8 to 2 mg/liter. Zu^{++}, Cu^{++} and Co^{++} were used in only one synthetic medium. These cations, with the exception of Co^{++}, are found in trace amounts in many peptones. Perhaps they are present in sufficient quantities to meet the requirements, if any, of the lactic streptococci. As no significant difference was obtained after adding these cations to a basal medium, it is assumed that the preceding statement is correct.

Only citrate, of the organic acids used in this investigation, had been reported on concerning its possible effect on lactic streptococci. Kizer and Speck (27) reported that citrate was inhibitory for *S. cremoris* but not for *S. lactis*. A synthetic medium was used in their study. In this study there were no significant differences in colony counts, compared with a basal medium, when any one of the organic acids was incorporated into the basal medium. If a synthetic medium and different amounts of the organic acids had been used, differences might have been detected.

As the lactic streptococci are facultative anaerobes, it was thought that an increase in the CO_2 concentration might favor their growth. This was not the case, as no significant differences were found when the plates were incubated under increased CO_2 concentration. Normal atmosphere contains

about 78% N_2, 21% O_2 and 0.03% CO_2. If the concentration of CO_2 is raised to 6% this constitutes a 200-fold increase or at the highest concentration of CO_2 used (14%) there would be almost a 500-fold increase. This is much in contrast to the decrease in O_2 content. At a concentration of 14% CO_2 the amount of O_2 would drop by about 14%. Apparently even great increases in CO_2 concentration are not going to effect the colony productivity of the lactic streptococci on the media tested.

An agar overlay has been used in plating methods for the enumeration of lactobacilli. Its use will, in many instances, result in higher colony productivity of a particular medium. In this investigation no significant difference was obtained between plates poured with or without an agar overlay. One possible effect of an agar overlay, though subtle it may be, is the possible retardment of the diffusion of O_2 into the base layer. This effect might only last for several hours but this is all that is needed for growth initiation or repair of cellular damage before conditions become less favorable.

Plant growth hormones used in this study are known to cause rapid growth of many of the higher plants. A chance existed that they might be stimulatory for the lactic strep-

tococci. This did not prove to be so, as none of the 15 plant growth hormones had any effect, either stimulatory or inhibitory. Results might have been different had a synthetic medium been used, different concentrations of plant growth hormones tried or even if combinations of the growth hormones had been tried. Biological specificity might be important here. Even though bacteria are classed as plants, they might not be stimulated by factors that affect the growth of higher plants.

Under the conditions of this investigation none of the 12 carbohydrates used gave any evidence of being stimulatory or inhibitory to lactic streptococci. If the test medium had not contained any carbohydrate, stimulation might have resulted. Various combinations of these carbohydrates in a suitable medium might have given different results.

One of the most interesting aspects of this investigation was the series of experiments concerned with comparative colony productivity of Eugonagar, BW agar and Lactic agar after the culture had undergone injury. Injury was affected by holding cultures at 4C for several days or holding cultures at 32C for 24 hr. This exposure to toxic agents of cell metabolism, primarily lactic acid in this case, could conceivably make the cells more demanding in their growth

requirements on a plating medium. In the manufacture of Cheddar cheese the culture is subjected to rapidly changing environmental conditions. These include: a change from a liquid (milk) to a solid menstruum (curd) with subsequent changes in moisture conditions, exposure to a cooking temperature of approximately 39C, increase in acidity and the addition of salt at milling. All are factors that either singly or in combination might cause the cells to become more fastidious in their growth requirements on a plating medium. A similar situation exists in the manufacture of Cottage cheese. Here the temperature stress that a culture is subjected to is much greater than in Cheddar cheese manufacturing. A cooking temperature of approximately 53C, together with a low pH, are most conducive to cell injury. Mattick and Nichols (33) and Watkins and Wilson (59) reported that as the temperature increased and the pH decreased the number of bacteria surviving decreased. The stress that a culture undergoes in the manufacture of Cottage cheese is much greater as compared to Cheddar cheese manufacturing, of holding cultures several days at 4C or holding cultures at 32C for 24 hr. It seems quite possible that the surviving cells in Cottage cheese would be even more demanding in their growth requirements on a plating medium.

The average plate counts of eight injured cultures (32C for 24 hr) obtained on Eugonagar, BW agar and Lactic agar varied widely. The plate counts obtained on Eugonagar were about four times greater than those on BW agar and about six times greater than those on Lactic agar. The average plate counts of Cottage cheese show that both Eugonagar and BW agar yielded counts that were about twice those on Lactic agar. The plate counts obtained at the various stages in the manufacture of Cheddar cheese show that Eugonagar and BW agar yielded essentially the same counts. Slightly lower, although significantly different, counts were obtained with Lactic agar.

What is the reason or reasons for the differences in colony productivity of Eugonagar, BW agar and Lactic agar? An examination of the peptones used in these three media show little difference. These media each contain a total of 20 g/ liter of a peptone or peptones. Tryptone, in Lactic agar, and Trypticase, in both Eugonagar and BW agar, are derived from casein by pancreatic digestion. Phytone, found only in Eugonagar, is a papaic digest of soybean oil meal. Black (8) indicated that the better peptones, although not defined, gave comparable results. It was stated by Nelson (39) that variation in Tryptone content influenced the colony produc-

tivity of a medium. As stated previously, the plate counts obtained with injured cultures (32C for 24 hr) on Eugonagar were much higher than on either BW agar or Lactic agar. With Cottage cheese, Cheddar curd and cultures held at 4C for several days no difference was obtained between Eugonagar and BW agar. Perhaps these different types of injury influence the demands of the culture differently. If the peptones alone are considered, it would appear that Phytone in Eugonagar might be responsible for the higher counts obtained after the cultures were held at 32C for 24 hr.

The carbohydrate content of the three media is different. Eugonagar with only dextrose, BW agar with dextrose and lactose and Lactic agar with dextrose, lactose and sucrose. The information (18, 47) available in the literature indicates that the addition of a carbohydrate to a plating medium used in the enumeration of heat injured cells is desirable. No mention was made of the possible effects of various combinations of carbohydrates.

Lactic agar contains ascorbic acid and sodium acetate while the other two media do not. Perhaps ascorbic acid creates an unfavorable oxidation-reduction potential that will prevent injured cells from initiating growth on Lactic agar. Or it might possibly be a combination of factors

brought about by ascorbic acid and sodium acetate in Lactic agar.

Whatever the reasons may be for these differences in colony productivity, it is apparent that Lactic agar is not well suited for maximum yield after cells have been injured by heat.

It appears from the one series of observations made on the cooking temperature of Cheddar curd that the final temperature attained is very critical as regards its influence on the subsequent bacterial population. Probably it is reasonable to assume that at the cooking temperature of Cheddar curd (39C) an increase of 1C will be more deleterious on the cell population than would a 7C rise from 32 to 39C. The cooking temperatures for the Cheddar curd in vat one (Table 25) and vat two (Table 26) were 39.2 and 39.7C, respectively, a difference of 0.5C. Yet the plate counts of the Cheddar curd, after overnight in the press, from vat one (Table 25) were about three times those of vat two (Table 26). On the basis of this one observed variable, the cheese-maker must control the cooking temperature precisely or the bacterial population might become so low that the vat would be termed "dead".

The results from this entire investigation certainly

indicate that choice of medium and temperature of incubation are both critical for obtaining maximum plate counts of lactic streptococci. This is true regardless of whether the cultures are fresh or injured cultures.

SUMMARY AND CONCLUSIONS

The effect of plate incubation temperature and length of incubation period on the enumeration of lactic streptococci was studied. Eugonagar was used as the plating medium. Of the plate incubation temperatures of 32, 28, 25, 21 and 18C, incubation at 21C for 4 days was found, on the average, to be optimum for obtaining maximum plate counts of lactic streptococci. This was true of both fresh and injured cultures.

Ten commercial dehydrated media and seven other media known to have been used or had been suggested as being suitable for the enumeration of lactic streptococci were evaluated for colony productivity at an incubation temperature of 21C. Of these media Eugonagar was found to be the best of the commercial dehydrated media and Lactic agar the best of the other media.

Several peptones were evaluated for their colony productivity in a basal medium. Trypticase, Tryptone and Phytone gave similar results. In a like manner, Trypticase at different concentrations in a basal medium was evaluated for colony productivity. A concentration of 20 g/liter gave optimum recovery, although there was little difference in

colony productivity at concentrations of 15, 20 and 25 g/ liter.

Various materials that were potentially capable of increasing colony productivity of a plating medium were studied. Of the many that were tried only filtered V-8 cocktail vegetable juice, yeast extract and beef extract gave any increase in colony productivity of a plating medium.

An agar overlay, increased CO_2 concentration, 15 plant growth hormones and 12 carbohydrates were evaluated as to their effect on colony productivity of a plating medium. None were found, under the conditions of this investigation, to increase the colony productivity of media tested.

Eugonagar, BW agar and Lactic agar were evaluated for their colony productivity with both fresh and injured cells. Although differences, for the most part, were slight on fresh cultures wide variations in plate counts were observed after the culture had been injured. Eugonagar and BW agar yielded virtually the same counts when used for plating Cottage cheese, Cheddar curd and culture that had been held at 4C for several days. These counts, however, were much higher than those obtained on Lactic agar. Eugonagar yielded much higher counts than either BW agar or Lactic agar when used for plating cultures held at 32C for 24 hr.

It is the opinion of this investigator that Eugonagar with its high colony productivity of both fresh and injured cells as well as its clarity, which makes for easy and accurate counting is the medium of choice for the enumeration of lactic streptococci. The optimum temperature of incubation is 21C.

LITERATURE CITED

1. American Public Health Association. Standard methods for the examination of dairy products. 11th ed. New York, N. Y. Author. 1960.

2. Anderson, A. W. and Elliker, P. R. The nutritional requirements of lactic streptococci isolated from starter cultures. I. Growth in a synthetic medium. J. Dairy Sci. 36: 161. 1953.

3. Anderson, A. W. and Elliker, P. R. The nutritional requirements of lactic streptococci isolated from starter cultures. II. A stimulatory factor required for rapid growth of some strains in reconstituted non-fat milk solids. J. Dairy Sci. 36: 608. 1953.

4. Anderson, A. W., Parker, R. B. and Elliker, P. R. The nutritional requirements of lactic streptococci isolated from starter cultures. III. Variation in growth promoting properties of fresh whole milks. J. Dairy Sci. 38: 1083. 1955.

5. Babel, F. J., Collins, E. B. Olson, J. C., Peters, I. I., Watrous, G. H. and Speck, M. L. The standard plate count of milk as affected by the temperature of incubation. J. Dairy Sci. 38: 499. 1955.

6. Baltimore Biological Laboratory. Products for the microbiological laboratory. 4th ed. Baltimore 18, Maryland. Author. 1956.

7. Barkworth, H. and Davis, J. C. A cheap and efficient medium for the plate count of milk. J. Hyg. 42: 218. 1942.

8. Black, L. A. Current investigations of laboratory methods used in milk and food utensil sanitation. J. Milk and Food Technol. 11: 5. 1948.

9. Black, L. A. Effects of contemplated changes in standard methods. J. Milk and Food Technol. 11: 200. 1948.

10. Bowers, C. S. and Hucker, G. J. The composition of media for the bacteriological analysis of milk. N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull. 228. 1935.

11. Briggs, Mary. An improved medium for lactobacilli. J. Dairy Res. 20: 36. 1953.

12. Collins, E. B., Nelson, F. E. and Parmalee, C. E. Acetate and oleate requirements of the lactic group of streptococci. J. Bacteriol. 59: 69. 1950.

13. DeMans, J. C. and Galesloot, Th. E. De invloed van een toevoeging van mangaan aande melk op de groei van zuurselbacteriën. Nederlands Melk-en Zuiveltijdschrift 16: 1. 1962.

14. Difco Laboratories. Difco manual of dehydrated culture media and reagents. 9th ed. Detroit, Michigan. Author. 1953.

15. Duncan, D. B. Multiple range and multiple F tests. Biometrics 11: 1. 1955.

16. Elliker, P. R., Anderson, O. W. and Hannesson, C. An agar culture medium for lactic streptococci and lactobacilli. J. Dairy Sci. 39: 1611. 1956.

17. Fabian, F. W., Fulde, R. C. and Merrick, J. R. A new V-8 medium for determining lactobacilli. Food Res. 18: 280. 1953.

18. Fay, A. C. Thermo-tolerant organisms as a cause of so-called pin point colonies. J. Bacteriol. 13: 347. 1927.

19. Foster, E. M., Nelson, F. E., Speck, M. L., Doetsch, R. N. and Olson, J. C. Dairy Microbiology. Englewood Cliffs, New Jersey, Prentice-Hall, Inc. 1957.

20. Galesloot, Th. E., Hassing, F. and Stadhouders, J. Agar media voor het isoleren en tellen van aromabacteriën in starter. Nederlands Melk-en Zuiveltijdschrift 15: 127. 1961.

21. Garvie, Ellen I. and Mabbitt, L. A. Acid production in milk by starter cultures; the effect of peptones and other stimulatory substances. J. Dairy Res. 23: 305. 1956.

22. Hunter, G. J. E. Growth requirements of lactic streptococci: Differences within the group. J. Dairy Res. 16: 152. 1949.

23. Hunter, G. J. E. A simple agar medium for the growth of lactic streptococci: The role of phosphate in the medium. J. Dairy Res. 14: 283. 1946.

24. Hussain, I. and McDonald, I. F. Amino acids and utilization of sodium caseinate by lactic streptococci. Can. J. Microbiol. 3: 487. 1957.

25. Kaufmann, O. W., Harmon, L. G., Pailthorp, O. C. and Pflug, I. J. Effect of heat treatment on the growth of surviving cells. J. Bacteriol. 78: 834. 1959.

26. Kennedy, H. E. and Speck, M. L. Studies on corn steep liquor in the nutrition of certain lactic acid bacteria. J. Dairy Sci. 38: 208. 1955.

27. Kizer, D. E. and Speck, M. L. Observations on the acetate and citrate metabolism of Streptococcus lactis and Streptococcus cremoris. J. Dairy Sci. 38: 96. 1955.

28. Kizer, D. E., Speck, M. L. and Aurand, L. W. The effect of methionine and methionine precursors on the growth of Streptococcus lactis. J. Bacteriol. 69: 16. 1955.

29. Koburger, J. A., Speck, M. L. and Aurand, L. W. Identification of growth stimulants for Streptococcus lactis. J. Bacteriol. 85: 1051. 1963.

30. Kulp, W. L. An agar medium for plating Lactobacillus acidophilus and Lactobacillus bulgaricus. Science 66: 512. 1927.

31. Kulp, W. L. and White, V. A modified medium for Lactobacillus acidophilus. Science 76: 17. 1932.

32. Maki, L. R. and Foster, E. M. Effect of roughage in the bovine ration on types of bacteria in the rumen. J. Dairy Sci. 40: 905. 1957.

33. Mattick, A. T. R. and Nichols, A. A. The effect of reaction (pH) of milk on the destruction of micro-organisms by heat. J. Dairy Res. 6: 125. 1935.

34. McLaughlin, C. B. A readily prepared medium for the cultivation of the lactobacilli. J. Bacteriol. 51: 381. 1946.

35. Metcalf, D., Hucker, G. J. and Carpenter, D. C. A growth factor in certain vegetable juices. J. Bacteriol. 51: 381. 1946.

36. Mull, L. E. Factors influencing organism-bacteriophage populations. Unpublished Ph.D. thesis. Ames, Iowa, Library, Iowa State Univ. of Sci. and Technol. 1950.

37. Murdock, D. I., Folinazzo, J. F. and Troy, V. S. Evaluation of plating media for citrus concentrates. J. Food Technol. 6: 181. 1952.

38. Nelson, F. E. Factors which influence the growth of heat-treated bacteria. I. A comparison of four agar media. J. Bacteriol. 43: 395. 1943.

39. Nelson, F. E. Factors which influence the growth of heat treated bacteria. II. Further studies on media. J. Bacteriol. 48: 473. 1944.

40. Nelson, F. E. and Baker, M. P. The influence of time and temperature of plate incubation upon bacterial counts of market milk and related products, particularly after holding under refrigeration. J. Milk and Food Technol. 17: 95. 1954.

41. Niven, C. F. Nutrition of *Streptococcus lactis*. J. Bacteriol. 47: 343. 1944.

42. Nurmikko, V. and Karha, E. Nutritional requirements of lactic acid bacteria. I. The calcium requirements of Streptococcus thermophilus strains. Ann. Acad. Sci. 104, Series A. Section 11. 1962.

43. Pollock, M. A. and Lindner, M. Glutamine and glutamic acid as growth factors for lactic acid bacteria. J. Biol. Chem. 143: 655. 1942.

44. Reiter, B. and Oram, J. D. A note on the CO_2 requirements of Streptococcus lactis strain ML3. J. Dairy Res. 28: 175. 1961.

45. Reiter, B. and Oram, J. D. Nutritional studies on cheese starters. I. Vitamin and amino acid requirements of single strain starters. J. Dairy Res. 28: 63. 1961.

46. Rowlands, A. and Provan, A. L. Pasteurized milk. III. The relationship between the colony count at various temperatures and keeping quality. Proc. Soc. Agr. Bacteriologists 1939: 23. 1939.

47. Sherman, J. M. The advantages of carbohydrate medium in the bacteriological examination of milk. J. Bacteriol. 1: 481. 1937.

48. Smith, F. R. Nutritional studies on Streptococcus lactis. I. An unidentified growth factor found in yeast extract. J. Bacteriol. 46: 369. 1943.

49. Snell, E. E. and Mitchell, H. K. Purine and pyrimidine as growth substances for lactic acid bacteria. Natl. Acad. Sci. Proc. 27: 1. 1941.

50. Speck, M. L., McAnelly, J. K. and Wilbur, Jeane P. Variability in response of lactic streptococci to stimulants in extracts of pancreas, liver and yeast. J. Dairy Sci. 41: 502. 1958.

51. Sprince, H. and Woolley, D. W. Relationship of new growth factor required by certain hemolytic streptococci to growth phenomena in other bacteria. J. Exptl. Med. 80: 213. 1947.

52. Stevens, J. W. Preparation of dehydrated media containing orange juice serum. Food Technol. 8: 88. 1954.

53. Stokes, J. L. Substitution for folic acid in the nutrition of lactic acid bacteria. J. Bacteriol. 48: 201. 1944.

54. Thomas, W. R. Enumeration of thermoduric bacteria in milk. Unpublished Ph.D. thesis. Ames, Iowa, Library, Iowa State Univ. of Sci. and Technol. 1961.

55. Thomas, S. B. and Jenkins, E. Colony counts on milk agar incubated at 25, 30 and 37C. Proc. Soc. Agr. Bacteriologists 1940. 38: 1940.

56. Thomas, R. C., Levine, B. S. and Black, L. A. Studies showing the effect of changes in the new (9th) edition of standard methods in relation to the bacteriological analysis of milk. Am. J. Public Health 38: 233. 1948.

57. Troy, V. S. and Beisel, C. G. Unpublished laboratory reports. Research Department, Continental Can Co. and Florida Citrus Canners Cooperative, Lake Wales, Florida. February, 1948. Original not available; cited by Murdock, D. I., Folinazzo, J. F. and Troy, V. S. Evaluation of plating media for citrus concentrates. J. Food Technol. 6: 181. 1952.

58. Vera, H. D. The ability of peptones to support surface growth of Lactobacilli. J. Bacteriol. 54: 14. 1947.

59. Watkins, J. H. and Wilson, C. E. A. Factors determining the rate of mortality of bacteria exposed alkalinity and heat. J. Bacteriol. 24: 243. 1932.

60. Websters New Collegiate Dictionary. Based on Websters New International Dictionary. 2nd ed. Springfield, Mass., G. and C. Merriam Co. 1953.

61. White, H. R. The heat resistance of _S_. _faecalis_. J. Gen. Microbiology 8: 27. 1953.

62. Whitehead, H. R., Jones, P. A. and Robertson, P. S. The influence of carbon dioxide on the growth of lactic streptococci. J. Dairy Res. 25: 24. 1958.

63. Wright, L. D. and Skeggs, H. R. The growth factor requirements of certain streptococci. J. Bacteriol. 48: 117. 1949.

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APPENDIX A

Composition of Bacteriological Media Expressed as Grams per Liter of Distilled Water. Media Were Sterilized at 121C for 15 Minutes, Unless Otherwise Stated

Laboratory Media

Agar medium, Hunter (23)

Ingredient	Amount
Peptone	10 g
Beef extract	10 g
Yeast extract	100 ml
Lactose	20 g
Disodium phosphate	6 g
Agar	20 g

pH 6.8

Lactic agar, Elliker et al. (16)

Ingredient	Amount
Tryptone	20 g
Yeast extract	5 g
Gelatin	2.5 g
Dextrose	5 g
Lactose	5 g
Sucrose	5 g
Sodium chloride	4 g
Sodium acetate	1.5 g
Ascorbic acid	0.5 g
Agar (Noble)	15 g

pH 6.8

Modified V-8 agar, Tittsler

Ingredient	Amount
Tryptone	5 g
Tryptose	5 g
Yeast extract	5 g
Dextrose	5 g
V-8 cocktail vegetable juice (filtered)	100 ml
Agar	15 g

Special Trypticase soy agar, Mull (36)

Ingredient	Amount
Trypticase	15 g
Phytone	5 g
Sodium chloride	4 g
Sodium citrate	1 g
L-cystine	0.2 g
Dextrose	5 g
Agar	15 g

TGV agar, Galesloot et al. (20)

Ingredient	Amount
Tryptone	10 g
Beef extract	10 g
Yeast extract	5 g
Tomato juice (filtered)	40 ml
Dextrose	20 g
Tween 80	1 g
Potassium phosphate	2 g
Agar	15 g

pH 6.8

Tomato juice agar, Briggs (11)

Ingredient	Amount
Neopeptone	15 g
Yeast extract	6 g
Tomato juice (filtered)	400 ml
Dextrose	20 g
Sodium chloride	5 g
Tween 80	1 g
Agar	15 g

pH 6.6

Tomato juice agar, Kulp and White (31)

Peptone	10 g
Peptonized milk	10 g
Tomato juice (filtered)	400 ml
Agar	15 g

pH 6.6

Triple sugar agar, McLaughlin (34)

Trypticase	20 g
Gelatin	2.5 g
Lactose	5 g
Dextrose	5 g
Sucrose	5 g
Agar	10 g

pH 6.8

V-8 agar, Fabian et al. (17)

Tryptone	10 g
Dextrose	5 g
V-8 cocktail vegetable juice (filtered)	125 ml
Agar	18 g

pH 6.8

Commercial Dehydrated Media

Difco Laboratories (14)

Standard methods agar

Tryptone	5 g
Yeast extract	2.5 g
Dextrose	1 g
Agar	15 g

pH 7.0

Penassay agar

Peptone	6 g
Casitone	4 g
Beef extract	1.5 g
Yeast extract	3 g
Dextrose	1 g
Agar	15 g

pH 6.6

Tomato juice agar

Peptone	10 g
Peptonized milk	10 g
Tomato juice (400 ml)	20 g
Agar	11 g

pH 6.1

Whey agar

Peptone	10 g
Whey	13 g
Sodium chloride	5 g
Agar	12 g

pH 6.5

Baltimore Biological Laboratory (6)

APT agar

Trypticase	10 g
Yeast extract	7.5 g
Sodium chloride	5 g
Potassium phosphate	5 g
Sodium citrate	5 g
Dextrose	10 g
Polysorbate 80	0.2 g
Manganous chloride	0.14 g
Ferrous sulfate	0.04 g
Sodium carbonate	1.25 g
Agar	13.5 g

pH 6.7

Brain heart infusion agar

Polypeptone	10 g
Calf brain, infusion from	200 g
Beef heart, infusion from	250 g
Sodium chloride	5 g
Disodium phosphate	2.5 g
Dextrose	2 g
Agar	15 g

pH 7.4

Eugonagar

Trypticase	15 g
Phytone	5 g
Sodium chloride	4 g
Sodium sulfite	0.2 g
L-cystine	0.7 g
Dextrose	5.5 g
Agar	15 g

pH 7.0

L agar

Lactalysate	20 g
Sodium chloride	2 g
Sodium citrate	3 g
Dextrose	9.5 g
Agar	13.5 g

pH 7.0

Sunkist Grower Exchange

Orange serum agar

Peptone	10 g
Yeast extract	3 g
Orange juice serum solids	12.6 g
Sucrose	6 g
Dextrose	8 g
Cysteine hydrochloride	0.001 g
Agar	15 g

pH 5.5

Composition of Experimental Media

The pH was 6.8 for All Media

1

Ingredient	Amount
Trypticase	15 g
Phytone	5 g
Yeast extract	5 g
Sodium chloride	4 g
Sodium sulfite	0.2 g
Cystine	0.7 g
Dextrose	5 g
Agar	15 g

2

Ingredient	Amount
Trypticase	15 g
Phytone	5 g
Beef extract	5 g
Sodium chloride	4 g
Sodium sulfite	0.2 g
Cystine	0.7 g
Dextrose	5 g
Agar	15 g

3

Ingredient	Amount
Trypticase	20 g
Phytone	5 g
Yeast extract	2.5 g
Sodium chloride	4 g
Sodium sulfite	0.2 g
Cystine	0.7 g
Dextrose	5 g
Agar	15 g

4

Ingredient	Amount
Trypticase	25 g
Yeast extract	2.5 g
Sodium chloride	4 g
Sodium sulfite	0.2 g
Dextrose	5 g
Agar	15 g

5

Ingredient	Amount
Copanase	15 g
Yeast extract	5 g
Sodium chloride	4 g
Dextrose	5 g
Agar	15 g

6

Ingredient	Amount
Trypticase	15 g
Yeast extract	5 g
Sodium chloride	4 g
Dextrose	5 g
Agar	15 g

7

Trypticase	20 g
Yeast extract	5 g
Gelatin	3 g
Sodium chloride	4 g
Dextrose	5 g
Lactose	5 g
Sucrose	5 g
Agar	15 g

8

Trypticase	20 g
Yeast extract	5 g
Gelatin	2 g
Sodium chloride	4 g
Dextrose	5 g
Lactose	5 g
Agar	15 g

9

Trypticase	25 g
Gelatin	2 g
Sodium chloride	4 g
Dextrose	5 g
Lactose	5 g
Agar	15 g

10

Trypticase	20 g
Yeast extract	5 g
Gelatin	3 g
Sodium chloride	4 g
Dextrose	5 g
Lactose	5 g
Agar	15 g

11

Trypticase	10 g
Tryptone	10 g
Yeast extract	5 g
Gelatin	2 g
Sodium chloride	4 g
Dextrose	5 g
Lactose	5 g
Agar	15 g

12

Trypticase	20 g
Yeast extract	3 g
Beef extract	2 g
Gelatin	2 g
Sodium chloride	4 g
Dextrose	5 g
Lactose	5 g
Agar	15 g

APPENDIX B

Table 30. Plate counts obtained at selected temperatures after different periods of time

Culture	Incubation time, days	Plating medium Eugonagar — Incubation temperature C: 21	25	28	32
		Plate count in millions/g[a]			
FL	2	520	580	460	200
	4	570	630	480	200
	7	570	630	490	210
	10	590	640	490	220
M-9	2	700	750	780	650
	4	730	770	830	650
	7	740	790	840	660
	10	740	800	840	660
6	2	850	900	810	720
	4	940	920	820	730
	7	950	940	830	730
	10	950	940	840	730
32	2	800	770	760	700
	4	910	800	760	710
	7	910	850	770	710
	10	920	860	770	720
41	2	910	940	800	710
	4	980	950	830	730
	7	990	950	830	730
	10	990	980	840	740

[a]Average of three replications.

Table 30 (Continued)

Culture	Incubation time, days	Plating medium Eugonagar			
		Incubation temperature C			
		21	25	28	32
		Plate count in millions/g[a]			
42	2	800	870	610	500
	4	860	890	630	520
	7	870	890	630	520
	10	870	900	640	530
W-2-6	2	910	950	820	700
	4	940	960	830	710
	7	940	960	850	720
	10	950	960	860	720

Table 31. Plate counts obtained with commercial dehydrated media

Culture	Eugon-agar	Standard methods agar	APT agar	Penassay agar	Trypticase soy agar	L agar	Brain heart infusion agar	Tomato juice agar	Whey agar	Orange serum agar
	Plate count in millions/g[a]									
FL-1	820	790	300	500	230	320	270	38	47	39
FL-3	750	670	390	450	250	380	220	85	36	25
K-11	890	880	670	460	480	340	380	72	82	27
Xi	870	670	680	290	390	490	370	52	40	30
Alpha	940	820	710	710	620	550	410	75	100	80
FC	810	850	590	780	400	430	380	90	100	130
8	780	500	480	610	410	360	340	47	70	42
15	670	660	390	530	290	220	110	85	71	65
18	830	580	450	370	310	250	160	73	46	56
32	840	850	460	680	500	530	390	50	56	38
41	710	260	480	350	300	210	180	45	55	34
42	850	860	680	640	440	410	310	65	75	50
M-9	920	840	760	680	470	600	540	81	110	63
M-16	730	410	480	470	390	360	370	63	76	60
5-K	760	690	440	470	290	420	320	70	40	50
FD	840	620	380	580	440	420	360	80	60	55
W-2-6	960	850	730	700	710	610	430	92	75	75

[a]Average of three replicates.

Table 32. Plate counts obtained with laboratory media

Culture	Lactic agar Elliker et al. (16)	TGV agar Galesloot et al. (20)	Tomato juice agar Briggs (11)	Modified V-8 agar Tittsler	V-8 agar Fabian et al. (17)	Tomato juice agar Kulp & White (31)	Special Trypticase soy agar Mull (36)
	Plate count in millions/g[a]						
FL-1	610	520	470	480	440	370	280
FL-3	770	710	410	580	470	600	390
Xi	740	770	530	410	500	550	470
Alpha	770	630	590	400	470	670	600
8	820	780	600	530	410	530	490
15	700	590	520	480	610	380	570
22	650	530	470	570	510	400	470
23	680	690	490	500	560	370	540
33	790	680	470	620	580	430	480
40	850	860	720	630	630	380	290
M-9	780	870	640	600	490	600	390
FD	900	830	780	670	730	480	570
W-2-5	910	770	700	710	650	590	620
W-2-6	700	730	630	550	480	600	570

[a]Average of three replicates.

Table 33. Plate counts obtained with Eugonagar and Eugonagar plus filtered V-8 cocktail vegetable juice

Culture	Plating medium: Eugonagar	Eugonagar plus 10% filtered V-8 cocktail vegetable juice	Eugonagar plus 20% filtered V-8 cocktail vegetable juice	Eugonagar plus 30% filtered V-8 cocktail vegetable juice
	Plate count in millions/g[a]			
6	720	850	870	810
15	650	760	800	750
22	780	890	850	870
23	850	930	930	900
33	830	870	890	910
41	750	910	870	880
FD	730	860	820	810
FL-3	810	890	900	850
M-9	820	920	930	900
Xi	810	860	930	910
W-2-5	870	960	950	940
W-2-6	890	980	990	960

[a]Average of three replications.

Table 34. Plate counts obtained with media containing various bacteriological peptones

Culture	Bacteriological peptone, 15 g/liter				
	Trypticase	Tryptone	Peptonized milk	Casamino acids	Phytone
	Plate count in millions/g[a]				
FL	490	520	420	470	480
M-9	690	660	590	600	620
6	630	670	530	510	640
33	530	510	440	540	560
Xi	570	570	520	510	540
W-2-6	680	640	590	600	610

[a]Average of three replications.

Table 35. Plate counts obtained with a basal medium containing different concentrations of Trypticase

	Concentration of Trypticase in g/liter					
	5	10	15	20	25	30
Culture	Plate count in millions/g[a]					
FL	440	510	690	710	680	410
M-9	490	550	640	670	680	550
6	590	650	690	760	720	590
Xi	530	610	640	680	660	520
W-2-5	570	660	780	830	870	610
W-2-6	550	600	700	760	630	500

[a]Average of three replications.

Table 36. Plate counts obtained when a basal medium was supplemented with compounds that have been reported as stimulatory for lactic streptococci

	Supplement added/liter							
	None	Beef extract 5 g	Yeast extract 5 g	Liver fraction 1 g	Gelatin 2 g	Stimilac 2 g	Sodium acetate 1.5 g	Tween 80 1 g
Culture	Plate count in millions/g[a]							
FL-1	510	630	670	530	500	470	530	490
M-9	630	710	790	600	670	620	600	590
6	680	740	830	700	650	640	720	700
Xi	620	690	700	630	590	640	560	570
W-2-5	690	760	800	730	680	770	640	670
W-2-6	550	610	700	510	580	490	570	600

[a]Average of three replications.

Table 37. Plate counts obtained with a medium supplemented with glutamine and/or asparagine

	Supplement added/liter			
	None	Glutamine 100 mg	Asparagine 100 mg	Glutamine and asparagine Each 100 mg
Culture	Plate count in millions/g[a]			
FL-1	600	560	620	550
M-9	530	480	550	500
6	670	630	710	660
W-2-6	790	840	740	760

[a]Average of three replications.

Table 38. Plate count obtained when BW agar was supplemented with inosine

Culture	None	Inosine added/liter 0.1 g	1 g
	Plate count in millions/g[a]		
FL-1	770	720	740
6	620	660	640
W-2-6	930	890	860

[a]Average of three replications.

Table 39. Plate counts obtained after cultures were held at 32C for 24 hours prior to plating on BW agar supplemented with inosine

Culture	Incubation temp. C	Inosine added/liter: None	0.1 g	1 g
		Plate count in millions/g[a]		
FL	21	120	120	110
	25	85	80	76
	28	58	55	57
	32	26	28	29
6	21	6.5	5.6	6.5
	25	5.6	5.6	5.3
	28	4.1	4.3	4.0
	32	2.7	2.5	2.4
W-2-6	21	8.7	8.2	8.4
	25	7.3	6.9	5.6
	28	5.0	4.8	4.0
	32	2.2	1.9	1.6

[a]Average of three replications.

Table 40. Plate counts obtained when BW agar was modified by the addition of carbonate and phosphate

Culture	Supplement added/liter						
	None	Na_2CO_3, 1.32 g; Cysteine HCl, 0.025 g	Na_2CO_3, 2.64 g; Cysteine HCl, 0.05 g	$NaHCO_3$, 1.0 g; $Na_2S \cdot 9H_2O$, 0.8 g	$NaHCO_3$, 2.0 g; $Na_2S \cdot 9H_2O$, 1.6 g	KH_2PO_4, 1 g	KH_2PO_4, 2 g
	Plate count in millions/g[a]						
FL-1	570	670	540	580	610	560	600
6	710	660	670	740	770	650	680
W-2-6	980	900	870	860	940	970	850

[a]Average of three replications.

Table 41. Plate counts obtained when a basal medium was supplemented with selected cations

Culture	None	Mg^{++}, 30 mg; Mn^{++}, 3 mg	Mg^{++}, 20 mg; Mn^{++}, 2 mg; Zn^{++}, 1 mg; Cu^{++}, 0.8 mg and Co^{++}, 0.5 mg
	Concentration of cations added/liter		
	Plate count in millions/g[a]		
FL-1	510	480	440
Xi	560	490	580
M-9	500	550	520
6	760	680	740
W-2-6	810	830	790

[a]Average of three replications.

Table 42. Plate counts obtained when a basal medium was supplemented with selected organic acids

Culture	None	Citrate 1 g	Citrate 2 g	Fumarate 0.1 g	Fumarate 0.2 g	Malate 0.1 g	Malate 0.2 g	Succinate 0.1 g	Succinate 0.2 g
	Plate count in millions/g[a]								
FL-1	450	490	410	460	420	400	430	500	480
Xi	570	560	520	580	470	490	510	590	620
M-9	790	720	760	820	760	750	740	770	730
6	660	670	620	590	610	640	680	610	600

Organic acid added/liter applies to all column headings.

[a]Average of three replications.

Table 43. Plate counts obtained with experimental media and Standard methods agar

Culture	Standard methods agar	1	2	3	4	5	6	7	8	9	10	11	12
	Plating medium												
	Plate count in millions/g[a]												
FL-1	670	810	730	500	830	860	880	960	930	670	860	890	830
M-9	660	890	800	590	810	790	730	800	780	750	920	850	790
6	700	830	730	610	760	830	840	900	910	760	890	920	860
W-2-6	750	750	640	670	920	930	900	890	920	590	870	900	890

[a]Average of three replications.

Table 44. Plate counts obtained on Lactic agar, TGV agar and BW agar

Culture	Plating medium Lactic agar	 TGV agar	 BW agar
	Plate count in millions/g[a]		
FL	540	510	510
M-9	590	580	620
W-2-6	780	780	860

[a]Average of four replications.

Table 45. Plate counts obtained after holding cultures at 32C for 24 hours

Culture	Incubation temp. C	Plating medium Eugonagar	BW agar	Lactic agar
		Plate count in millions/g[a]		
FL	18	150	140	60
	21	150	60	55
	25	110	40	33
	28	80	20	22
6	18	69	2.8	2.5
	21	60	2.5	2.2
	25	42	2.0	1.8
	28	32	1.6	1.4
W-2-6	18	56	8.7	5.8
	21	50	7.9	5.6
	25	30	5.9	4.5
	28	19	3.6	2.8

[a]Average of two replications.

Table 46. Plate counts obtained from fresh cultures and after holding at 32C for 24 hours

Culture	Incubation temp. C	Eugonagar Fresh	Eugonagar Injured	BW agar Fresh	BW agar Injured	Lactic agar Fresh	Lactic agar Injured
		Plate count in millions/g[a]					
FL	18	390	170	380	100	410	80
	21	410	170	420	58	410	59
	25	400	110	300	42	370	41
	28	370	66	310	40	230	18
6	18	930	43	810	1.8	850	1.6
	21	940	32	930	1.4	870	1.5
	25	860	15	850	1.2	800	1.2
	28	840	7	720	0.67	740	0.74
W-2-6	18	850	43	970	0.83	820	0.43
	21	870	28	1100	0.77	870	0.28
	25	830	23	890	0.49	830	0.23
	28	620	12	790	0.49	620	0.12

[a]Average of three replications.

Table 47. Plate counts obtained from fresh cultures and after holding at 32C for 24 hours

		Plating medium					
		Eugonagar		BW agar		Lactic agar	
		Treatment of cultures					
Culture	Incubation temp. C	Fresh	Injured	Fresh	Injured	Fresh	Injured
		Plate count in millions/g[a]					
FL-1	18	440	140	430	140	390	110
	21	430	130	440	110	430	92
6	18	880	72	880	2.5	850	1.8
	21	890	58	850	2.2	810	1.6
W-2-6	18	1000	55	1000	0.92	900	0.51
	21	1100	51	1000	0.80	850	0.51
C-11	18	1100	310	1000	260	930	150
	21	980	290	930	230	910	150
C-26	18	1300	160	1200	74	1100	52
	21	1300	160	1300	74	1100	52
H-21	18	850	180	880	140	880	57
	21	820	180	850	150	780	47

[a]Average of three replications.

Table 47 (Continued)

Culture	Incubation temp. C	Eugonagar Fresh	Eugonagar Injured	BW agar Fresh	BW agar Injured	Lactic agar Fresh	Lactic agar Injured
		Plate count in millions/g[a]					
P-1	18	950	160	1000	130	630	65
	21	920	170	1000	150	610	60
P-1-A	18	1300	140	1300	140	900	72
	21	1200	140	1300	100	960	72

Table 48. Plate counts obtained after holding lactic cultures at 4C for two, four and seven days

Culture	Time of sampling	Eugonagar 18	Eugonagar 21	BW agar 18	BW agar 21	Lactic agar 18	Lactic agar 21
		Plate count in millions/g[a]					
FL	Fresh	450	460	470	480	440	460
	2	420	430	440	440	330	380
	4	360	390	420	360	290	330
	7	280	300	250	260	210	222
6	Fresh	880	890	910	840	880	850
	2	760	800	720	750	750	780
	4	660	650	62	620	580	610
	7	520	500	41	430	280	360
W-2-6	Fresh	920	930	980	960	870	850
	2	770	760	900	790	740	710
	4	610	630	590	630	500	570
	7	530	520	430	630	240	320
C-11	Fresh	1100	960	1000	900	920	940
	2	910	860	910	900	840	770
	4	720	750	670	730	550	560
	7	500	480	500	520	370	400
C-26	Fresh	1200	1300	1300	1200	1000	1100
	2	1100	1000	1100	1100	930	970
	4	840	870	840	880	650	680
	7	650	610	580	620	480	500
H-21	Fresh	910	870	940	900	990	970
	2	860	820	760	730	740	720
	4	610	620	570	640	520	490
	7	380	440	420	410	330	270

Plating medium; Incubation temperature C.

[a]Average of four replications.

Table 48 (Continued)

Culture	Time of sampling	Plating medium: Eugonagar, Incubation temperature C: 18	Eugonagar 21	BW agar 18	BW agar 21	Lactic agar 18	Lactic agar 21
		Plate count in millions/g[a]					
P-1	Fresh	1000	950	1100	1100	810	780
	2	840	870	870	910	650	630
	4	680	630	690	650	510	500
	7	590	550	510	470	400	370
P-1-A	Fresh	1300	1300	1300	1400	1200	1100
	2	1300	1200	1200	1200	1100	1000
	4	990	930	940	970	870	870
	7	740	690	750	710	600	620

Table 49. Plate counts obtained from Cottage cheese

Age of cheese, days	Incubation temp. C	Plating medium: Eugonagar	BW agar	Lactic agar
		Plate count/g[a]		
Fresh	18	3800	3300	1300
Fresh	21	3900	3500	1500
2 days	18	3600	3400	1500
2 days	21	3900	3500	1500
4 days	18	3600	3500	1800
4 days	21	3500	3200	1200
7 days	18	3200	2700	1700
7 days	21	3600	3400	2100

[a]Average of three replications.

Table 50. Plate counts obtained with and without an agar overlay

	Plating medium					
	Eugonagar			Eugonagar plus 10% filtered V-8 cocktail vegetable juice		
	without overlay	with overlay of		without overlay	with overlay of	
Culture		Eugonagar	Plain agar		Eugonagar plus V-8 juice	Plain agar
	Plate count in millions/g[a]					
FL-1	690	650	660	830	810	800
Alpha	750	720	690	910	880	930
Xi	830	760	800	870	900	890
M-9	790	830	810	860	880	890
6	650	630	600	770	790	750
32	810	840	780	870	860	900
41	760	750	710	870	860	820
FD	830	850	860	890	900	850
W-2-5	800	830	770	910	890	920
W-2-6	880	840	920	920	900	930

[a]Average of three replications.

Table 51. Plate counts obtained with and without added CO_2

	Plating medium			
	Eugonagar		Eugonagar plus 10% filtered V-8 cocktail vegetable juice	
	Normal atmosphere	with 6% CO_2	normal atmosphere	with 6% CO_2
Culture	Plate count in millions/g[a]			
FL-1	850	810	870	840
Alpha	810	790	750	810
Xi	720	680	780	790
M-9	700	660	840	860
M-16	830	850	890	910
6	650	700	760	720
23	750	760	820	830
33	810	800	930	910
W-2-5	840	820	950	930
W-2-6	890	930	1000	960

[a]Average of three replications.

Table 52. Plate counts obtained with and without added CO_2

Culture	Eugonagar: Normal atmosphere	Eugonagar: with 10% CO_2	Eugonagar plus 10% filtered V-8 cocktail vegetable juice: Normal atmosphere	Eugonagar plus 10% filtered V-8 cocktail vegetable juice: with 10% CO_2
	Plate count in millions/g[a]			
FL-1	750	790	840	830
Alpha	770	790	910	860
Xi	830	800	850	870
M-9	780	810	870	910
M-16	750	790	860	840
6	810	770	840	850
23	830	780	900	870
33	850	810	880	900
W-2-5	870	840	930	950
W-2-6	800	830	910	870

[a]Average of three replications.

Table 53. Plate counts obtained with and without CO_2.

Culture	Plating medium: Eugonagar, Normal atmosphere	Plating medium: Eugonagar, with 14% CO_2	Plating medium: Eugonagar plus 10% filtered V-8 cocktail vegetable juice, Normal atmosphere	Plating medium: Eugonagar plus 10% filtered V-8 cocktail vegetable juice, with 14% CO_2
	Plate count in millions/g[a]			
FL-1	830	850	920	910
Alpha	870	880	930	890
Xi	700	660	800	750
M-9	730	770	890	850
M-16	870	850	910	930
6	660	630	710	730
23	770	710	830	810
33	680	730	780	770
W-2-5	880	890	930	900
W-2-6	740	710	870	840

[a]Average of three replications.