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The Lactic Acid Bacteria: A Literature Survey

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ABSTRACT: The purpose of this review article on the lactic acid bacteria grew from an early curiosity and a desire to convey and impart the broad scope of literary information on their functions as starter cultures, in the manufacture of fermentation products such as dairy products and alcoholic beverages, as well as their contribution to better health. This review article is an attempt to empower the reader and to circumvent the difficult task in acquiring and elucidating a large body of information. The intent is to familiarize the reader with the various lactic species, their habitat or source, associated food, physiological characteristics, colonial morphology, biochemical characteristics, culture media (enrichment, nonselective, and selective), classic description, and taxonomy. This review provides information on *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Carnobacterium*, and *Enterococcus*. Trends are presented, such as the use of nisin to extend food shelf-life and the current research premise that Probiotic strains may alter the intestinal flora and thus prevent intestinal wall penetration by pathogens.

KEY WORDS: Betabacteria, Thermobacteria, Streptobacteria, Lactic Acid Bacteria, Homofermenter, Heterofermenter, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, *Streptococcus*, meat, vegetable, dairy, fermented products, wine, preservative, bacteriocin, Probiotic and Probiotic therapy, and Vancomycin resistance.

I. INTRODUCTION

The article has been written in order to make available many of the biochemical tests, culture media, and cultural characteristics reported by the original authors for the Lactic Acid Bacteria (LAB).

In the attempt to compile the information, it was necessary to overcome a number of obstacles. Many of the original authors had retired or were unavailable for personnal comment. Many of the publications were difficult to find because these were unlisted in library computer databases, and furthermore were stored out of ready access. Another problem was that articles that update older data frequently provided only bits and pieces of the original information.

Much of the emphasis in the fermentation industry has shifted from research to quality control to meet more stringent governmental regulations. Personal contacts with the scientific community indicate that a considerable amount of research is occurring, but it is not for publication. Another factor, besides the time spent on quality control, might include the reluctance of the food and dairy industries to publish data due to its proprietary nature. Therefore, this article has been written to provide a compilation of physically scattered information accumulated over the years from articles written during the 1950s to the present. Much original research work occurred during the early 1950s and tapered off in the late 1970s. The investigators included such authors as D.M.

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Wheater, M.E. Sharpe, P. Elliker, M. Rogosa, J.B. Evans, L.V. Holdeman, W.E. Moore, E.I. Garvie, W.F. Harrigan, M.E. McCance, W.E. Sandine, and others.

The LAB, frequently termed "the Lactics", constitute a diverse group of microorganisms associated with plants (cabbage, corn, barley, mashes, kale, and silage), meat, and dairy.[1,2,3,4,5,6] LAB are best known for their use as starter cultures in the manufacture of dairy products such as acidophilus milk, yogurt, buttermilk, cottage cheese, hard cheeses (Cheddar, Provolone, Romano, and Edam), and soft cheeses (Brie and Camembert).[2] The Lactics are also important commercially in the processing of meats, alcoholic beverages, and vegetables. These products include sausage, cured hams, wines, beer, fortified spirits, pickles, and saukerkraut.[1,2,3,4,5,7,8,9] Although the LAB have beneficial effects in the food industry, they can be a nuisance as contaminants by producing off-flavors.[3,4,10,11,12]

LAB have been implicated in patients with impaired immune systems, as etiologic agents of such diseases as endocarditis, bacteremia, and septicemia.[1,2,3,7,8,10,13,14,15] More interest and cooperative research by both the medical and food industries are necessary in order that appropriate treatment may be administered expediently.[10,15,16,17,18,19] Current research suggests the idea of using LAB to provide a transient intestinal flora to compete with potentially harmful organisms, thereby preventing disease from being established.[3,20,21]

Even before fermentation chemistries were understood, the Lactics were highly prized by the food and dairy industries for the ability to produce aromatic and flavor-enhancing compounds. The type of lactic acid and the amount of lactic acid formed during fermentation is critical in food manufacture and plays a prominent role in taxonomic classification of the Lactics.[2,3,22,23,24,25]

The Lactics have been characterized primarily by their ability to form various isomers of lactic acid from the fermentation of glucose. Lactic acid may be extracted from the fermentation product and a determination made of the ability to optically rotate light. If the rotation is to the right, it is termed Dextrorotary (D); if to the left, it is termed Levorotary (L), or, if there is a mixture of both D and L, it is termed racemic (DL).[2,22,25,26]

The Lactic Acid bacteria, although consisting of a number of diverse genera, are grouped as either Homofermenters or Heterofermenters based on the end product of their fermentation. The Homofermenters produce lactic acid as the major product of fermentation of glucose. The Heterofermenters produce a number of products besides lactic acid, including carbon dioxide, acetic acid, and ethanol from the fermentation of glucose. The Homofermenters possess the enzyme aldolase and are able to ferment glucose more directly to lactic acid than the Heterofermenters. The Heterofermenters use the alternate pentose monophosphate pathway, converting six carbon sugars (hexoses) to five carbon sugars (pentoses) by the enzyme phosphoketolase, producing in the process both aldehyde and diacetyl—highly desirable aromatic and flavor-enhancing substances. The Heterofermenters are often used in dairy industry because of these flavor-enhancing substances.[2,22,27,28]

The Homofementative Lactics include the genera *Streptococcus* and *Pediococcus*. *Streptococcus* produces L(+) lactate and *Pediococcus* produces DL lactate. The Heterofermentative Lactics consist of the genus *Leuconostoc* and a subgroup of the genus *Lactobacillus*, the Betabacteria. *Leuconostocs* produce D(-) lactate; Betabacteria produce DL lactate, Table 1.[1,2,22,23,25]

II. CLASSICAL NOMENCLATURE

The LAB consist of a number of genera: *Lactobacillus, Leuconostoc, Pediococcus,*

TABLE 1
Differentiation of the Lactic Acid Bacteria

Genus	Gram stain Morphology	Type of Lactic Acid formed	Carbon dioxide From Glucose	Ribose fermented	Gluconate fermented	Arginine hydrolyzed
Homofermenter						
Streptococcus	Cocci/chains	L+	-	(-/+)[46]	ND	V
Pediococcus	Cocci/tetrad	DL, L+	-	(V)[42]	-	V
Lactococcus	Cocci/chains	L+	-	-	-	V
Lactobacillus	Bacilli/ pairs	D- , L+ DL	-	(+/-)[a,b]	(+/-)[a,b]	-
Heterofermenter						
Betabacteria (Lactobacillus)	Pair/chains	DL	+	+	+	+
Leuconostoc	Pair/chains	D(-)	+	(V)	(-/+)	-

*+ indicates a positive test result; -. Indicates a negative test result; (+/-) indicates most strains positive, an occasional strain negative; (-/+) indicates most strains negative, an occasional strain positive; V indicates variable, some produce (+) or (-) results; L(+) indicates levo-lactic acid; D(-) indicates dextro-lactic acid; DL indicates a mixture of D and L lactic acid; ND indicates no data is available.

[a]Streptobacteria and Betabacteria (subgroups of Lactobacillus) are positive for the substrates ribose and gluconate.

[b]Thermobacteria (subgroup of Lactobacillus) are negative for both ribose and gluconate.

Data from Barre 1978 (36), Bergan 1984 (228), Brock 1974 (206), Dellaglio 1995 (95), Doring 1988 (128), Farrow 1989 (199), Frobisher 1974 (28), Garvie 1984 (51), Hardie et al. 1995 (46), Harrigan 1976 (39), Jay 1986 (2), Jay 1992 (9), Kandler 1986 (3), Schleifer 1985 (43), Schleifer 1987 (187), Sharpe 1979 (23), Sharpe 1979 (219), Sharpe 1981 (4), Simpson 1995 1995 (42), Singleton 1987 (22), Stamer 1976 (25), Teuber 1995 (86).

and *Lactococcus* (Lactic *Streptococci*). In general, the LAB may be characterized as Gram-positive, aerobic to facultatively anaerobic, asporogenous rods and cocci which are oxidase, catalase, and benzidine negative, lack cytochromes, do not reduce nitrates to nitrite, are gelatinase negative, and are unable to utilize lactate.[1,2,3,22,25,27,28,29,30,31,32,33,34,35]

The *Lactobacilli* were originally classified by Orla and Jensen into the Homofermenters and Heteroferementers based on the amount of lactic acid formed during glucose fermentation. Orla and Jensen also divided the *Lactobacilli* into the three groups (the Thermobacteria, Streptobacteria, and the Betabacteria) based on growth temperature and biochemical reactions. Although those three groups have been replaced for the most part, the three names are still in common use and are defined according to growth temperature, ability to ferment pentoses, ability to produce carbon dioxide from glucose or gluconate, requirement for thiamine, formation of lactic acid as a major product of fermentation, Homofermentative or Heterofermentative type of fermentation, reduction of fructose to mannitol, and hydrolysis of arginine, (Table 2).[1,3,4,22]

The Betabacteria are Heterofermenters and are unique from other *Lactobacilli* in that they form carbon dioxide by glucose fermentation, and most hydrolyze arginine.[1] Both Betabacteria and Streptobacteria ferment ribose, a pentose; however, the Thermobacteria do not. The Streptobacteria produce carbon dioxide from gluconate but not from glucose. The Thermobacteria are thermophilic and Homofermentative. The Thermobacteria neither form carbon dioxide from glucose nor gluconate nor grow at 15°C, but grow at

TABLE 2
The Lactobacillus Subgroups (Orla and Jensen)
Streptobacteria, Betabacteria, and Thermobacteria

	Growth Temperatures 15°C	45°C	Gas from glucose	Gas from gluconate	Arginine hydrolysed	Ferment pentose substrates	Thiamine requirement	Lactic acid from glucose
Thermobacteria	-	+	-	-	-	-	-	+
Streptobacteria	(+)	(V)[25,40]	-	+	(-)[3]	+	-	+
Betabacteria	(+)	(V)[25,40]	+	+	+	+	+	+

	Predominant Lactic acid type	Fructose fermented & reduced to mannitol
Thermobacteria	L(+), D(-), DL,	-
Streptobacteria	L(+), D(-), DL,	-
Betabacteria	DL	+

*+ indicates a positive test result; -. indicates a negative test result; (+/-) indicates most strains positive, an occasional strain negative; (-/+) indicates most strains negative, an occasional strain positive; V indicates variable, some produce (+) or (-) results; L(+) indicates levo-lactic acid; D(-) indicates dextro-lactic acid; DL indicates a mixture of D and L lactic acid; ND indicates no data is available.

Data from Barre 1978 (36), Facklam et al. 1995 (40), Harrigan & McCance (39), Kandler 1986 (3), Law & Sharpe 1978, (186), Schillinger 1987 (1), Sharpe 1977 (23), Sharpe 1979 (26), Sharpe 1979 (219), Sharpe 1981 (4), Singleton 1987 (22), Stamer 1976 (25), Topley 1982 (37).

45°C.[22] They also fail to hydrolyse arginine. The Thermobacteria differ from the Betabacteria and Streptobacteria by growing at 45°C or greater, not fermenting ribose, nor hydrolysing arginine and by not growing at 15°C, the optimium temperature for both Streptobacteria and Betabacteria.[1,4,22,23,36] The Betabacteria grow optimally at 15°C and generally hydrolyze arginine. In contrast to the Betabacteria, Streptobacteria are Homofermentative and form CO_2 from gluconate not glucose, and grow optimally at 15°C (mesophilic).[1,22,23,36,37] To further contrast the three subgroups, the Thermobacteria and the Streptobacteria are both Homofermentative, while the Betabacteria are Heterofermentative and often hydrolyze arginine.[3,22,25] The Thermobacteria and Streptobacteria differ mainly in that the former are thermophiles, while the latter are mesophiles.[3] The Betabacteria, however, not only differ from the other two groups by being Heterofermentative (forming CO_2 from glucose) but also differ by hydrolyzing arginine, and require thiamine for growth, (Table 2).[1,3,4,22,23,29,36,37,38]

III. HOMOFERMENTATIVE GRAM-POSITIVE COCCI

Homofermentative Gram-Positive cocci include three genera, the Streptococci, the Enterococci, and the Pediococci. The Streptococci and the Pediococci are placed into their genera based on gram stain morphology, the type and amount of lactic acid formed (D,L,or DL), growth in 6.5% NaCl, arginine hydrolysis, and temperature of optimium growth (Figure 1).[1,2,22,25,39,40] The Pediococci are readily distinguished from the other Homofermentative LAB by the former's tetrad morphology in broth media and by its formation of predominately L(+) and DL lactate. The Pediococci also differ from the other Lactic Streptoccoci (Lactococci) by not reacting to Lancefield Group N Streptococcal antisera.[1,3,22,25,39,40,41,42]

The genus Enterococcus has the same gram stain morphology as the Lactococci and may be differentiated from these by its ability to grow at both 10°C and 45°C. By definition, the Enterococci are Gram-positive, facultative anaerobic cocci with growth at 10°C and 45°C, growth in broth with 6.5% NaCl, growth at pH 9.6, and reduction of 0.1% Methylene Blue Milk Medium.[1,43,44,45,46,47,48] The Enterococci may also be distinquished by Lancefield Group N and Group D serogroupings. The Lactococci are Group N, while most Enterococci are Group D. Lactococci grow optimally at 10°C and are only capable of growing between 10°C and 40°C. Enterococci, unlike the Lactococci and the Streptococci, are capable of growth in broth with a 6.5% salt concentration and at temperatures of 10°C and 45°C (Figure 1; Table 3).[1,22,41,43,47]

IV. MEDIA AND BIOCHEMICAL TESTS

The cultivation of the LAB may be accomplished using enrichment broths and selective or nonselective media, depending on a need to isolate either a particular genus from a mixture of microorganisms or to maintain isolates in culture.[49] When processing samples for LAB, samples should not be refrozen prior to plating; chilled 0.1% peptone water should be used for dilution; and blender cups should be chilled prior to blending. Phosphate buffer diluent results in lower recovery rates.[50]

Most LAB are acid tolerant but have an optimium growth pH; therefore, a common approach to selective isolation is to adjust the pH for a particular genus or group.[4,50,51,54] A pH of less than 4.5 may be used to differentiate *Lactobacillus* and *Pediococcus* from *Leuconostoc*; the latter requires a pH of 4.5

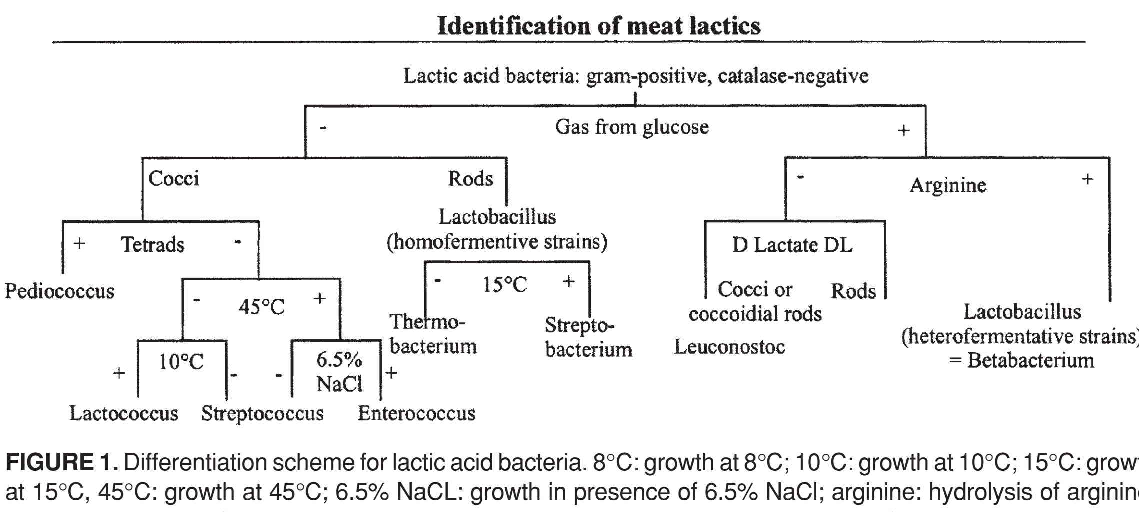

FIGURE 1. Differentiation scheme for lactic acid bacteria. 8°C: growth at 8°C; 10°C: growth at 10°C; 15°C: growth at 15°C, 45°C: growth at 45°C; 6.5% NaCL: growth in presence of 6.5% NaCl; arginine: hydrolysis of arginine; lactate: production of lactate: D or L: the isomere recorded makes up 90% or more of the total lactic acid: D(L) or L(D): the isomere given in brackets makes up 15 to 20% of total lactic acid; DL: 25 to 75% of total lactic acid are of the L-configuration; ribose: production of acid from ribose; mDpm: presence of meso-diaminopimelic acid in cell wall. (Reprinted with permission from Dr. Schillinger, the author and from the article *Identification of Lacobacillie from Meats*. Figures 1 to 3, 4:199–208. Copyright 1987. Academic Press, London.)

TABLE 3
Differentiation of the Lactococcus and Similiar Gram-Positive cocci

Microorganism	Growth at 10°C	45°C	Growth in 6.5 % NaCl	Type Lactate Formed	Gas from Glucose	Growth in Broth at pH 9.6	Arginine hydrolysis	0.10% Methylene Blue Reduction
Enterococcus	+	+	+	ND	-	+	+	+
Lactococcus	+	-	-	L	-	-	V	V
Streptococcus	-	+	-	L	-	-	V	-
Leuconostoc	+	+	-	D	+	ND	-	ND

*+ indicates a positive test result; -. indicates a negative test result; (+/-) indicates most strains positive, an occasional strain negative; (-/+) indicates most strains negative, an occasional strain positive; V indicates variable, some produce (+) or (-) results; L(+) indicates levo-lactic acid; D(-) indicates dextro-lactic acid; DL indicates a mixture of D and L lactic acid; ND indicates no data is available.

Data from Barre 1978 (36), Brock 1974 (206); Deibel & Steeley 1978 (245), Frobisher 1974 (28). Hardie et al. 1995 (46), Harrigan & McCance 1976 (39), Huggins 1984 (90), MacFaddin 1980 (34), Schillinger 1987 (1), Schleiffer 1985 (43), Sharpe 1965 (27), Sharpe 1966 (239), Sharpe 1966 (239), Stamer 1976 (25).

or greater.[39,50,51] Both *Lactobacillus* and *Pediococcus* will grow at a pH of less than 4.5.[39,49,51,52]

A common approach to LAB isolation is to use a general purpose medium such as APT Agar, Rogosa Agar, or MRS Agar and then to employ a Homofermentative Heterofermentative Differential Medium (HHD) or a Modified MRS *Lactobacillus* Heteroferm Screen Broth (at pH 4.5) for differentiating the Heterofermenters from the Homofermenters.[4,51,52,53,54,55,56] APT Medium may also be used for the cultivation of *Lactobacillus viridescens*.[4,27,53,55,56,57]

Homofermentative Heterofermentative Differential Agar has been recommended for the differentiation of heterofermenters from homofermenters; heterofermenters appear as white colonies with a drop in pH to 4.6 (fructose metabolized to mannitol), whereas homofermenters form blue to green colonies, resulting from a drop in pH to 5.6.[4,27,52,53,54,57,58,59]

An agar medium that detects the type of lactic acid formed exists.[60] A red halo around colonies indicates the production of either D(-) or DL lactate; colonies without halos have formed the L(+) isomer of lactic acid.[54,60,61]

The use of MRS broth or agar has gained acceptance as the all-purpose media because of its ability to support a variety of LAB. The MRS name originates from the formula of deMan, Rogosa, and Sharpe (1960).[39,49,51,52,54,62]

MRS broth may be used as a general culture medium and also as a basal medium for performing tolerance tests such as temperature, pH, alcohol, salt, and Teepol concentration. Other biochemical tests based on MRS include arginine hydrolysis and acetoin tests.[1,19,27,39,63,57] The glucose normally found in MRS broth may be replaced with 4% sodium gluconate (Modified MRS) for the detection of gas from the fermentation of gluconate.[1,19,27,39,57,63] MRS broth with 0.4% Teepol may be used as a base medium to test for inhibition of growth in the presence of 0.4% Teepol-610, an anionic detergent consisting of alkyl sulfate.[26,64] Teepol was added to MRS broth by Nayor and Sharpe as a substitution for Wheaters bile salts tolerance test.[26,39,64,65,66,67]

One general purpose medium used for cultivating and selectively isolating the *Lactobacilli* based on low pH, the presence of Tween 80, and acetate is SL agar, an acetate medium by Rogosa, Mitchell, and Wiseman.[4,52,55] Sharpe warns that some cold meat *Lactobacilli* may not be recovered when SL agar is the only primary isolation medium. SL Agar may be used for selective isolation of the *Lactobacilli* from the oral and intestinal tract.[4,55,56]

The test for hydrogen peroxide production may be performed with Manganese Dioxide Agar (MDO Agar), a Modified MRS Agar that includes 0.75% manganese dioxide and 0.5% xanthan gum[1] or by using Modified APT with Tween. Tetramethylbenzidine Medium (TMB) has been used for the detection of hydrogen peroxide producing microorganisms from clinical sources.[1,68,69,70,71,72]

Boehringer Mannheim Biochemicals has developed a commercial test kit for the detection of the isomeric forms of lactic acid. MRS Broth may also be used for the detection of the type of lactic acid produced during glucose fermentation.[1,4,19,26,27,39,57,73,74]

The traditional method for determining gas from glucose fermentation by heterofermenters has been by either of two methods: (1) the method of Gibson and Malek[27,39,51] using a semisolid tomato juice skim milk agar, or (2) using MRS glucose broth with a vaspar overlay.[1,4,26,27,39,51] The use of Durham tubes in MRS broth with 5% glucose is a more practical method for the detection of gas formation [1,4,26,27,39,51].

Arginine hydrolysis is used as a test to separate the Heterofermentative LAB (Betabacteria) from arginine negative *Leuconostocs* and other arginine negative Streptobacteria.[39] The test may be performed with MRS base without glucose or meat extract. In addition, the 0.2% sodium citrate normally found as a ingredient in MRS broth must be replaced by ammonium citrate.[1,39] Arginine hydrolysis is detected by the addition of Nessler's reagent to 1 ml of an arginine broth culture after 2 to 7 days of incubation. This test is used primarily for *Streptococcus* spp. or *Lactobacillus* spp. and detects the presence of free ammonia, with a positive test resulting in a orange to brown color.[1,33,39,51,75] Moeller's decarboxylase test medium, designed primarily for Enterobacteriaceae, may be used for LAB.[39,76]

Sandine (personal communication, 1996) recommends King's modification of the Voges-Proskauer (VP) test for the production of acetylmethylcarbinol.[39,40,76,77,78] Facklam et al. recommends VP testing by the Coblentz method.[1,39,40,76,77,78,79,80]

The Lactic *Streptococci* may be cultivated on a number of culture media such as Semisolid Citrate Milk Agar, Arginine Tetrazolium Agar, Glucose Lemco broth, and Elliker's Lactic Agar.[39,56,81,82] The use of Elliker's Lactic agar is employed in the dairy industry for the cultivation of the two important dairy starter cultures, *Streptococcus lactis* and *Streptococcus cremoris*.[81] M17 culture medium, with a pH of 6.8, is selective for isolating *Streptococcus thermophilus* from yogurt.[83,84] This microorganism forms white, lens-shaped colonies.[56,83,84,85,86] M17 may be used as a general cultural medium for isolating Mesophilic Streptococci when the pH is adjusted to 7.2.[56,83,84,86] Yeast Glucose Agar (YGA) may also be used as a general culture medium for the Mesophilic Streptococci.[56,83,86] Methylene Blue Milk Medium (MBM) may be used to differentiate the Enterococci from Streptococcus species.[34,39,46,56,81,87]

M17 Agar with Beta-Glycerophosphate by Terzaghi and Sandine supports a more rapid growth of *Streptococcus thermophilus* than Yeast Glucose agar.[83] Another medium for isolating *Streptococcus thermophilus* from yogurt is Yeast Lactose Agar (YLA), with lactose substituted for glucose.[83] Thallous Acetate Tetrazolium Glucose Agar by Barnes[39,83,88] was originally devised for intestinal Streptococci (differentiates *Enterobacter faecalis* and *Enterobacter faecium*) but may also be used to cultivate Lactococcus spp., Streptococcus spp. other than Group D, and Lactobacillus spp. Streptococci form colonies that vary from white to pale pink or maroon.[88] Lactococci form small maroon colonies when cultured on this media.[39,71,83,84,88]

When attempting to differentiate *Streptococcus lactis* and *Streptococcus cremoris*, Reddy's agar medium provides differentiation based on colony color. *Streptococcus lactis* colonies are white; *Streptococcus cremoris* forms yellow colonies due to the fermentation of lactose.[50,89,90] Matalon and Sandine reviewed the recovery efficiency of Elliker's agar and described Elliker's Lactic agar as being well suited for the recovery of the dairy Streptococci and Lactobacilli.[81,89,91,92] Their subsequent modification of Elliker's lactic agar, called Yogurt Lactic Agar, is particularly useful in the differentiation of *Lactobacillus bulgaricus* from *Streptococcus thermophilus*. *Lactobacillus bulgaricus* produces large white colonies on YLA, whereas *Streptococcus thermophilus* forms smaller colonies that are red.[50,56,81,89,91,92,93]

The Leuconostocs may be isolated as recommended by Garvie with Yeast Glucose Phosphate Peptone Broth.[51,94] Garvie also notes that MRS broth or MRS agar are adequate for cultivating both the Leuconostocs and Pediococci.[51] The most common Leuconostocs that form dextran from sucrose are *L. mesenteroides* ssp. *mesenteroides* and ssp. *dextranicum*. They may be tested for dextran production on Trypticase Soy Agar or MRS Agar with the addition of 5% sucrose. Incubation is 3 to 5 days in an aerobic atmosphere.[1,39,51,94,95]

Some isolates of *Leuconostoc citrovorum (L. cremoris)* and *Leuconostoc oenos* may require the addition of 0.05% cysteine for growth in MRS Agar or broth.[51,87] Although all the Leuconostocs and some Pediococci grow aerobically in broth, all Leuconostocs display enhanced growth under reduced atmospheric conditions, while Pediococci produce a luxuriant growth under anaerobic conditions.[42,51,94,96,97,98,99]

V. THE STREPTOBACTERIA

The Streptobacteria consist of a large group of organisms used extensively in the manufacture of food products and industrial fermentations.[100,101,102] The Streptobacteria are found naturally associated with various plants such as cabbage (*Lactobacillus plantarum*), corn (*Streptococcus lactis*), maize silage (various Streptobacteria and Betabacteria), milk (*Lactobacillus casei*), and cheeses (*Lactobacillus plantarum* and *Lactobacillus lactis* for Cheddar cheese). The Streptobacteria are also found associated with red meat (usually atypical Streptobacteria) and with summer sausage and vacuum-packaged meats (*Lactobacillus curvatus* and *Lactobacillus sake*).[1,2,3,82,100,101,102,103,104,105,106,107]

The term Atypical Streptobacteria was reintroduced by Cavett in 1963.[108,109,110] He described these microorganisms as atypical because of their tendency to form coccoid cells when grown on low carbohydrate media, inability to grow at pH 3.9, and fermentation of both lactose and maltose but not mannitol. The Atypical Streptobacteria ferment both lactose and maltose but are unable to ferment mannitol. Typical Streptobacteria such as *Lactobacillus plantarum* and *L. casei* ferment both lactose and maltose, but differ from Atypical Streptobacteria by fermenting mannitol. Hitchner, however, found no strains of Atypical Streptobacteria capable of fermenting both lactose and mannitol.[104,108]

Over the years, various investigators have used different criteria to describe the Atypical Streptobacteria. It is difficult to compare and draw conclusions from their research.[104] Although the classic description for the Atypical Streptobacteria has little taxonomic value today, Klein et al.[102] has used the parameters of coccoid cell shape on a low carbohydrate medium, growth at 2°C but not 40°C to 42°C, and inability to grow at pH 3.9, as criteria for characterizing the Atypical Streptobacteria.[102,110]

Recently, the Streptobacteria have been found to release factors that inhibit other microrganisms, and by this process establish the desired effect of preserving some food products. These factors, called bacteriocins, inhibit the growth of *Staphylococcus aureus, Salmonella* species, *Escherichia coli*, and other microorganisms.[111] These bacteriocins may be isolated and used with or without the presence of the Streptobacteria. Two species capable of releasing such factors, *Lactobacillus helveticus* ssp. *jugurti* and the yeast *Candida utilis*, display a synergistic activity when grown together in sour milk. These two species have been shown experimentally to inhibit cell division of colon tumors in rats.[111] This gives some credence to the theory that certain food products with their indigenous flora might help to promote good health in humans.[58,111,112,113,114,115,116]

The Streptobacteria are Lactobacillus spp. which are Homofermentative and may be tentively grouped and identified as follows. They predominately grow at the temperature of 15°C and not 45°C.[1,22,26] There are a few exceptions. *Lactobacillus fermentum* is capable of growth at 45°C.[26] *L. sake*, *L. curvatus*, and *L. plantarum* are capable of growing at temperatures of 2°C to 8°C and so should be considered psychrophilic, although they grow only slowly at those temperatures.[3] The Streptobacteria ferment pentose sugars. They produce carbon dioxide from gluconate hydrolysis; however, being homofermenters, they do not produce carbon dioxide from the fermentation of glucose.[22,37]

The Streptobacteria may be differentiated from the Betabacteria by colony color when grown on Homofermentative Heterofermentative Differential Medium (HHD) containing fructose.[1,3,22,58] Homofermentative LAB form colonies on HHD that are blue to green, while heterofermenters remain white due to limited reduction of fructose to mannitol.[54,58] This is due to the ability of Homofermentative Lactics to form more acid from fructose and any available hexose. Therefore, they are capable of producing more acid, lowering the pH to 4.6, resulting in a color shift to blue to blue green. The heterofermenters, however, are only capable of reducing the pH to 5.6, and thus they form only white colonies.[54,58] The Streptobacteria ferment ribose (as do Betabacteria); however, none of the Thermobacteria do this.[1,4,22,29,30,54]

Schillinger et al. divided the Streptobacteria into two major groups based on the ability to ferment mannitol.[1] The mannitol positive Streptobacteria group contains the following: *Lactobacillus carnis*, *L. piscicola*, *L. coryniformis*, *L. plantarum*, *L. casei* spp. *casei*, *L. casei* spp. *rhamnosus*, *L. maltaromicus*, *L. casei* spp. *pseudoplantarum*, and *L. agilis*. The mannitol negative group of Streptobacteria consists of the following: *Lactobacillus bavaricus*, *L. sake*, *L. curvatus*, *L. alimentarius*, *L. casei* spp. *tolerans*, *L. yamanashiensis*, *L. amylophilus*, *L. farciminis*, and *L. sharpeae*. Those that ferment mannitol may be further separated by the production of ammonia from arginine.[1,22]

Lactobacillus plantarum, although naturally occurring in dairy products, is an important cause of spoilage in the pickle industry, where some strains are capable of decarboxylating malate to form carbon dioxide during fermentation. These strains may be detected by the use of a MD (Malate Decarboxylating) medium.[2,54,108,117,118] The malolactic fermentation is critical to the acidity as well as the sensory aspects important in the flavor of wine.[119] Certain strains of *L. plantarum* can cause ropiness,[4] acetic acid in wine, or off-flavors through aldehyde formation.[3,4,54,101,108,109,119,120]

VI. RECLASSIFIED STREPTOBACTERIA

Due to DNA Homology and physiological studies, *Lactobacillus divergens*, *Lactobacillus piscicola*, and some rod-shaped psychrophilic microorganisms were installed in a new genus, Carnobacterium.[121] This group was called "atypical meat lactics" because they were psychrophilic (produced growth at refrigeration temperatures) and had characteristics that differed from other lactobacillus spp.[1,121]

The Carnobacteria have been predominantly associated with meats, particularly poultry; investigators have also isolated these microrganisms from refrigerated, vacuum-packaged, unprocessed beef and lamb. Growth of some fastidious strains is enhanced on D-MRS Agar, pH 8.0 to 8.5.[52,55] MRS Agar, modified by substituting sucrose for glucose, adjusting the pH to 8.5, and increasing $MnSO_4$ to 0.4% improves recovery.[52] Tryptone Glucose Yeast Extract Agar, pH 7.0, is recommended for total counts from prepackaged refrigerated meats and packed meat products.[52,55,122] This medium should be incubated aerobically at 25°C to 28°C for 3 days in room air or in a reduced atmosphere (5% CO2 + H2).[3,52]

Standard I Nutrient Agar, with sucrose substituting for glucose and a pH of 8.0, or CASO-Agar, with a pH of 7.5 to 8.0, is recommended for nonselective or partial selective recovery of Carnobacteria.[52] When conventional tubed tests are used for identification, the basal medium by Shaw and Harding[55,124] or of Holzapfel and Gerber may be used for identification.[52,55,123] Carbohydrates should be filter sterilized and used at 0.5%.[55,124] The following carbohydrates are considered important for differentiation of the Carnobacterium species: amygdalin, galactose, gluconate, inulin, mannitol, melibiose, alpha methyl-D-glucoside, D-turanose, and D-xylose (Table 4.0).[52,55,59,122,124]

The common meat Lactics, *Lactobacillus carnis*, *Lactobacillus piscicola,* and *Lactobacillus divergens* differ from other Streptobacteria with the following characteristics: ability to grow at 0°C, production of ammonia from arginine, meso-diaminopimelic type peptodoglycan in their cell wall, production of L-lactate only, cellular fatty acids, fermentation of gluconate, and by the inability to grow on acetate agar (Rogosa SL Agar) or in acetate broth (Figure 2).[1,3,4,29,121,124,125] *L. divergens* is unable to grow below a pH of 6.0[123] and has an unusual optimium pH of 8 to 9.[52]

L. carnis produces shiny white colonies, 1.5 to 2.0 mm in size, on agar-based media.[123,124] *L. piscicola* was isolated from diseased rainbow trout and chinook salmon. Growth occurs on MRS Agar, Trypticase Soy Agar, and Brain Heart Infusion Agar. On Trypticase Soy Agar, *L. piscicola* forms white colonies that are pinpoint, convex, circular, and with an entire edge.[126] *L. divergens* (related to *L. piscicola*) was originally isolated from raw, vacuum-packaged, and minced meats and forms cream-colored to white, convex, shiny colonies when grown on either Standard I-Agar or MRS Agar without acetate.[52,55,123,126,127] *L. divergens*, *L. carnis,* and *L. piscicola* may be differentiated on the basis of fermentation of inulin, mannitol, and gluconate (Table 4).[121] *L. divergens*, unlike the other two species, does not ferment either inulin or mannitol. *L. carnis* and *L. divergens* both differ from *L. piscicola* in that they produce L-lactate, in contrast to *L. piscicola,* which produces DL lactate.[106,121,123,124, 125,126,127,128]

When the Carnobacteria are in the minority, Cresol Red Thallous Acetate Sucrose Agar (CTAS), with an incubation temperature of 30°C for 24 to 48 h or 25°C for 72 h may be used. Elevated pH values of 8 to 9 help promote the growth of *Carnobacterium* colonies while inhibiting *Lactobacillus* spp. from growing. The selectivity of CTAS

TABLE 4
Selected Physiological Characteristics of Species of Carnobacterium

Characteristic	*C. divergens*	*C. gallinarum*	*C. mobile*	*C. pisicola*	*C. carnis*
Growth at:					
0° C	+	+	+	+	(+)[d]
45°C	-	-	-	-	(-)[e]
Motility	-	-	+	-	(-)[e]
Voges-Proskauer test	(+/-)	+	(-/+)	+	(-)[e]
Acid produced from:					
Amygdalin	+	-	+	+	(+)[e]
L-Arabinose	-	-	-	-	(v)e
Galactose	-	+	+	+	(v)[e]
Gluconate	+	+	-	+	(+)[d]
Inulin[b]	-	-	+	+	(+)[d]
Lactose	-	-	+	+	(+)[d]
Mannitol	-	-	-	+	(+)[d]
Melezitose	(+/-)	+	-	(+/-)	(+)[d]
Melibiose	-	-	-	+	(+)[d]
α-methyl-d-glucoside	-	+	-	+	(+)[d]
α-methyl-d-mannoside	-	-	-	+	(+)[d]
Raffinose	-	-	-	(-/+)	(v)[d,e]
Sorbitol	(-/+)[c]	-	-	(-/+)	(-)[e]
d-Tagatose	-	+	(-/+)	-	(-)[d]
d-Turanose	-	+	-	(+/-)	(+)[d]
d-Xylose	-	+	-	-	(-)[e]
Growth in presence of (%):					
Cd acetate (0.1)	(-/w)	ND	ND	+	ND
Co nitrate (0.5)	-	ND	ND	+	ND
Cu sulfate (0.01)	-	ND	ND	+	ND
Mercury(II) chloride (0.5)	-	ND	ND	(w/-)	ND
Mn sulfate-7-hydrate (3.0)	-	ND	ND	+	ND
Na selenite (0.5)	w	ND	ND	w	ND
K tellurite	+	ND	ND	+	ND
Pb acetate	(-/w)	ND	ND	+	ND
Sensitivity to (in micrograms)					
Erthromycin (15)	+	ND	ND	+	ND
Gentamycin (10)	(+/-)	ND	ND	-	ND
Kanamycin (30)	(-/+)	ND	ND	-	ND
Methicilin (10)	-	ND	ND	-	ND
Nalidixix acid (30)	-	ND	ND	-	ND
Tetracycline (50)	+	ND	ND	+	ND
GC content (mol%)	33.0-36.4	34.3-36.4	35.5-37.2	33.7-36.4	34-36%

Symbols +/-, occasional strain negative, -/+, occasional strain positive; ND, no data; w. weak positive reaction.

[a]According to Feresu and Jones (1988), more than 80% of their isolates resembling *C. divergens* and *C. piscicola* grew at 45°C! [b]Hiu et al. (1984) reported that all 17 *C. piscicola* strains were negative for inulin fermentation.[c]Some strains were positive according to Feresu and Jones (1988). Compiled from Borch and Molin, 1988; W. Bosch and W.H. Holzapfel, 1985, unpublished observations; Collins et al., 1987; Feresu and Jones, 1988, Hiu et al. 1984; Holzapfel and Gerbe 1983; Shaw and Harding 1985. **Data for** C. carnis from (121)[d] & (124)[e]. Reprinted with Permission from W.P. Hammes, N. Weiss, and W. Holzapfel; the autho A. Balows, H.G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer, editors, Chapter 70, Table 9.0 p. 1577, ***The Genera Lactobacillus and Carnobacterium,*** and Copyrighted 1992, by Springer Verlag.

medium is based on the presence of thallium acetate, nalidixic acid, a high concentration of sodium citrate, and an alkaline pH. *Carnobacterium divergens* produces only pinpoint colonies on CTAS medium, and this reduced recovery may be compensated for by reducing the thallous acetate concentration to 0.5 g/L or less. This Modified CTAS is not completely inhibitory to the Leuconostocs and Enterococci. The substitution of 2% inulin for sucrose in CTAS Medium differentiates *C. piscicola* from the

Enterococci. *C. piscicola* ferments inulin, whereas the Enterococci do not. *C. piscicola* produces small, yellowish to pinkish colonies with a metallic bronze sheen, a yellow color change of the medium, and a clearance of precipitate. *C. piscicola* forms what has been described as a umbolate or beta type colony when inosine is substituted for sucrose in CTAS Agar. *C. divergens* strains form sparser growth, a metallic bronze sheen, and often no color change of the medium. The Enterococci also produce a yellowing of the medium and a clearing of the precipitate but do not have a metallic sheen.[52]

VII. REPRESENTATIVE STREPTOBACTERIA

Mannitol Fermenters

The first major branch of the Streptobacteria ferments mannitol, whereas the second major branch is comprised of mannitol nonfermenters. The mannitol fermenters consist of *Lactobacillus coryneformis, L. plantarum, L. casei* ssp. *casei, L. casei* ssp. *rhamnosus, L. maltaromicus, L. casei* ssp. *pseudoplantarum, Lactobacillus carnis*, and *L. piscicola*.[1]

L. coryneformis has pear-shaped cells and has been isolated with fermented sausage.[1,30] Although similiar to *L. divergens* and *L. carnis*, *L. coryneformis* produces D(L) lactate and is readily differentiated from the previously mentioned meat Lactics by its inability to ferment ribose (Table 5). This species produces colonies on agar media that are raised, smooth, compact, and white.[1,2,3,30,103,128,129]

A common milk Streptobacterium, *L. plantarum*, is not only found in milk but also in meats, fermented sausage, pickles, and pickled cabbage.[26] *L. plantarium* ferments melibiose, raffinose, and grows in 0.4% teepol.[3,26,30,43] *L. plantarium* produces colonies on Blood Agar and Nutrient Agar that are raised, smooth, circular, with an entire edge, and may be slight to dark yellow in color.[3,30,43,130] This species is unable to produce gas from citrate, unlike the similar Streptobacterium, *L. casei* ssp. *casei*.[26,30,64] Two subspecies of *Lactobacillus casei* are *L. casei* ssp. *casei* and *Lactobacillus casei* ssp. *pseudoplantarum*.[1,30] *L. casei* ssp. *casei* produces L-lactate, and *L. casei* ssp. *pseudoplantarum* produces DL-lactate [1,3].

Lactobacillus casei ssp. *casei* forms short to long rods with square ends, whereas *L. plantarum* forms small chains with short round ends.[30] *L. casei* is found in milk, cheese, other dairy products, in the dairy environment and is also found in meats.[1,26,30,43] *L. casei* ssp. *casei* forms two colony types, lens or disc shaped, and a type that upon age, forms filaments that radiate outward from the colony.[26,30,43,66,131] *Lactobacillus casei* ssp. *alactosus* differs from *L. casei* ssp. *casei* by the former's inability to ferment lactose.[3,30,128,132] Bergey's[3] indicates that *L. casei* ssp. *alactosus* is no longer recognized as a separate taxon and instead should be considered *L. casei* ssp. *casei*, as originally described by Orla-Jensen.[3,26,132] Bergey's previously differentiated *L. casei*, *L. alactosus*, and *L. rhamnose* by their ability to grow at 15°C and 45°C and by the fermentation of lactose and rhamnose.[1,3,26,30,128,132] *Lactobacillus casei* ssp. *rhamnosus* grows at both 15°C and 45°C and may be identified biochemically by its negative raffinose fermentation, ability to ferment rhamnose unlike *L. plantarium*, and has meso-diaminopimelic acid in its cell wall.[1,3,26,30]

Collins et al.[133] tried to resolve confusion over the subspecies of *Lactobacillus casei*. Collins proposed that the subspecies *L. casei* ssp. *alactosus, L. casei* ssp. *pseudoplatarum, L. casei* ssp. *rhamnosus, L. casei* ssp. *tolerans* be raised to species status, based on their low homology with the type strain NCDO 161. They proposed a new species, *L. paracasei* ssp., *paracasei,*

TABLE 5

Streptobacteria

	n-acetyl-glucosamine	amydallion	arabinose	Arginine	cellobiose	m-DAP	esculin	fructose	galactose	glucose	gluconate	D-lactate	L-lactate	DL-lactate	lactose	maltose	mannose	mannitol	melibiose	10%NaCl	12%NaCl	raffinose	rhamnose	ribose	salicin	starch	sucrose	trehalose	xylose	15°C	45°C
L. agilis	-	+	-	+	+	(+)	+	+	+	+	-	+	+	+	(+)	+		+	(+)			+	-	+	+		+	+	-	-	+
L.alimentarius	(+)[a]	+	-	-	+	-	+	+	+	+	+	+	+	-	(+)[w]	+	+	-	-	+	-	-	-	+	+	(+)[w]	+	+	-	+	-
L. amylophilus		-	-		-	-	-	+	+	+	-	-	+	-	-	+	+	-	-			-	-	-	-	+	-	-	-	+	-
L. barvaricus		-	-	-	+		+	+	+	+	+	-	+	-	+	+	+	-	+			-	-	+	+		+	-	-	+	-
L. bifermentans		-	-	-	-	-	-	+	(+)	+	-	-	-	+	-	+	+	+	-			-	+	+	-	-	-	-	-	+	-
L. casei ssp. *casei*	+	+	-	-	+	-	+	+	+	+	+	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	-
L. casei ssp. *casei* *pseudoplantarum*		+	-	-	+	-	+	+	+	+	(+)	-	+	+	+	+	+	+	-			-	-	+	+	-	+	+	-	+	-
L. casei ssp. *tolerans*		-	-	-	-	-	-	+	+	+	(-)	-	+	-	+	-	-	-	-			-	-	-	-	-	-	-	-		
L. coryneformis ssp. *coryneformis*		-	-	-	-	-	-	(+)	(+)	+	(+)	+	+	-	+	(+)	+	+	d	+	-	d	+	-	d		+	-	-	+	-
L. coryneformis ssp. *torquens*	-	-	-	-	+		-	(+)	(+)	+	-	+	-	-	+	-	-	-	-			-	-	-	-		-	-	-		
L. curvatus	(+)[a]	-	-	-	+	-	+	+	+	+	+	-	-	+	(d)	+	+	-	(d)	-	-	-	-	+	+	(-)[a]	-	-	(-)[a]	-	-
L. farciminis		+	-	+	+	-	+	+	+	+	-	+	+	-	+	+	+	-	-	+	-	-	-	-	+	(-)[a]	+	+	-	+	-
L. graminis		+	-	-	+	-	+	+	+	+	(-)	-	-	+	+	-		-	-			-	-	-	+		-	+	+	+	-
L. hamsteri		+	+		+	-	+		(+)		+	-	-	+		+		-	+			+		+	+	ND	+	d	d	-	ND
L. homohiochii		-	-	-	d	-	+	+	-	+	-	-	-	+	-	+	+	-	-			-	-	+	d	-	-	d	-	+	-
L. intestinalis		-	-		d	-	-				ND	-	-	+				+	d			d		d			+		-	-	+
L. maltaromicus		+	-		+	+		+	+	+	+	-	-	-	+	+		+	+			-	-	+	+		+	+	-	+	-
L. murinus		d	+	-	+	-	+	+	+	+	+	-	+	-	+	+	+	d	+			+	-	+	d		+	d	-	-	+
L. paracasei spp. *paracasei*	+	+	-	-	+	-	+	+	+	+	(+)	-	+	+	+	+	+	+	-			-	-	+	+	-	+	+	-	+	d
L. paracasei ssp. *tolerans*	+	-	-	-	-	-	-	+	+	+	-	-	+	-	+	-		-	-			-	-	-	-	-	-	-	-	+	-
L. pentosus	+	+	-	-	+	+	+	+	+	+	(+)	+	+	-	+	+	+	+	+	-	-	+	-	+	+	-	+	+	+	-	
L. plantarum	+	+	d	-	+	+	+	+	+	+	(+)	-	-	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	-	+	-
L. rhamnosus	+	+	-	-	+	-	-	+	+	+	(+)	-	+	-	+	+	+	+	-			-	+	+	+	-	+	+	-	+	+
L. sake	+	+	+	-	+	-	+	+	+	+	+	-	-	+	+	+	+	-	+	+		-	-	+	+		+	+	-	+	-
L. sharpeae		+	-	-	+	+	+	+	+	+	(-)	+	+	(-)	+	+	+	-	-			-	(-)	-	+		-	-	-	+	-
L. yamanashiensis		+	-	-	d	+	+	+	d	+	(-)	-	+	+	-	(-)	+	-	-	-	-	-	d	-	+		+	+	-	+	-

*+, 90% or more of the strains positive; -, 90% or more of the strains negative, d, 11-89% of strains positive; (d), delayed reaction, ND, no data, w = weak positive.

Data from Atlas 1993 (56), Beck 1988 (276), Bringel 1996 (280), Carr 1977 (143), Collins 1989 (133), Cox et al. 1990 (268), Curk 1996 (279), Facklam 1995 (40), Hammes 1992 (55), Kandler 1986 (3), Kandler 1983 (11), Klein 1996 (102), [a]Maida 1999 (296), Miller 1974 (135), Reuter 1983 (146), Sharpe 1981 (4), Stetter 1980 (137), Weiss 1981 (148), Wood & Holzapfel 1995 (134), and Zanoni 1987 (278).

based on low homology with the type strain *L. casei* ssp. *casei* (strain NCDO 161).[55,133,134]

L. paracasei ssp. *paracasei* is nonmotile and grows at 10°C and 40°C. It produces L-(+) lactic acid. It forms acid from amygdalin, arbutin, cellobiose, D-fructose, galactose, D-glucose, maltose, mannitol, D-mannose, melezitose, *N*-acetylglucosamine, salicin, D-turanose, and trehalose. It fails to hydrolyze arginine, esculin, or urease.[55,133,134]

Lactobacillus rhamnosus is a Heterofermenter and forms rod-shaped cells that are nonmotile. Most strains grow at 10°C, some at 15°C and 45°C. It produces L-Lactic acid and ferments amygdalin, arbutin, cellobiose, D-fructose, galactose, beta-gentiobiose, gluconate, D-glucose, lactose, mannitol, D-mannose, maltose, melezitose, *N*-acetylglucosamine, rhamnose, ribose, salicin, sorbitol, D-turanose, and trehalose. *L. rhamnosus* hydrolyses neither arginine nor urea but hydrolyzes esculin.[133]

Lactobacillus maltaromicus is isolated from milk. It forms both smooth and rough colonies, with smooth types forming colonies that are white, opalescent, and with an entire edge or slightly fimbriate margin. The rough colony types are fimbriate with a ground glass appearance.[135] It ferments a number of carbohydrates, including ribose, but is negative for raffinose and rhamnose.[135] The malty aroma formed by *L. maltaromicus* is believed due to the production of aldehydes.[1,3,37,128,135,136]

VIII. REPRESENTATIVE STREPTOBACTERIA

Mannitol Nonfermenters

The second major branch of the Streptobacteria are mannitol nonfermenters; they are *L. bavaricus*, *L. sake*, *L. curvatus*, *L. alimentarius*, *L. casei* ssp. *tolerans*, *L. yamanashiensis*, *L. amylophilus*, *L. farciminis*, and *L. sharpeae*. The first subbranch all ferment ribose and differentiated by their ability to grow at 8°C form L or DL lactate, and fermentation of a variety of sugars such as ribose, melibiose, maltose, sucrose, trehalose. The second subbranch, the ribose nonfermenters, are differentiated by the fermentation of maltose, lactose, cellobiose, and arginine hydrolysis (Table 5; Figure 2).[1,137]

Lactobacillus bavaricus, the sauerkraut bacillus, is used for the production of sauerkraut and produces L(+) lactic acid. *L. bavaricus* forms smooth colonies that lack pigmentation on agar, grows optimally at 30°C to 34°C, and can grow from 2°C to 37°C but not at 45°C. Both *L. sake* and *L. curvatus* are similiar to *L. bavaricus* in that all three grow psychrotrophically, but the two former species produce DL-lactate in contrast to *L. bavaricus,* which produces L lactate.[28,137] Both *L. sake* and *L. curvatus* are the dominant bacterial flora of fermenting sausages and vacuum-packaged meats.[1,3,22,28,107,138,139] *L. sake and L. curvatus* are capable of growing anaerobically at refrigeration temperatures and are important in the spoilage of vacuum-packaged meats.[1,3,139]

L. sake is Homofermentative, producing D- and L-Lactic acid, with growth at 4°C, 15°C, but not at 45°C. This microorganism will also grow at pH 3.9, and in 8% NaCl but not 10% NaCl.[102,107,139,140,141]

L. curvatus occurs in pairs, has a curved shape, and may form unusual closed rings consisting of four cells making up a horseshoe form.[3,102] *L. curvatus* may initially be motile but may lose its motility after subculture.[3,30] Its colonies are similiar in morphology to *L. plantarum* but are smaller in size.[30] *L. curvatus* hydrolyzes esculin, ferments salicin, ribose, mannose, maltose, cellobiose, fructose, and galactose, and does not form gas from glucose or gluconate fermentation.[1,3,102,139]

The alcohol-tolerant (10% to 20%) *L. yamanasheinsis* is mainly associated with wine and other alcoholic beverages. *L. yamanasheinsis* forms colonies that are white, smooth, and glistening.[3,4,130,142,143,144,145]

L. alimentarius is a meat species that produces acetoin, forms internal granules, is nonmotile, microaerophilic, Homofermentative, and produces predominately L(+) lactic acid.[146] When grown on MRS Agar, it forms greyish white, circular, convex colonies, 2 mm in diameter, with an irregular margin.[146] This species grows at 15°C but not 42°C, and grows in 10% NaCl (Table 5).[1,3,52,101,110,146]

Lactobacillus paracasei ssp. *tolerans* (formerly *L. casei* ssp. *tolerans*) is found in dairy products and forms cells that occur singly or in chains.[30,133] It is nonmotile and grows at 10°C and 37°C, but not at 40°C. This microorganism ferments D-fructose, galactose, D-glucose, lactose, D-mannose, *N*-acetylglucosamine, and tagatose. It neither hydrolyzes arginine, esculin, nor urea.[55,133,134]

L. amylophilus forms smooth, white, convex colonies that are translucent with an entire edge. This species demonstrates starch hydrolysis on modified MRS medium with starch or on LBS-Corn Starch Agar.[38,147]

L. farciminis may serve as a sausage starter culture. *L. farciminis* is a microaerophile that produces greyish white pinpoint colonies with irregular margin on MRS Agar and forms metachromatic granules. It grows in the presence of 10% NaCl and tolerates up to 12% salt.[1,134,146] *L. farciminis* hydrolyzes arginine, *L. sharpeae* and *L. amylophilus* do not.[1]

L. sharpeae grows at 15°C and forms colonies on agar that are greyish and flat. Microscopically, *L. sharpeae* exhibits elongated chains of cells which have been termed "snakes"; wrinkled chains are produced after prolonged incubation.[148] The important biochemical tests for *L. sharpeae* are ribose (–), arginine (–), lactose (+), and cellobiose (+).[1]

L. agilis is a relatively new species. This species, as well as *L. sharpeae*, was originally isolated from sewage.[148] *L. agilis* is one of the few Streptobacteria that grows well at 45°C. *L. agilis* forms colonies on agar that are flat and rough.[148]

Lactobacillus homohiochii is a facultative heterofermenter.[55,145] It may be cultured on Liver Infusion Sake Medium and on Rogosa SL broth with the addition of DL-mevalonic acid (30 mg/liter).[3,55,56,145,149] Modified Homohiochii medium may be used for Obligately Heterofermentative Lactobacilli.[55,150] This microorganism does not grow in MRS Broth.[1,3,55,56,145,147]

In summary, the Streptobacteria grow at 15°C and produce gas from the fermentation of gluconate but not glucose.[1,38] They may be subdivided based on mannitol fermentation and still further differentiated by ribose and arginine hydrolysis. The differential biochemical characteristics of the Streptobacteria are presented in Table 5. The presence of meso-diaminopemilic acid in the cell wall is helpful in the differention of the species.[1,38,55,56,145]

IX. REPRESENTATIVE STREPTOBACTERIA

Culture Media

Streptobacteria isolation may be enhanced by a number of enrichment broths, depending on the type of food. In general, the Streptobacteria may be enriched by the use of MRS Broth. The addition of cycloheximide inhibits yeast.[49,59,152,153,154,195] Media for the enumeration and differential selective culture of the Streptobacteria are shown in Table 12 and 13.

Tomato Juice Medium, Apple Juice Medium (Carr and Davis, 1970), Fructose and Tween 80 Medium, Modified Homohiochii Medium, Sucrose Agar, Lactic Bacteria Broth, and MRS Agar have all been used for the isolation of Lactobacillus spp. from wines (Tables 12 and 13).[3,42,49,52,55,56,59,87,150,152,154,195,197,203] Liver Infusion Sake Medium has been used to cultivate the rice wine isolate, *Lactobacillus homohiochii*, and MRS Agar with 20% tomato juice, for Austrian wines.[55,56,87,145,203]

For the isolation of Lactobacilli from beer, MRS with Beer,[55] NBB, Raka Ray No.3 Medium, and Sucrose Agar have been recommended.[42,52,55,194,203]

Lactobacillus Heteroferm Screen Agar and Modified MRS Agar with 0.5% fructose may be used to facilitate the growth of Heterofermentative Lactobacilli from foods, for example, salad dressings.[56,158] The Association for Dressings and Sauces recommends the use of Lactobacillus Selective Agar, Rogosa, and Modified Rogosa (LBS Agar with 0.5% fructose) for the isolation of Lactobacillus from cheeses, dairy products, relishes, sweetners, tomato products, vegetables, and eggs.[4,39,52,54,56,158,159]

Lactobacillus spp. may be isolated from meat with a variety of culture media such as MRS Broth or Agar, Lactobacillus Agar, 0.04% Sorbate (LaS), Nitrite Polymyxin Agar, Brain Heart Infusion Yeast Extract Agar, MRS with pH 5.5, Acetate Agar, APT, SL Medium, MRS with 0.1% sorbate.[4,49,52,55,59,71,108,160] *L. sanfrancisco* (sourdough), *L. fructivorans, L. fermentum, L. brevis, L. pontis,* and *L. reuteri* may be cultivated from sourdough with Sanfranciso medium.[161,162,163]

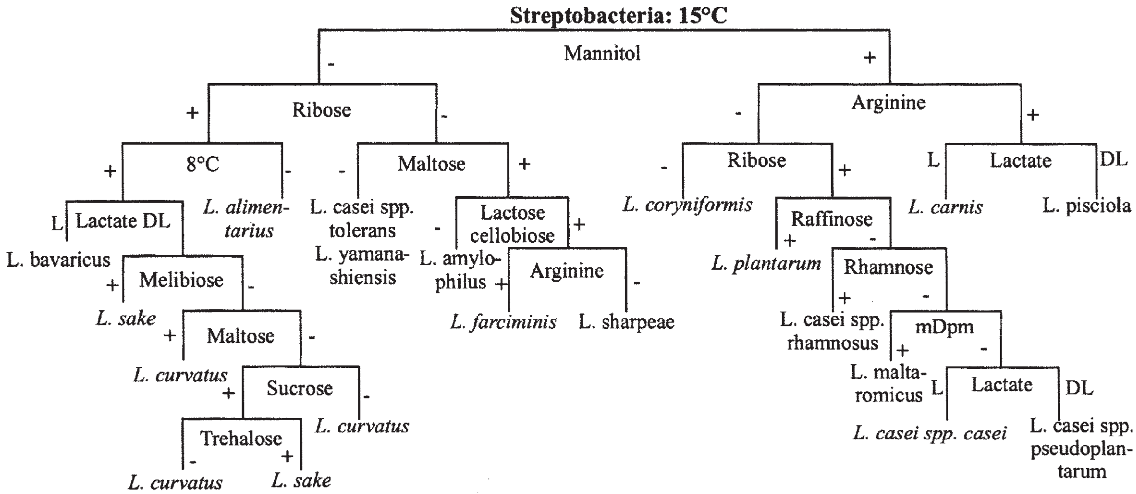

FIGURE 2. Scheme for rapid identification of streptobacteria from meat and meat products. 8°C: growth at 8°C, 15°C: growth at 15°C; arginine: hydrolysis of arginine; lactate: production of lactate: D or L: the isomere recorded makes up 90% or more of the total lactic acid; D(L) or L(D): the isomere given in brackets makes up 15 to 20% of total lactic acid; DL: 25 to 75% of total lactic acid are of the L-configuration; ribose: production of acid from ribose; mDpm: presence of meso-diaminopimelic acid in cell wall. Names of species most common on meats are italicized. (Reprinted with permission from Dr. Schillinger, the author and from the article *Identification of Lacobacilli from Meats*. Figures 1 to 3, 4:199–208. Copyright 1987. Academic Press, London.)

X. REPRESENTATIVE BETABACTERIA

The Betabacteria, Figure 3, constitute the Lactobacillus species that produce carbon dioxide from the fermentation of glucose and often hydrolyze arginine. These microorganisms require thiamine for their nutrition.[3,22,55,56,87,145] *Lactobacillus brevis* and *Lactobacillus fermentum* are important in the production of fermented feeds, *L. brevis*, and *L. carnis* in fermented meats, *L. sanfrancisco* in sour dough, and *Lactobacillus helviticus* and *Lactobacillus brevis* in milk and cheese products.[1,3,22,101,161] *L. brevis*, *L. buchneri*, and *L. fermentum* are also important spoilage microorganisms in the beer industry where they produce off-flavors and unwanted beer turbidity.[164] *Lactobacillus viridescens* causes greening of meats,[30] and *L. brevis* causes spoilage of marinated herring.[3,22,30,55,56,145,164] Generally, the Betabacteria may be cultivated in MRS broth, Betabacterium medium, or on MRS Agar.[1,52,55,56]

XI. REPRESENTATIVE BETABACTERIA

Major Groups

The Betabacteria according to Schillinger[1] may be divided into two major groups based on the ability to ferment ribose and hydrolyze arginine. The arginine and ribose negative group include the following: *L. sanfrancisco*, *L. fructosus*, and *L. viridescens*. The second group ferments ribose and hydrolyzes arginine and includes: *L. confusus*, *L. minor*, *L. carnis*, *L. divergens*, *L. kandleri*, *L. halotolerans*, *L. hilgardii*, *L. fructivorans*, *L. buchneri*, *L. brevis*, *L. kefir,* and *L. collinoides*.

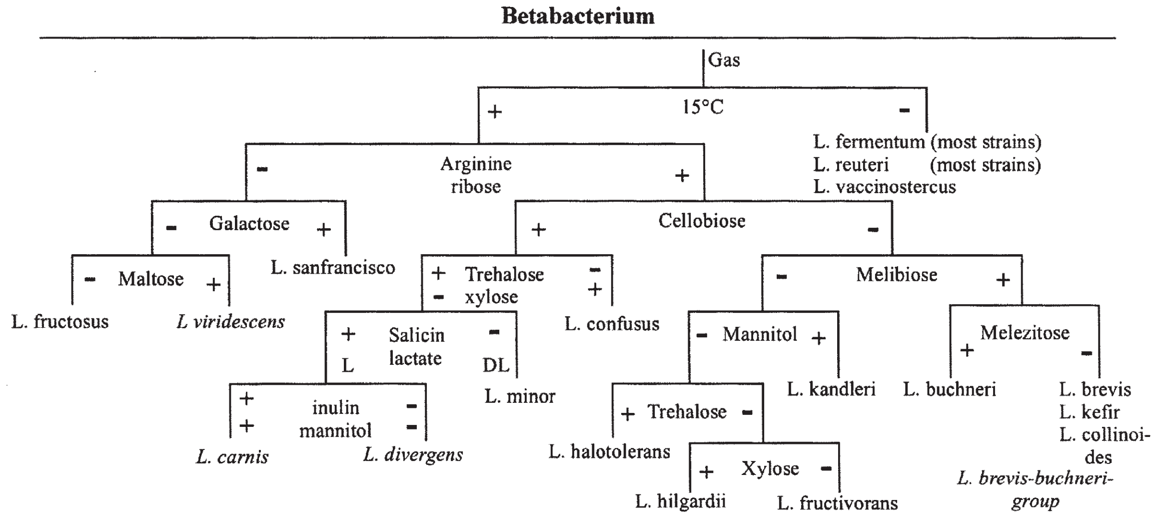

FIGURE 3. Scheme for rapid identification of heterofermentative lactobacilli from meat and meat products. 15°C: growth at 15°C; arginine: hydrolysis of arginine; lactate: production of lactate: D or L: the isomere recorded makes up 90% or more of the total lactic acid; D(L) or L(D): the isomere given in brackets makes up 15 to 20% of total lactic acid; DL: 25 to 75% of total lactic acid are of the L-configuration; ribose: production of acid from ribose; mDpm: presence of meso-diaminopimelic acid in cell wall. Names of species most common on meats are italicized. (Reprinted with permission from Dr. Schillinger, the author and from the article *Identification of Lacobacilli from Meats*. Figures 1 to 3, 4:199–208. Copyright 1987. Academic Press, London.)

XII. BETABACTERIA

Arginine and Ribose Negative

Lactobacillus sanfrancisco (sourdough), *Lactobacillus fructosus* (grape and wine) and *Lactobacillus viridescens* (spoilage of meats), unlike other Betabacteria, are unable to ferment ribose or hydrolyse arginine.[1,3,4,63,162,165,166,167] These three species may be differentiated based on the fermentation of galactose and maltose. *L. sanfrancisco* differs from the others by fermenting galactose, whereas *L. viridescens* ferments maltose and *L. fructosus* do not. *L. sanfrancisco* is unique from the other two species because its need for fresh yeast extractives (FYE) and may be cultured on a Modified MRS Medium with maltose substituted for glucose, with fresh 15% FYE and pH of 5.5.[1,3,63,161,162,165] *L. viridescens* forms white colonies on MRS Agar that are smooth, compact, and nonpigmented.[1,30] It may be cultivated with AOAC Lactobacilli Broth or Agar.[1,26,30,56,63,87,128]

XIII. BETABACTERIA

Arginine and Ribose Positive

Cellobiose Fermenters

The cellobiose fermenters may be divided further into species based on their fermentation pattern of trehalose and xylose, salicin and lactate formed (L or DL), and inulin with mannitol. They include the following: *L. confusus*, *L. minor*, *L. carnis*, and *L. divergens*. The cellobiose nonfermenters are identified by the fermentation of melibiose, mannitol, trehalose, xylose, and melibiose coupled with melezitose and include: *L. kandleri*, *L. halotolerans*, *L. hilgardii*, *L. fructivorans, L. buchneri, L. brevis*, *L. kefir,* and *L. collinoides* (Figure 3).

Lactobacillus confusus, which originally was named because it was confused with a Leuconostoc species, may be isolated from sugar cane, carrot juice, and juice-producing fruits.[51,55,145] *L. confusus* forms dextran slime from sucrose; the slime ruins the juice product.[1,4,51,145,168]

Lactobacillus minor was originally isolated from milk sludges.[1,138] *L. minor* is noted for its irregular swollen, tapered rods.[1,55,138,145,168] The differential characteristics are shown in Table 6.

Both *L. carnis* and *L. divergens* produce L-lactate from glucose and ferment salicin, whereas *L. minor* produces solely DL lactate and is negative for salicin.[1,3,30,121,123,124,125,138,145,168] *L. carnis* and *L. divergens* may be separated by inulin and mannitol; *L. carnis* fermenting both inulin and mannitol, whereas *L. divergens* is negative for both, Table 4.[1,3,121,123,124,125,168]

Most Betabacteria do not grow at 45°C; however, *L. fermentum*, *L. reuteri*, and *L. vaccinostericus* grow at this temperature and may be readily differentiated from other Betabacteria by their colony morphology.[1,22] *L. fermentum* forms small, spherical colonies that are translucent like “droplets of water”.[26,30,64,169] Other *L. fermentum* characteristics are given in the following references.[1,3,26,30,55,121,145,170,171,276] *Lactobacillus vaccinostericus* was originally isolated from cow dung. This species, when cultured on a

TABLE 6

Betabacteria

	n-acetyl-glucosamine	amydallion	arabinose	arginine	cellobiose	esculin	fructose	galactose	glucose	gluconate	inulin	D-lactate	L.lactate	DL-lactate	maltose	mannose	mannitol	melibiose	melezitose	raffinose	rhamnose	ribose	salicin	10 % NaCl	sucrose	trehalose	xylose	15°C	45°C
L. brevis	-	-	+	+	-	d	+	d	+	+	-	-	-	+	+	-	-	+	-	d	-	(+)	-	-	d	-	d	+	-
L. buchneri	+	-	+	+	-	d	+	d	+	+	-	-	-	+	+	-	-	(+)	(+)	d	-	+	-		d	-	d	+	-
L. collinoides	+	-	+	+	-	+	+	+	+	+	-	-	-	+	-	-	-	(+)	(-)	-	-	+	d	-	-	-	+	+	-
L. confusus	-	+	-	-	+	+	+	+	+	+		-	-	+	+	+	-	-	-	-	-	+	+		+	-	+	+	+
L. fermentum	-	-	d	+	d	-	+	+	+	+	-	-	-	+	+	w	-	+	-	+	-	(+)	-		+	(d)	(d)	-	+
L. fructivorans	-	-	-	+	-	-	+	-	+	d	-	-	-	+	d	-	-	-	-	-	-	(+)	-		(d)	-	-	+	-
L. fructosus			-	-	-	ND	+	-	+	-		+	+	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
L. halotolerans		-	-	+	-	-	+	-	+	+	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	+	-	+	-
L. hilgardii		-	-	+	-	-	+	d	+	(+)	-	-	-	+	+	-	-	-	d	-	-	(+)	-	-	(d)	(+)	+	+	-
L. kandleri	+	-	+	+	-	-	+	+	+	+	-	-	-	+	-	-	+	-	-	-	(+)	+	-		-	-	-	+	-
L. kefir		-	(d)	+	-	-	+	-	+	+	(-)	-	-	+	+	-	-	+	-	-	-	+	-	-	-	-	-	+	-
L. malefermentan	+	-	-	+	-	-	-	-	+	(d)	+	-	-	+	(+)	-	-	-	-	-	-	+	-		-	-	-	+	-
L. minor		-	-	+	+	+	+	-	+	+	-	-	-	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	-
L. oris	-	+	-	-	-	d	+	+	+	+	-	+	+	-	+	-	-	+	-	+	-	+	-		+	-	(+)	-	-
L. parabuchneri	-	-	(+)	+	-	-	+	+	+	+	-				+	ND	+	+	+	+	-	+	-		+	-	-	+	ND
L. pontis	-		-	+	-	-	+	+	+	-		+	+	-	+	-	-	+	-	+	(+)	+			-		-	+	+
L. reuteri		-	+	+	-	ND	+	+	+	+		-	-	+	+	-	-	+	-	+	-	+	-		+	-	-	-	+
L. sanfrancisco			-	-	-	-	-	+	+	+		-	-	+	+	-	-	-	-	-	-	-	-		-	-	-	+	-
L. suebicus		-	+		(+)	-	-	+	+			-	-	+	+		-	+	-	-		+	-		d	-	+	+	d
L. vaginalis	-	-	-	ND	-	d	+	+	+	-	-	+	+	-	+	+	-	+	-	+	-	+	-		+		-	-	+
L. vaccinostercus	ND	-	+	-	(+)	-	(-)	(+)	+	+	-	-	-	+	+	-	-	-	-	-	-	+	-		-	-	+	-	-
L. viridescens		-	-	-	-	-	+	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	+	-

*+, 90% or more of the strains positive; -, 90% or more of the strains negative, d, 11-89% of strains positive; (d), delayed reaction, ND, no data, w= weak positive reaction.

Data from Atlas 1993 (56), Beck 1988 (276), Carr 1972 (155), Collins 1989 (133), Curk 1996 (279), Doring 1988 (128), Entani 1986 (258), Hammes 1992 (55), Holzapfel 1983 (123), Kandler 1983 (138), Kandler 1983 (170), Kandler 1986 (3), Miller 1974 (135), Nakamura 1979 (38), Nonomura 1983 (144), Reuter 1983 (146), Schillinger 1987 (1), Sharpe 1981 (4), Stetter 1980 (137), Weiss 1981 (148), Weiss 1983 (167), Zanoni 1987 (278).

Xylose-YP Agar with $CaCO_3$, completely dissolves the media around the colonies.[1,56,145,172]

L. buchneri forms colonies on agar that are adherent, rough, flat, and white to yellowish.[1,3,30,145,169,170] *L. buchneri* ferments both melezitose and melibiose.[3,26]

XIV. BETABACTERIA

Arginine and Ribose Positive

Cellobiose Negative and Melibiose Positive

Betabacteria that hydrolyze arginine and ferment ribose may be divided further into subgroups based on cellobiose and melibiose fermentation. The branch of the cellobiose negative Betabacteria is often described as the Lactobacillus brevis-buchneri group. These species ferment melibiose, with *Lactobacillus buchneri* and *L. collinoides* being separated from the others by its fermentation of melezitose.[1,3,26,30] *L. collinoides* is found with apple cider.[197,145] This microorganism forms heaped colonies on Apple Juice Yeast Extract Agar and may be cream to buff in color, Table 6, Table 12, and 13.[3,4,26,55,145,148,197]

XV. BETABACTERIA

Arginine and Ribose Positive

Cellobiose and Melibiose Negative

The other branch of the Betabacteria cellobiose negative group are melibiose negative and may be separated further with mannitol, trehalose, and xylose.[1] The slime-producing *L. kandleri* was originally described by Holzapfel and Van Wyh.[168] *L. kandleri*, when grown on MRS Agar, forms colonies that are cream colored to translucent, flat, and 1 mm to 2 mm in diameter.[1,168] *L. halotolerans* was originally described as a subspecies of *L. viridescens* and microscopically tends to form coiling chains that lump together.[1,3,138,168] *L. halotolerans* and *L. hilgardii*, *L. viridescens*, and *L. fructivorans* are like the majority of the Heterfementative Lactobacillus spp. in fermenting maltose[168] but not fermenting mannitol Table 6. On MRS media, *L. halotolerans* forms greyish white, smooth colonies whose growth is enhanced anaerobically. The temperature range is 10°C to 40°C; optimium growth occurs at 30°C. It tolerates 12% NaCl, and weak growth occurs at 14% NaCl.[1,3,30,138,168]

Lactobacillus hilgardii and *Lactobacillus fructivorans* are associated with grapes and preserves. Both fail to ferment mannitol or trehalose.[1,4] *L. fructivorans* forms curved and or coiled filaments that may reach a length of 20 µm.[1,30,167] It forms colonies that are circular, raised, smooth, white, and with an entire edge. This species exhibits a unique "snowflake growth" when inoculated into freshly boiled broth prior to inoculation.[173] *L. hilgardii* ferments xylose, while *L. fructivorans* does not.[1] *L. hilgardii* is capable of tolerating up to 20% alcohol.[30,163] When cultured on Tryptone Yeast Extract Glucose Agar or similiar medium, *L. hilgardii* forms colonies that are raised and vary from hyaline to white to cream colored, with an entire edge.[1,8,30,39,49,1307,163,174] *L. fructivorans* may be cultivated on a Lactobacillus Sake Medium.[3,30,56,87]

The wine isolates, *L. fructivorans*, *L. desidiousis* (reclassified as *L. kefir*), *L. hilgardii*, and *L. brevis,* may be differentiated by their fermentation patterns for arabinose and xylose: – –, – +, + –, ++, respectively.[1,3,4,170] *L. kefir* forms greyish, smooth, flat colonies on MRS Agar.[3,30,170] With *L. brevis*, some strains are pigmented orange to red and are rough to intermediate, flat, and almost transparent.[1,3,4,30,163,170,175] *L. desidiosus*, a heterofer-menter, was redescribed by

Kandler and Kunath[170] as *L. kefir* because of its isolation from kefir.[1,3,4,30,170,174] The term kefir is derived from the Turkish noun that means "Caucasian sour milk".[170] *L. kefir* frequently produces volutin granules and forms smooth greyish colonies that are flat and 2 to 4 mm in diameter when cultured on MRS Agar (Table 6, Table 12, and 13.[3,165,170]

XVI. REPRESENTATIVE THERMOBACTERIA

The thermobacteria are Gram-positive bacilli grow at or above 45°C and are important in fermented beverages (alcoholic and nonalcoholic) and in various cheeses and other dairy products. They were originally described by Orla-Jensen in 1919.[1,2,3,22,26,30,36,37] The colonial morphology of the thermobacteria vary depending on the type of media used. Two colonial types that may occur are the smooth or rough forms.[1,3,22,26,30,36,37]

Most thermobacteria are long straight bacilli; however, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *L. delbrueckii* ssp. *lactis* may palisade ("line up like fence posts").[22,26,37] *Lactobacillus delbrueckii* ssp. *bulgaricus* develops a bizarre microscopic morphology described as "corkscrew forms".[37] Thermophilic Lactobacilli are commonly found in potatoes, mash grains, wort, rye, bourbon, and dairy products (yogurt, kefir, and cheeses).[1,3,4,26,30,36,37]

SL medium with 0.5% meat may be used for the Thermophilic Lactobacilli, particularly *L. delbrueckii* ssp. *bulgaricus* from yogurt.[55,145] The thermobacteria are Homofermentative, do not ferment pentoses or gluconate, and ferment glucose without the formation of carbon dioxide.[22,37] Thermobacteria have been subdivided into species based on type and amount of lactic acid formed from glucose, the presence of metachromatic granules, the fermentation of amygdalin, cellobiose, maltose, salicin, sucrose, and the hydrolysis of esculin.[3,22,26,30,37,176] The differential biochemical characteristics for these species are shown in Table 7. Metachromatic granules are prominently produced by *Lactobacillus lactis*, *L. leichmanni,* and *L. bulgaricus* when grown on a low glucose agar.[26,176] The presence of metachromatic granules may be observed microscopically when a slide specimen is heat fixed and then stained with a dilute methylene blue stain.[22,26,37,176,177] Sharpe advanced the study of the thermobacteria by reviewing the characteristics of many of the species.[26]

XVII. REPRESENTATIVE THERMOBACTERIA

Differential Characteristics

L. helviticus is microaerophilic and is found associated with dairy products.[3,30,169,178] *L. helviticus* forms colonies on agar that vary in color from white to light gray and rough to rhizoid in appearance.[30,178] Both *Lactobacillus jugurti* and *L. helviticus* produce large amounts of acid in milk.[26,30] *L. jugurti* is considered a biotype of *Lactobacillus helviticus*, differing only from *L. helviticus* by not fermenting maltose.[26,30] *L. bulgaricus* forms colonies that are small, flat, and yellowish. Its habitat includes the surface of plants, animals, and grains, as well as normal oral, gut, and vaginal flora.[37] The colonies of *L. lactis* are white to light gray and are usually rough in appearance.[26,30,169]

L. acidophilus produces factors inhibitory to tumor cell proliferation.[111] *L. acidophilus* is believed to exert a beneficial effect by restoring intestinal balance during disease processes or antibiotic treatment.[3] In a study with gnotobiotic chickens, *L. acidophilus* inhibited *Staphylococcus aureus*, *Salmonella typhimurium*, and demonstrated similiar results in another study against enteropathogenic *Escherichia coli*.[111] The consumption of *L. acidophilus* is also believed

TABLE 7

Thermobacteria

Phylogenetic grouping and key characteristics of Group A lactobacilli (obligate homofermentative)

	Phygenetic grouping	Peptidoglycan type	G+C content (mol(%))	Lactic acid isomer(s)	growth (oC) 15/45	NH3 from arginine	Carbohydrates fermented: amygdalin	cellobiose	galactose	lactose	maltose	mannitol	mannose	melibiose	raffinose	salicin	sucrose	trehalose	fructose**	glucose**	sorbitol**	esculin**
L. acidophilus	a	Lys-Dasp	34-37	DL	(-/+)	-	+	+	+	+	+	-	+	d	d	+	+	d	(+)[174,268]	(+)[174,268]	(+)[174,268]	(+)[174,268]
L. amylophilus	a	Lys-Dasp	44-46	L	(+/-)	ND	-	-	+	-	+	-	+	-	-	-	-	-	(+)[11]	(+)[11]	(+)[11]	(+)[11]
L. amylovorius	a	Lys-Dasp	40-41	DL	(-/+)	ND	+	+	+	-	+	-	+	-	-	+	+	+	(+)[11]	(+)[11]	(+)[11]	(+)[11]
L. crispatus	a	Lys-Dasp	35-38	DL	(-/+)	-	+	+	+	+	+	-	+	-	-	+	+	-	(+)[11,268]	(+)[11,268]	(+)[11,268]	(+)[11,268]
L. debrueckii ssp. *bulgaricus*	a	Lys-Dasp	49-51	D	(-/+)	-	-	-	-	+	-	-	-	-	-	-	-	-	(+)[11,268]	(+)[11,268]	(+)[11,268]	(+)[11,268]
L. delbrueckii ssp. *delbrueckii*	a	Lys-Dasp	49-51	D	(-/+)	d	-	d	-	-	d	-	+	-	-	-	+	d	(+)[11,268]	(+)[11,268]	(+)[11,268]	(-)[11]
L. delbrueckii ssp. *lactis*	a	Lys-Dasp	49-51	D	(-/+)	d	+	d	d	+	+	-	+	-	-	+	+	+	(+)[11,268]	(+)[11,268]	(+)[11,268]	(+)[11,268]
L. gallinarum	a	Lys-Dasp	36-37	DL	(+/+)	ND	+	+	+	d	+	-	+	+	+	+	+	-	(+)[174]	(+)[174]	(+)[174]	(+)[174]
L. gasseri	a	Lys-Dasp	33-35	DL	(-/+)	-	+	+	+	d	d	-	+	d	d	+	+	d	(+)[11,184]	(+)[11,184]	(+)[11,184]	(+)[11,184]
L. helveticus	a	Lys-Dasp	38-40	DL	(-/+)	-	-	-	+	+	d	-	d	-	-	-	-	d	(+)[258,268]	(+)[258,268]	(+)[258,268]	(+)[258,268]

*Group **a** species belong to the Lb. delbrueckii group; Group **b** organisms to the Lb. casei-Pediococci group. Symbols:+, 90% or more strains are positive; -, 90% or more of the strains negative, d, 11-98% of strains positive; (d), delayed reaction, ND, no data available, w = weak; DAP, diaminopimelic acid. Parenthesized isomers indicate<15% of total lactic acid. Reprinted with Permission from W.P. Hammes and R.F. Vogel, the authors; B.J.B. Wood and W. Holzapfel; editors Chapter 3, *The Genus Lactobacillus*. Tables 3.2, from The Genera Of Lactic Acid Bacteria. Vol. 2., pp. 23 and 24. Copyright 1995, and published by Aspen Publishers. **Data from:** [143]Carr 1977, [268]Cox et al. 1990, [128]Doring 1988, [258]Entani 1986, [6]Fuzisawa 1984, [61]Hartman 1992, [11]Kandler 1983, [3]Kandler 1986, [184]Lauer 1980, [146]Reuter 1983, [174] Takizawa 1994, [148]Weiss 1981.****Data from** other authors.

to improve lactose tolerance in humans.[111] *L. acidophilus* was shown to lower cholesterol levels in germ-free animals.[111] *L. acidophilus* was also reported to reduce the risk of cancer by reducing the conversion of procarcinogens to active carcinogens in experimental animals.[111] *L. helviticus* ssp. *jugurti* has been found, in a combined culture with *Candida utilis* in sour milk, to reduce colon tumors in rats.[3,111]

L. acidophilus is a microaerophile, ferments amydalin and cellobiose, and hydrolyzes esculin.[26,56,131,179,180] *L. acidophilus* forms a "feathery" or "crab-like" colony type on MRS Agar or on Blood Agar, as well as producing a greening of the blood.[23,30,49,51,130] *L. acidophilus* is sensitive to penicillin but resistant to sulfonamides.[3,7,30,37,180]

Johnson et al.[179] placed 78 strains of *L. acidophilus* into six DNA homology groups (A_1–A_4, B_1, and B_2). Physiological testing demonstrated that all strains of *L. acidophilus* fermented cellobiose, fructose, glucose, and maltose, hydrolyzed esculin, but could not provide differentiation of *L. acidophilus* strains based on phenotypic characteristics alone.[3,26,55,145,179,181]

L. leichmanii may be isolated from compressed yeast, fermenting milk, and other dairy products.[3,169,9] It produces metachromatic granules and forms colonies that are small, clear, and with white centers.[169] The biochemical characteristics are given in Table 7.

L. delbrueckii ferments amygdalin and sucrose but not trehalose, salicin, or cellobiose. *L. delbrueckii* unlike other thermobacteria, does not ferment lactose or galactose.[26,37,169] It does not hydrolyze esculin, and it grows at 45°C. Its colonies are small, flat, and with crenated edges.[7,26,37,169] *L. delbrueckii* is found associated with plants such as potato and grain mash.[3]

TABLE 7 (continued)

Phylogenetic grouping and key characteristics of Group A lactobacilli (obligate homofermentative)

	Phygenetic grouping	Peptidoglycan type	G+C content (mol(	Lactic acid isomer(s)	growth (°C) 15/45	NH3 from arginine	Carbohydrates fermented: amygdalin	cellobiose	galactose	lactose	maltose	mannitol	mannose	melibiose	raffinose	salicin	sucrose	trehalose	fructose**	glucose**	sorbitol**	esculin**
L. *jensenii*	a	Lys-Dasp	35-37	D	(-/+)	+	+	+	+	-	d	d	+	-	-	+	+	+	(+)[258]	(+)[258]	(+)[258]	(+)[258]
L. *johnsonii*	a	Lys-Dasp	33-35	DL	(+/+)	ND	+	+	+	d	+	-	+	d	d	+	+	d	(+)[258]	(+)[258]	(+)[258]	(+)[258]
L. *kefiranofaciens*	a	ND	34-35	D(L)	(-/-)	ND	-	-	+	+	+	-	ND	+	+	-	+	-	(+)[174]	(+)[174]	(+)[174]	(+)[174]
L. *aviarius* L ssp. *araffinosus*	b	Lys-Dasp	39-43	L(D)	(-)/ND	ND	d	d	-	-	+	-	+	-	-	d	+	+	(+)[6]	(+)[6]	ND	(+)[6]
L. *aviarius* L ssp. *aviarius*	b	Lys-Dasp	39-43	DL	(-)/ND	ND	d	+	d	d	+	-	+	d	+	+	+	+	(+)[6,61]	(+)[6,61]	ND	(+)[6,61]
L. *farciminis*	b	Lys-Dasp	34-36	L(D)	(+/-)	+	+	+	+	+	+	-	+	-	-	+	+	+	(+)[128]	(+)[128]	(+)[128]	(+)[128]
L. *salivarius* L ssp. *salicinus*	b	Lys-Dasp	34-36	L	(-/+)	-	-	-	+	+	+	+	-	+	+	+	+	+	(+)[258]	(+)[258]	(+)[258]	(w+)[258]
L. *salivarius*	b	Lys-Dasp	34-36	L	(-/+)	-	-	-	+	+	+	+	-	+	+	-	+	+	(+)[258]	(+)[258]	(+)[258]	(+)[258]
L ssp. *salivarius*	b	Lys-Dasp																				
L. *mali*	b	DAP	32-34	L	(+/-)	-	+	d	d	-	-	-	+	-	-	+	+	+	(+)[143]	(+)[143]	(+)[143]	(+)[143]
L. *ruminis*	b	DAP	44-47	L	(-/d)	-	+	+	+	d	+	-	+	+	+	+	+	-	(+)[3,128]	(+)[3,128]	(+)[3,128]	(+)[3,128]
L. *sharpeae*	b	Lys-Dasp	53	L	(+/-)	-	+	+	+	+	+	-	+	-	-	+	-	-	(+)[3,148]	(+)[3,148]	(+)[3,148]	(+)[3,148]

*Group **a** species belong to the Lb. delbrueckii group; Group **b** organisms to the Lb. casei-Pediococci group. Symbols:+, 90% or more strains are positive; -, 90% or more of the strains negative, d, 11-98% of strains positive; (d), delayed reaction, ND, no data available, w = weak; DAP, diaminopimelic acid. Parenthesized isomers indicate<15% of total lactic acid. Reprinted with Permission from W.P. Hammes and R.F. Vogel, the authors; B.J.B. Wood and W. Holzapfel; editors Chapter 3, *The Genus Lactobacillus*. Tables 3.2, from The Genera Of Lactic Acid Bacteria. Vol. 2., pp. 23 and 24. Copyright 1995, and published by Aspen Publishers. **Data from:** [143]Carr 1977, [268]Cox et al. 1990, [128]Doring 1988, [258]Entani 1986, [6]Fuzisawa 1984, [61]Hartman 1992, [11]Kandler 1983, [3]Kandler 1986, [184]Lauer 1980, [146]Reuter 1983, [174] Takizawa 1994, [148]Weiss 1981.****Data from** other authors.

L. delbrueckii is important in the manufacture of alcohol beverages such as beer, ale, bourbon, scotch whiskey, and rum.[3,9] *L. delbrueckii*'s importance stems from its ability to lower the pH of fermenting malt and thus facilitate the digestion of soluble starches in the mashing process. By increasing the acidity, not only are the starches solubilized, but the acidity also contributes to reducing the level of contaminating microorganisms.[3,9]

L. lactis was originally described by Orla and Jensen 1919.[145] This microorganism is used as a starter culture in the manufacture of Swiss cheese because of its heat tolerance (the Swiss cheese process includes high heat).[3,4,30] It forms long cells that tend to grow in threads.[169] *L. lactis* forms colonies that appear rough, 1 to 3 mm in diameter, and white to gray.[3,30]

L. salivarius was originally described by Rogosa in 1953 when he found large numbers of this microorganism in the gut of chickens and in saliva of hamsters. *L. salivarius* may be differentiated from other thermobacteria by its strong fermentation of mannitol.[26] *L. salivarius* was orginally described as having two varieties, *salivarius* and *salicinus,* differing in salicin and rhamnose fermentation.[3,26,131]

Turner and Martley[177] classified the thermobacteria based on the amount of acid produced in milk, the production of D or DL lactate, the presence or absence of metachromatic granules, as well as the fermentation of galactose. The ability to ferment galactose by *L. helviticus* may be used to differentiate *L. helviticus* from *L. bulgaricus* and *L. lactis*. Both *Lactobacillus lactis* and *L. bulgaricus* are galactose negative and produce methylene blue staining granules. *L. helveticus* also produces both D(–) and L(–) acid but lacks granules, while *L. lactis* and *L. bulgaricus* produce only D-lactic acid but are granule producing.[177]

Turner and Martley concluded that *Lactobacillus* strains that ferment galactose should be considered belonging to the *L. bulgaricus–L. lactis* group regardless whether they produce D- or DL-Lactate or metachromatic granules. Galactose fermentation should be used as a major criterion for characterizing a strain as belonging to the *L. helveticus* group, regardless of its ability to produce both D(–), or DL(–) lactate, and or metachromatic granules. It should be considered belonging to the *L. bulgaricus* group if it ferments galactose, regardless of its current species designation.[26,177,182]

The Taxonomic Subcommittee of the Lactobacilli[182] reclassified *Lactobacillus cellobiose* based on DNA homology studies as *Lactobacillus fermentum*.[182] *Lactobacillus jugurti* has been merged into *L. helveticus*.[182] *L. bulgaricus*, *L. leichmanii, and Lactobacillus lactis* have been reclassified as subspecies of *Lactobacillus delbrueckii*.[3,55,177,182,183]

A more recently named thermobacterium, *Lactobacillus gasseri*, named in honor of Francis Gasser, forms rough, flat colonies on agar media, and ferments a number of carbohydrates.[184] Like other thermobacteria, it does not ferment pentoses. It forms DL-lactic acid from the homofermentation of glucose.[22,49,55,87,177,182,184]

XVIII. THERMOBACTERIA

Culture Media

The thermobacteria are fastidious and require numerous vitamins in order to fulfill their nutritional requirements.[3,30,56] Tables 12 and 13 lists a number of media for culture and enumeration that meet those nutritional requirements as well as the additional microorganisms that may be co-isolated. The list of media for the thermobacteria is long; however, a few commonly used types are listed here. Lactic Broth AOAC, pH 6.8, may be used for the culture and maintenance of *Lactobacillus buchneri*, *L. delbrueckii*, and *Pediococcus damnosus*. *L. casei* ATCC 7469, *L. fermentum* ATCC 9338, *L. leichmannii* ATCC 4797, and *L. viridescens* ATCC 19706 are used for vitamin assays in Lactobacilli Broth. Lactobacilli MRS Broth, pH 6.5, may be used for the cultivation of Lactobacilli and cultivation of *Aerococcus viridans*, *Bifidobacterium coryneforme, Lactococcus plantarum*, *Leuconostoc* spp. and *Pediococcus* spp.[56] Lactic Bacteria Broth, pH 5.1 to 5.3, is used for the cultivation of *L. buchneri*, *L. delbrueckii,* and *Pediococcus damnosus* from dairy products and the brewing industry.[3,56]

XIX. THE LACTOCOCCUS GENUS

(Lactic Streptococci)

The Lactococci have been used primarily as starter cultures for various dairy products (yogurt, Cheddar, and hard cheeses). For the most part, they have been limited to the N and D Streptococci and the Leuconostocs: *L. cremoris* and *L. dextranicum*. By definition, the Lactococci are Gram-positive cocci, nonmotile, grow at 10°C and 40°C but not 45°C, grow in 4% NaCl (except for *L. cremoris*); some species grow in 0.1% methylene blue milk medium.[2,23,28,43,56,100,187,245] Several reports discuss culture media and biochemical tests (including microplates).[37,39,51,61,86,94,185]

The Lactococci do not grow in 6.5% NaCl or a pH of 9.6.[44,90] They ferment glucose by the Hexose Diphosphate pathway with the formation of L(+) lactic acid.[186] The differential biochemical characteristics are shown in Table 8.[23,25,43,44,90,100,186,187,188]

In general, *Lactococcus* spp. produce smooth colonies with an entire edge on agar media. They are nonmotile, Gram-positive, and form ovoid cells with the tendency to chain in one direction. They are facultatively anaerobic, catalase negative, and do not form endospores.[22,43,86,189]

TABLE 8

Lactococcus

	amydallion	arabinose	cellobiose	fructose	galactose	glucose	gluconate	maltose	mannitol	melibiose	melezitose	raffinose	rhamnose	ribose	salicin	sucrose	trehalose	xylose	sorbitol	arginine hydrolysis	n-acetyl-glucosamine	0.1 % methylene blue	10°C	40°C	45°C	2 % NaCl	4 % NaCl
L. cremoris	-	ND	-	ND	+	ND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	+	+	-	-	+	-
L. garviae	+	-	+	+	+	+	-	V	V	-	-	-	-	+	+	V	+	-	-	+	+	r	+	+	-	+	+
L. hordinae	-	-	-	+	-	+	-	-	-	-	-	-	-	-	+	+	+	-	-	+	ND	ND	ND	-	ND	+	-
L. lactis	V	-	+	+	+	+	(-)	+	V	-	-	-	-	+	+	-	+	-	-	+	+	+	+	+	-	+	+
L. piscium	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	ND	ND	-	ND	ND	ND
L. plantarum	+	-	+	+	-	+	-	+	+	-	+	-	-	-	+	+	+	-	+	-	+	-	+	-	-	+	+
L. raffinolactis	V	-	+	+	+	ND	-	+	-	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	-	+	-

Data From *+, indicates a positive test result, -. indicates a negative test result, (+/-) indicates that strains may produce differing, test results depending on the strain used.., ND = no data, r, reduction 0.1% methylene blue milk. V; variable, some produce + or -.results. Collins 1983 (189), Deibel and Steeley 1978 (245), Hardie and Whiley 1995 (46), Huggins 1984 (90), Lalorre-Guzman 1977 (190), Schleifer 1985 (43), Teuber 1995 (86), Willams 1990 (191).

In 1985, *Streptococcus lactis* and its subspecies, *Lactobacillus xylosus*, *Lactobacillus hordniae*, *Streptococcus garviae*, *Streptococcus plantarum*, and *Streptococcus raffinolactis,* were transferred to the genus *Lactococcus* based on DNA hybridization, immunological relationships, lepteichoic acid structure, fatty acid profiles, meaqunone composition, and cultural phenotypes.[22,43,129,187]

XX. REPRESENTATIVE LACTOCOCCUS SPP.

Differential Characteristics

Streptococcus thermophilus is used in dairy products and grows at 50°C.[23,43,186] It may be isolated on Thallous Acetate Tetrazolium Glucose Agar,[39,83] where this species forms red colonies.[39,83] *Streptococcus thermophilus* may be also isolated on M17 medium with pH 6.8 where its colonies are lens shaped.[23,43,83,84] *L. garviae*, *L. raffinolactis*, *L. plantarum*, and *L. hordinae* are not used for the manufacture of dairy products.[23,43,83,100,187]

Lactococcus garvieae was named in honor of the British microbiologist E.I. Garvie. This species produces a smooth, circular colony with an entire edge on Nutrient Agar or Blood Agar.[43,86,130] Like other Lactococci, it has ovoid cells that tend to chain in one direction.[43,86,130]

Lactococcus lactis ssp. *lactis (*formerly *Lactobacillus lactis*) was originally found as a milk-souring isolate, but it is also associated with plants. This species is used in the manufacture of Colby Barrel cheese. *Lactococcus lactis* ssp. *cremoris* is used as a starter culture for the manufacture of Cheddar cheese, where it contributes a highly prized flavor.[1,2,4,23,101]

Of *L. garviae, L. lactis* ssp. *hordniae*, and *L. raffinolactis*, only *L. raffinolactis* ferments raffinose, whereas the other two hydrolyze arginine.[43] *L. raffinolactis* forms colonies that are circular, smooth, and with an entire margin. *L. hordiae* does not ferment galactose, lactose, maltose, is ribose negative, and hydrolyzes arginine.[43] *L. raffinolactis* differs from other *Lactococci* by fermenting both melibiose and raffinose.[43,187] *Streptococcus lactis* and *S. raffinolactis* were the only legitimate Lactic Streptococci, according to Mundt.[44,190]

L. plantarium forms colonies that are circular, smooth, and with an entire margin on Blood or Nutrient Agar.[30,43,86,130] *L. plantarium* ferments a number of carbohydrates, such as salicin, sucrose, sorbitol, trehalose, mannitol, and melezitose. Both *L. plantarium* and *L. piscium* ferment melezitose, but *L. piscium* differs from *L. plantarium* by fermenting melibiose.[43,191] *L. plantarium* may be differentiated from other Lactococci by its fermentation of melezitose and sorbitol.[3,43,187,191]

Both *S. lactis* and *S. diacetylactis* (current members of genus Lactococcus) ferment maltose, whereas *S. cremoris* and *S. thermophilus* do not.[44,90] *S. diacetylactis*, although previously not recognized taxonomically as a legitimate species, is considered a legitimate name by the dairy industry. It forms carbon dioxide and diacetyl during the fermentation process.[2,39,44,90,192] The salt tolerance is much greater (4% to 6.5%) for both *S. lactis* and *S. diacetylactis* than for *S. cremoris* and *S. thermophilus (*which tolerates less than 2% salt).[23,25,43,44,90,186,187] Resistance to salt by both *Lactococcus* ssp. *lactis* and *Lactococcus lactis* ssp. *diacetylactis* is important because some dairy fermentations require exposure to high salt levels.[23,25,43,44,90,100,186,187]

XXI. REPRESENTATIVE LACTOCOCCI

Culture Media And Cultural Characteristics

Reddy's Medium is a differential agar for the separation of *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris, and Lactococcus lactis* ssp. *diacetylactis*.[50,89,193] This media detects arginine hydrolysis, acid and diacetyl from lactose, acetoin, and CO_2 from citrate. *L. lactis* ssp. *cremoris* produces acid from lactose, and therefore produces yellow colonies on the agar.[50,89,193] Culture plates are inoculated 0.1 ml aliquots; all plates are incubated at 32°C for 36 to 40 h in a candle jar. A total colony count is performed, and a separate colony count is made for all yellow colonies (*L. cremoris*). All plates are reexamined at 32°C, for an additional 4 days in a candle jar. All yellow colonies are considered *L. cremoris*. Prior to final counting, all plates are exposed to air for 1 h. First a total count is determined and then a count of all colonies surrounded by clear zone due to clearing of the calcium citrate. All plates are reincubated at 32°C, for an additional 4 days in a candle jar. Prior to final counting, all plates are exposed to air for 1 h. First a total count is determined and then a count of all colonies surrounded by clear zones (*L. lactis* ssp. *diacetylactis*) due to clearing of calcium citrate. The *L. lactis* ssp. *cremoris* and *L. lactis* ssp. *diacetylactis* counts are subtracted from the original total count in order to obtain a *L. lactis* count. The *L. lactis* ssp. *cremoris* and *L. lactis* ssp. *diacetylactis* are subtracted from the original total count in order to obtain a *L. lactis* count. If a more accurate *L. lactis* ssp. *cremoris* count is required, all yellow colonies (after 36 to 46 h) are marked with a smudge-proof pen. When the final count is taken, marked colonies that show clearing (*L. lactis* ssp. *diacetylactis*) are counted. The *L. lactis* ssp. *diacetylactis* count is subtracted from the original yellow colony count in order to obtain the *L. lactis* ssp. *cremoris* count.[50,84,194]

L. lactis ssp. *diacetylactis* differs from *L. lactis ssp. lactis* and *L. lactis* ssp. *cremoris* by its ability to form carbon dioxide from citrate.[50,84,86,186,194,195] Citrate media is incubated at 30°C and observed for 24 to 72 h.

L. lactis ssp. *lactis* and *L. lactis* ssp. *diacetylactis* both hydrolyze arginine to form a dark purple. *L. lactis* ssp. *diacetylactis* hydrolyzes arginine (producing ammonia) with a more intense purple (alkaline) and with gas from citrate fermentation by 48 h. *L. lactis* ssp. *lactis* may at first turn the broth yellow (acid), the violet hue returns due to ammonia formation during the 72-h incubation period. *L. lactis* ssp. *cremoris* produces acid from its fermentation of lactose without ammonia from arginine and so the bromcresol purple remains yellow. The indicator never reverts back to its original purple color and remains yellow. Arginine hydrolysis can also be determined by testing a small aliquot culture of Reddy's Broth, with Nesslers reagent; a positive test result is indicated by a deep red precipitate.[50,59,97,194]

Isolates suspected of being either *Lactococcus lactis* ssp. *lactis* or *Lactococcus lactis* ssp. *cremoris* are presumptively identified with semisolid citrate milk agar.[39] Litmus milk, arginine hydrolysis, growth at 40°C, growth in the presence of 4% NaCl, growth at a 9.2 pH, production of acetoin in Glucose Lemco broth, and arginine hydrolysis on Arginine Tetrazolium Agar (ATA) are also useful.[34,39] On ATA, colonies of *Lactococcus lactis* ssp. *lactis* are characteristically red due to arginine hydrolysis, while *Lactococcus* ssp. *cremoris* colonies remain white (arginine negative).[34,39,193] Arginine Tetrazolium Agar is not useful, however, for differentiating mixed cultures containing both *Lactococcus* ssp. *lactis* and *Lactococcus* ssp. *cremoris*.[39,81,83,89,90,92,186,193,196]

Sandine and Matalon[91] developed Yogurt Lactic Agar (YLA) by modifing Elliker's Lactic Agar for the recovery of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The additional ingredients, Tween 80 and 2,3,5-triphenyltetrazolium, were added. On YLA Agar, *Streptococcus thermophilus* colonies appear as small red colonies; *Lactobacillus bulgaricus* produces large white colonies with a cloudy halo. The medium provides good rod-coccus differentiation for *S. thermophilus* and *L. bulgaricus*.[81,91,196]

M17 Broth, formulated by Terzaghi and Sandine, Elliker's Broth, or skimmed milk may be used as an enrichment for Lactococcus species. After enrichments, either Kempler and McKay Agar (KM Agar) or other selective medium is employed. Isolated Lactococcal colonies on KM Agar appear blue or with blue centers; colonies on M17 Agar are white.[3,49,52,56,79,84,85,86,186,195,196,197] Lactobacillus Streptococcus Differential medium may be used for the enumeration and differention of *Lactobacillus* spp. and *Streptococcus* spp. in yogurt. On this agar, *Lactobacilli* form red colonies, which are irregular and surrounded with a white, opaque zone. Streptococci colonies also appear red but are surrounded by a clear zone.[52,56] *Leuconostoc* spp. and *Lactococcus lactis* ssp. *diacetylactis* from starter cultures and dairy products may be enumerated by Galesloot's Agar.[59,86,198] A flow chart identifying the important dairy lactics is given in Figure 4 and Figure 5.[50,59,75,79,86,90,97] The differential biochemical characteristics of the Lactococci are shown in Table 8.

XXII. REPRESENTATIVE LEUCONOSTOCS

Leuconostoc was originally described in 1878.[41] Leuconostocs are Gram-positive cocci, occurring in pairs and chains, and form D(–) lactic acid and carbon dioxide from the fermentation of glucose.[22,41] These microorganisms form small, grey, flat colonies on agar media.[7,41] The genus is commonly found on the surface of and inside of fruits and vegetables, in dairy products, and have been implicated as a cause of disease in humans.[51,199,200] The genus plays a prominent role in the fermentation of numerous dairy products, the production of sauerkraut, pickles, and various meats.[1,9,22,51,109,201]

The Leuconostocs are Heterofermentative, but, unlike the similiar Betabacteria, produce D(–) lactate and are unable to hydrolyze arginine to form ammonia. They have complex nutritional requirements and are considered vancomycin resistant.[1,9,22,39,41,51,54,95,109,201,202,203]

Although *Leuconostoc mesenteroides* ssp. *mesenteroides* and *Lactococcus plantarum*

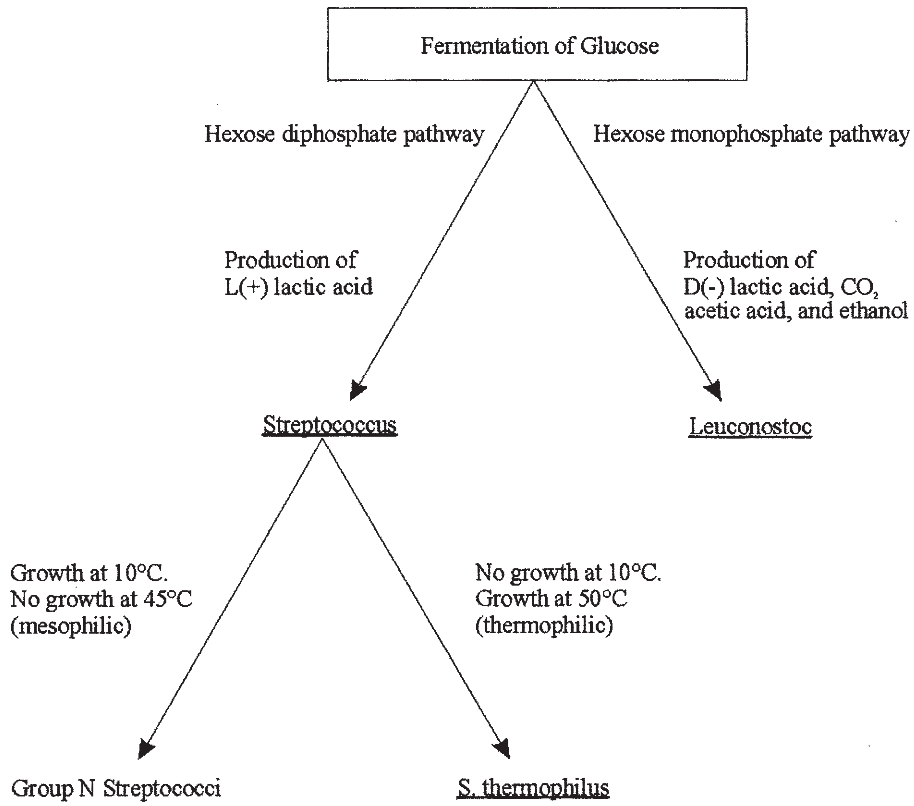

FIGURE 4. Differentiating the Group D streptococci from the lactic strepotococci. Based on Deibel and Seeley (1978). (Reprinted with permission from Allan R. Huggins, the author and from his article entitled *Progress in Dairy Starter Culture Technology.* Figures 3 to 4 *Food Technology* 38:41–40. Copyright 1984, Institute of Food Technologies.)

play the major role in cucumber fermentation, *Enterobacter* spp., *Lactobacillus* spp., and *Pediococcus* spp. have also been responsible for gas formation in pickles ("bloaters").[2,9,28,59,204]

In the dairy industry, *Leuconostoc dextranicum* and *Leuconostoc cremoris* are of vital importance as starter cultures. They produce diacetyl, a flavor compound, in the manufacture of such dairy products as butter, buttermilk, and cheeses.[2,9,205] *Leuconostoc mesenteroides* ssp. *mesenteroides* has been used in the manufacture of dextrans, which have been used as blood plasma extenders.[87,206,207,208]

In meats, the Leuconostocs may cause spoilage, which may be accompanied with the production of dextran slime.[9,63,95] Most slime-producing Leuconostocs may be differentiated from the meat contaminant, *Lactobacillus viridescens,* by Leuconostoc's failure to ferment trehalose or hydrolyze arginine.[63,110]

The Leuconostocs have caused serious problems in the sugar cane industry by the formation of dextran slime. These dextrans affect not only the clarity of the refined sugar but may affect the appearance, quality, and quantity of manufactured product. This problem is particularly important in regions dependent on sugar for their economy.[98,207,209] *Leuconostoc mesenteroides* ssp. *mesenteroides* is the dominant species causing this damage, however *Leuconostoc dextranicium* may play a minor role.[2,4,9,59,95,209]

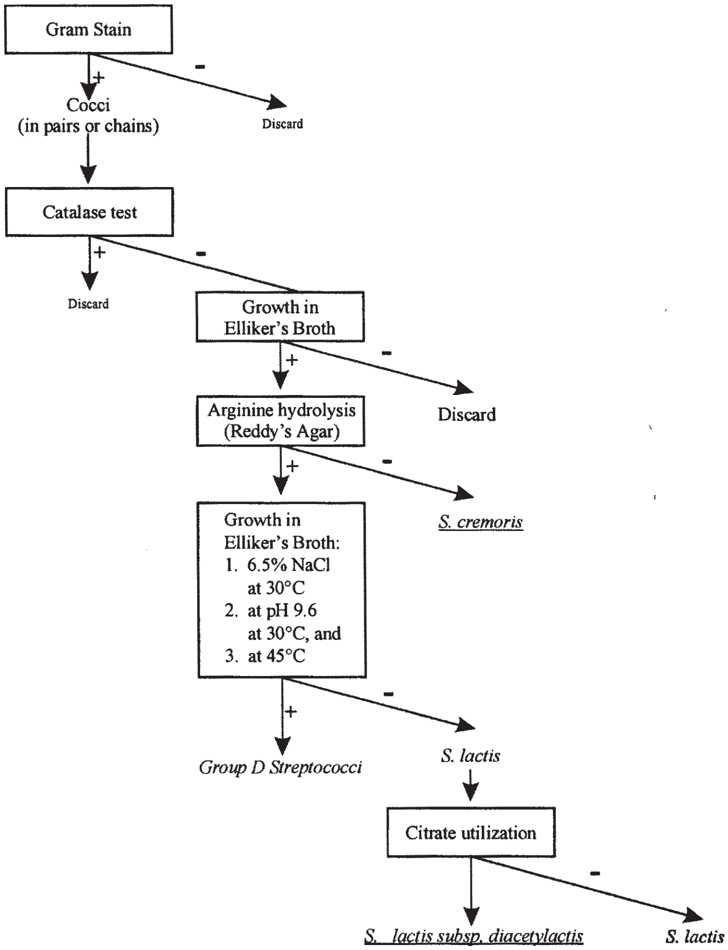

FIGURE 5. Differentiating the Group D streptococci from the lactic streptococci. Based on Deibel and Seeley (1978). (Reprinted with permission from Allan R. Huggins, the author and from his article entitled *Progress in Dairy Starter Culture Technology*. Figures 3 to 4 *Food Technology* 38:41–40. Copyright 1984, Institute of Food Technologies.)

XXIII. REPRESENTATIVE LEUCONOSTOC

Culture Media and Differential Characteristics

The Leuconostocs may be separated into two groups, the wine isolate *L. oenos* and nonwine isolates (all other species), by the ability to grow well in Citrate Malate Broth at pH 4.2 and 4.7, as well as growth in the presence of 10% ethanol ("wine isolates versus nonwine isolates"). Enrichment of nonwine Leuconostocs may be obtained in Yeast Glucose Citrate Broth (YGCB).[51,59,94,210]

The Leuconostocs have a Heterofermentative-type metabolism similiar to the Heterofermentative Lactobacilli in that they produce carbon dioxide from the fermentation of glucose.[1,22] The Leuconostocs and Pediococci differ from the Lactobacilli by the former species' relative inactivity in litmus milk, rarely producing enough acid in order to cause milk coagulation.[39,51,94,211] The Leuconostocs are differentiated from the similiar heterolactic Betabacteria by hydrolysis of arginine, the formation of D(-) lactic acid, and trehalose fermentation (Figure 1, and Table 13).[1,22,23,63]

In general, the Leuconostocs require a slightly more alkaline pH when compared with their more acidophilic relatives, Lactobacillus and Pediococcus.[51] Leuconostocs are generally inhibited below a pH of about 4.5, whereas both the Lactobacilli and the Pediococci are capable of growing at or below pH of 4.[51,94]

MRS Agar is a general media for culturing the majority of lactic acid bacteria, including the majority of Leuconostocs and Pediococci.[51,59] The Leuconostocs may be selectively inhibited by using Modified MRS Broth below the pH of 4.5. Lactobacilli and Pediococci are capable of growth at a lower pH.[51,59]

For cultivating the Leuconostocs, Garvie[51] recommends Yeast Glucose Phosphate Peptone Broth (YGPG) to which 0.1% thallium acetate and 0.0005% crystal violet is added to inhibit spore formers and Gram-negative bacilli.[42,51,59,98] The use of Acetate Agar is acceptable for cultivating *L. mesenteroides* and *L. paramesenteroides*, but *L. cremoris* is inhibited by Acetate Agar's 5.4 pH.[39,51,59,68,94,203]

The differential biochemical characteristics of the Leuconostocs are shown in Table 9; the enrichment and culture media are found in Tables 12 and 13. Figure 7 describes the differentiation of Leuconostoc.

Leuconostoc mesenteroides ssp. *mesenteroides* may be cultivated on Leuconostoc Mesenteroides Medium. Acidic Tomato Broth may also be used for the enrichment of Leuconostocs from wines.[51,52,56,59,94,95]

Tomato Juice Agar and MRS Agar supplemented with cycloheximide (20 to 50 μg) may be employed for direct isolation of *Leuconostoc oenos* from wines, barley malt, and grain whiskey. Other useful media are Fructose Tween 80 Medium (FT-Medium), Leuconostoc oenos Medium, and Acidic Grape Broth. Nakagawa and Sucrose Agars have been used for the enumeration of the LAB from wines.[42,51,52,55,56,59,87,94,154,195,203,210,212,213,214,215]

Yeast Glucose Citrate Broth may be used for cultivation of the nonwine Leuconostocs.[94,59] Other enrichment broths for Leuconostocs include MRS Broth, HP medium, and Yeast Glucose Phosphate Broth (YGPB).[49,51,55,59,94,210,216]

Tetrazolium Sucrose Agar may be used for thawed frozen peas and vegetables and Thallous Acetate Tetrazolium Sucrose agar for plant materials. In the case of TATS Agar, Leuconostocs are selected by their resistance to thallous acetate.[51,52,55,56,59,87,194,203,210,212,213,215]

WAACA Agar was developed for the enumeration of Leuconostocs and *Lactococcus lactic* ssp. *diacetylactis* in fermented dairy products.[51,59,86,198]

Orange Serum Agar (OSA) is used for the enumeration of Lactobacilli and Leuconostoc from citrus products. Leuconostoc colonies are gummy on this medium, whereas Lactobacilli produce small white pinpoint colonies.[52,55,154,210,217] LUSM Medium may be used for selective isolation of Leuconostocs from vegetable and dairy products.[202]

The growth of *L. cremoris* and *L. oenos* may be enhanced at 22°C on MRS Agar with 0.05% cysteine with a reduced atmosphere. Although both *L. cremoris* and *L. oenos* are capable of growing aerobically in broth, their growth is improved by the addition of reduced atmospheric conditions.[3,51,98,198] Leuconostocs may be tentatively differentiated from its nearest neighbors, the Heterofermentative Lactobacilli, by the former's ability to produce gas from glucose and the inability to hydrolyze arginine.[1,22,39,51,198,202]

L. oenos differs from other *Leuconostocs* by its inability to ferment lactose, maltose, and sucrose.[95] *L. cremoris* in most cases is set apart by its ability to assimilate citrate in Acidic Tomato Broth with triammonium citrate as the substrate.[94] *L. cremoris* ferments glucose, galactose, and lactose.[51,59,94,95,218]

TABLE 9
Diagnostic Characteristics of the Subspecies and Species Belonging to the Genus Leuconostoc*

Characteristics	*Leuc. mesenteroides* subsp. *mesenteroides*	*Leuc. mesenteroides* subsp. *dextranicum*	*Leuc. mesenteroides* subsp. *cremoris*	*Leuc. paramesenteroides*	*Leuc. lactis*	*Leuc. oenos*	*Leuc. gelidum*	*Leuc. carnosum*	*Leuc. pseudomesenteroides*	*Leuc. citreum*	*Leuc. amelibiosum*	*Leuc. argentinum*	*Leuc. fallax*
Acid from													
Arabinose	+	-	-	d	-	d	+	-	d	+	+	d	-
Arbutin	d	-	-	-	-	ND	+	-	d	+	ND	-	-
Cellulose	d	d	-	(d)	-	d	ND	ND	ND	ND	ND	ND	ND
Cellobiose	d	-	-	d	-	d	+	d	d	d	+	d	-
Fructose	+	+	-	+	+	+	+	+	+	+	+	d	+
Galactose	+	d	+	+	+	d	-	-	d	-	ND	+	-
Lactose	d	+	d	d	(+)	-	-	-	d	-	-	+	-
Maltose	+	+	-	+	+	-	d	-	+	+	+	+	+
Mannitol	d	-	-	-	-	-	-	-	-	d	-	d	(d)
Mannose	d	d	-	+	d	d	+	d	+	+	ND	+	+
Melibiose	d	d	-	+	d	d	+	d	d	-	-	+	-
Raffinose	d	d	-	d	d	-	+	-	d	-	-	+	-
Ribose	d	ND	-	+	-	d	d	d	+	-	-	-	+
Salicin	d	-	-	-	d	d	+	d	d	+	+	-	-
Sucrose	+	+	-	+	+	-	+	+	d	+	+	+	+
Trehalose	+	+	-	+	-	+	+	+	+	+	+	d	(d)
Xylose	d	d	-	-	-	d	+	-	+	-	-	d	-
Citrate[c]	d	d	+	d	d	d	ND	ND	ND	ND	ND	ND	ND
Gluconate	w[a]	w[a]	(-)[a]	(+)[b]	(-)[a]	ND	w[a]	w[a]	w[a]	w[a]	(+)[b,e]	(+)[f]	(-)[a]
Hydrolysis of esculin	+	d	-	d	-	+	+	d	d	+	ND	-	ND
Dextran formation	+	+	-	-	-	-	+	+	ND	ND	+	-	ND
Growth at pH 4.8	-	-	-	-	-	+	ND	ND	ND	ND	-	ND	ND
Requirement for TJF	-	-	-	-	-	d	-	-	-	-	-	-	-
Growth in 10% ethanol	-	-	-	-	-	+	ND	ND	ND	ND	ND	ND	ND
NAD-dependent G6PDH present	+	+	+	+	+	-	ND	ND	ND	ND	ND	ND	ND
Growth at 37° C	d	+	-	d	+	d	-	-	+	d	ND	+	+
Peptidoglycan type	Lys-Ser Ala_2	Lys-Ser Ala_2	Lys-Ser Ala_2	Lys-Ala_2	Lys-Ala_2	Lys-Ser_2 Lys-Ala-Ser	ND	ND	Lys-Ser Ala_2	ND	Lys-Ala_2	ND	Lys-Ala

*+, 90% or more of the strains positive; -, 90% or more of the strains negative, d, 11-98% of the strains positive; (d), delayed reaction, ND, no data, (w) = weak reaction. Data for *Leuc. mesenteroides*, *Leuc. paramesenteroides*, *Leuc. lactis* and *Leuc. oenos* from Garvie (1986). Data for *Leuc. gelidum and Leuc. carnosum* from Shaw and Harding (1989), *Leuc. pseudomesenteroides and Leuc. citreum* from Farrow et al. 1989, *Leuc. amelibiosum* from Schillinger et al. (1989), *Leuc. argentinum* from Dicks et al. (1993), and *Leuc. fallax* from Martinez-Murcia and Collins (1991). **Data from** [a]Cai (12), [b]Farrow (199), [c]Garvie (51), [d]Kandler (3), [e]Shaw & Harding (220), and [f]Takahaski (221). Reprinted with Permission from F. Dellaglio, L.M.T. Dicks and S. Torriani; the authors; B.J. B. Wood and W. Holzapfel; editors; Chapter 7, The genus Leuconostoc, Table 7.1 p. 240-241 and Copyrighted 1995, and published by Aspen Publishers.

L. cremoris and *L. oenos* have an enhanced growth at 22°C, with 0.05% cysteine in the media. *L. oenos* has the unique property of tolerating 10% ethanol in Citrate Malate Agar.[51,56,59,94,210,219]

L. cremoris is biochemically differentiated by its inability to ferment either trehalose or sucrose, as well as its ability to grow at pH 4.2.[51,94,199]

L. citreum produces a lemon yellow pigment, ferments arabinose and gluconate, and may be differentiated from *L. mesenteroides* ssp. *mesenteroides* and *L. pseudoesenteroides* by its inability to ferment ribose and raffinose.[95,199] *Leuconostoc amelibiosum* differs from *L. mesenteroides* ssp. *mesenteroides* by not fermenting melibiose or raffinose.[95]

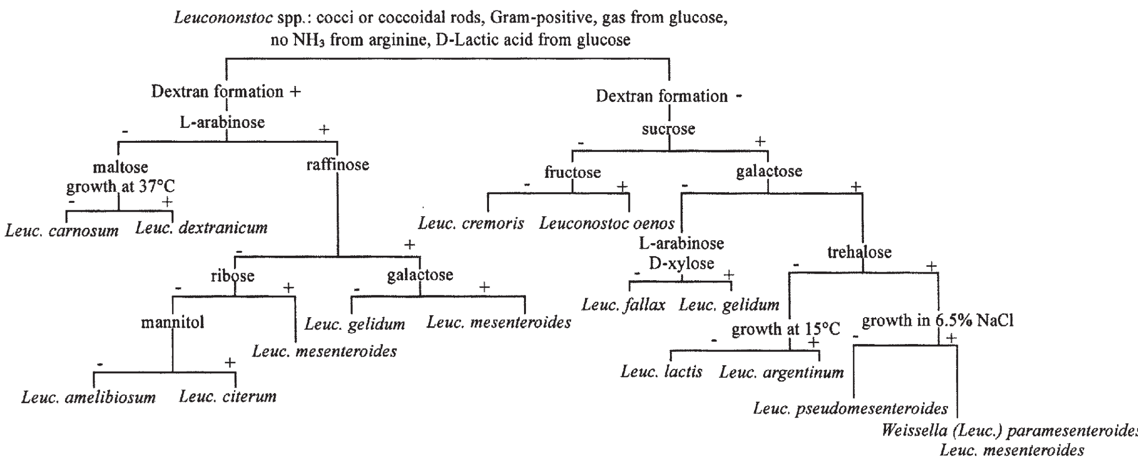

FIGURE 6. Scheme for presumptive identification of *Leuconostoc* spp. (Reprinted with permission from Salvatore Coppola et al., the author and from his article entitled *Characterization of strains of Leuconostoc mesenteroides by analysis of soluble whole-cell protein pattern, DNA fingerprinting and restriction of ribosomal DNA.* Figure 1 *Appl. Microbiol.* 82:p.582 Copyright 1997, The Society for Applied Bacteriology.)

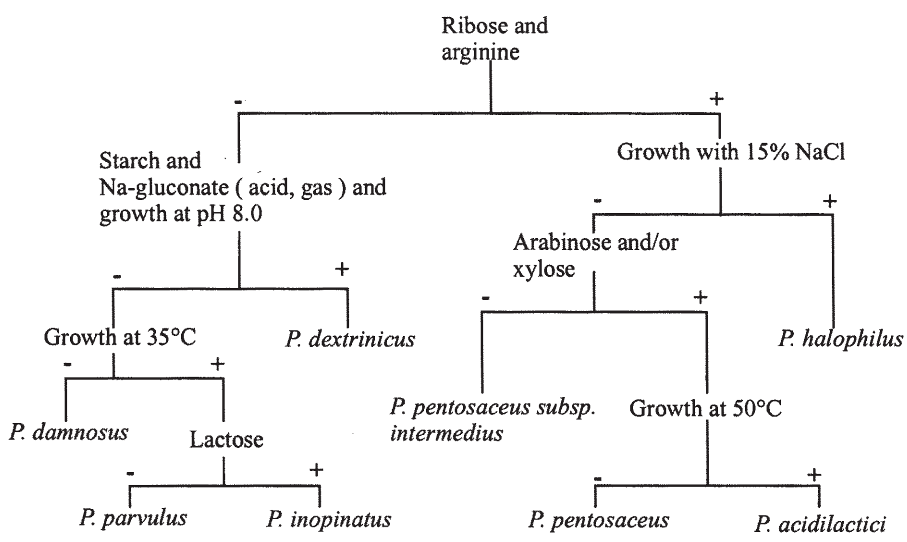

FIGURE 7. Flow chart for identification of aerocci and pediococci. Results should be verified by comparison with Table VII to utilize more reactions for identification. (Reprinted with permission from T. Bergan, the author and from the article entitled *Fatty Acid Carbohydrate Cell Composition in Pediococci and Aerocci and Idenification of Related Species*. Figure 9. In. T. Bergans (ed.) *Methods in Microbial.* 16:p. 210, and Copyright 1984, and published by Academic Press.)

The two new psychrophilic species *L. gelidum* and *L. carnosum* grow at 1°C. Both produce dextran and DNAse, and most strains fail to grow at 37°C.[41,51,95,218,199,220,221,222,223]

L. mesenteroides ssp. *mesenteroides* and *L. mesenteroides* ssp. *dextranicum* are most recognized from other similar Leuconostoc species, by the ability to produce dextran when grown on 5% sucrose agar.[51,59,95]

Both *L. mesenteroides* spp. *mesenteroides* and *L. mesenteroides* spp. *pseudomesenteroides* differ from *L. citreum* by utilizing ribose and raffinose.[95] *L. mesenteroides* spp. *mesenteroides* ferments arabinose and ribose, but, unlike *L. citreum* and *L. paramesenteroides*, does not hydrolyze gluconate.[95,199] *L. paramesenteroides* is the most likely organism to be confused with the wine isolates; however, any isolate that ferments maltose, sucrose, salicin, and esculin should be considered *L. paramesenteroides*.[94,203] *L. paramesenteroides* also ferments fructose and mannose and differs from *L. lactis* by fermenting trehalose.[51,94,95,98,199,200,224,225,226]

The common milk isolate *L. lactis* ferments lactose readily and is biochemically differentiated by its ability to utilize citrate, ferment sucrose but not trehalose, and its inability to grow at pH 4.2.[94,95]

XXIV. PEDIOCOCCUS

The genus Pediococcus was first described by Wochnschre F. Balcke in 1884.[41] The Pediococci are Gram-positive, nonmotile cocci that divide at right angles in two planes, resulting in a tetrad morphology especially when isolates are cultured in a broth medium. These microorganisms lack cytochromes and are catalase negative. The Pediococci require complex nitrogen compounds, and therefore, unlike *Bacillus* spp., are unable to use ammonia salts as a sole nitrogen source or to reduce nitrates to nitrites. They are aerobic to microaerophilic, ferment glucose by the Embden Meyerhof Pathway, and form either DL- or L-lactate without the production of carbon dioxide from glucose. Both *P. pentosaceus* and *P. acidilactici* are aerobic; *P. dextrinicus* is a facultative anaerobe, while others are microaerophilic.[1,2,22,28,39,41,99,207,227]

XXV. REPRESENTATIVE *PEDIOCOCCUS* SPP.

The Pediococci are found commonly associated with various plants and their products such as cabbage and saukeraut, cucumbers and pickles, grapes and wine, and wort and grain mashes.[2,108,228] *P. damnosus* and *P. acidilactici* are useful in the manufacture of fermented sausage, the fermentation of Lebanon bologna (summer sausage), bacon, as well as pickles and olives.[9] The Pediococci also play a prominent role in marinated products, particularly in marinated herring.[2,9,22,28,108,109,164,227,228,229]

P. damnosis is of economic importance as a contaminant in beer, where it may be responsible for a cloudy, acid-tasting beer due to the production of diacetyl (sarcina-sick) beer.[42,164] Pediococci may also be a problem in the pickle industry where they can cause swelling of cucumbers ("bloaters").[2,42,99] The hop-tolerant species (resistance to humulone and colupulone) formerly referred as *P. cerevisiae* has been reclassified as *P. damnosus*.[2,42,227,230]

Pediococci (and other bacteria) may be detected by a filter technique in conjunction with fluorescent dyes.[42,99,227,231] The most useful dye according to Kirsop and Dolezel[231] is Euchrysine 2GNX, which fluoresces a vivid orange for living cells, while dead cells appear as a weak green.[231] This dye is stable in culture media, stains rapidly, and is nontoxic. The membrane technique is very sensitive, detecting as low as 100 cells/membrane. Magnesium is added to the culture medium to stimulate chain cell formation, thus making cells more readily detectable for microscopic observation.[231,227] Other detection and quantification techniques include DNA probe methods and immunochemical methods with antibodies.[2,39,42,99,227,231]

XXVI. REPRESENTATIVE *PEDIOCOCCUS* SPP.

Culture Media and Differential Characteristics

The Pediococci require folic acid (Citrovorum factor) as a nutritional requirement. They may be cultivated using MRS broth and MRS agar adjusted to pH 5.2 for enrichment. ATCC Medium 562, with the addition 0.003% mevalonic acid, may also be used for the cultivation of Pediococci.[42,51,56,87,99,227] The Institute of Brewing recommends the use of a sucrose agar for the routine isolation of Pediococci.[227] Acetate or Lee's Differential Agar may be used at pH 5.6 to 5.8 for selective isolation.[4,227,232] *P. damnosus* and *P. parvulus* require more anaerobic conditions than other Pediococci.[56,87,99,227] For these two species, growth is enhanced by the addition of 0.05 to 0.1% cysteine.[99,233]

Lactobacilli and Pediococci grow on MRS Agar at pH 4.0; Leuconostoc are inhibited at pH 4.5.[51,227] Pediococci recovery in the presence of Lactobacilli may be enhanced if the 1% glucose in MRS Agar is substituted by other sugars, such as mannose, cellobiose, or salicin. Other additives for selectivity include cycloheximide, crystal violet, 2-phenylethanol, sorbic acid, and acetic acid for controlling yeasts and Gram-negative bacteria. The addition of vancomycin may be used for selectively isolating Pediococci and Leuconostocs.[39,42,51,99,227,234]

Several media are useful in culturing the Pediococci. MRS may serve as an enrichment broth for the Pediococci when the pH is adjusted to a pH of 5.2. Pediococcus Medium with 0.003% mevalonic acid is used for Pediococci from beer, particularly *P. damnosus*, and for those beer spoilage Pediococci which require it.[42,51,56,87,59,194,215] Glucose Yeast Broth with 4 to 5% NaCl is recommended for the cultivation and maintanence of *P. halophilus*.[39,42,56,87,99] GYP-CaC0$_3$ Medium is used for the detection of Pediococci from food and beverages.[42,235] For monitoring beer production, a number of media have been formulated; NBB (Nachweismedium für Bierschädliche Bakterien) is excellent.[30,42,55,56,76,207,236] Other culture media include Universal Beer Agar, Sucrose Agar, Tomato Juice Agar, W-L Differential Agar, and Kirin-Ohkochi-Taguchi.[42,52,55,56,59,130,215,237] Another medium for the detection of beer spoilage is Glucose Yeast Extract Agar, which supports Pediococci and Leuconostoc.[56,59,235] Both the American Society of Brewing Chemists and the European Brewing Congress Analytica Microbiologica recommend the use of Raka-Ray No. 3 Medium for the cultivation of Lactobacilli and Pediococci. Lactobacillus colonies appear white, moist, and about 1 mm in diameter on this agar.[52,56,59,130,194,215,232] *Pediococcus cerevisiae* produces small, greyish white colonies on Raka-Ray No.3 agar.[215,232] Wallerstein Laboratory Nutrient Broth may be used for brewery surveillance.[56,130]

On Lee's Multidifferential Agar (LMDA), the Lactobacilli appear as greenish to brownish yellow, round colonies, some with dark green centers and may vary from smooth to rough, depending on the Lactobacillus species. Lactobacillus species also form a clear halo, which is different from halos of other brewery microbes. Pediococci appear as small colonies with a yellowish-green color. Pediococcus halos are restricted to the edge of the colony. The colonies themselves are round with a smooth surface. Flavobacteria form colonies that are round and bluish-green, with a rough edge and surface and a slightly darker center. Both *Escherichia coli* and *Enterobacter aerogenes* are the dominant coliforms in beer.[42,227,232] *Escherichia* form colonies that are translucent round, blue-green to yellowish-green, smooth, shiny, and without a halo. Colonies of *Enterobacter* are much larger and do not form a halo. *Enterobacter aerogenes* may be distinguished from *E. coli* on LMDA by its "fried egg" appearance.[42,56,215,232]

The differential biochemical characteristics of the Pediococci are shown in Table 10, and the culture media are listed in Table 12 and 13. The respiration of Pediococci is microaerophilic to facultative anaerobic.[62,99] Pediococci do not acidify milk and are nonproteolytic. They are negative for indole and nitrate reduction but decarboxylate gluconate.[39,99,234] Pediococci may be separated into three groups based on ribose fermenta-

TABLE 10
Physiological Characteristics of the Pediococci*

Character	*P. acidilactici*	*P. damnosus*	*P. dextrinicus*	*P. halophilus* (syn. '*Tetra-togenococcus halophilus*')	*P. inopinatus*	*P. parvulus*	*P. pento-saceus*	*P. pento-saceus subsp. intermedius'*	P. urinae-equi
Growth at 35°C	+	-	+	+	+	+	+	+	+
40°C	+	-	+	(+/-) (weak)	(+/-) (weak)	-	(+/-) (weak)	(+/-) (weak)	+
45° C	+	-	(+/-) (weak)	-	-	-	(+/-) (weak)	(+/-) (weak)	(+/-) (weak)
50° C	+	-	-	-	-	-	-	-	-
Maximum NaCI concentration for growth	10%	5%	6%	>18%	8%	8%	10%	10%	10%
Growth at pH 4.5	+	+	(+/-)	-	+	+	+	+	-
pH 5.0	+	+	+	-	+	+	+	+	-
pH 7.5	+	-	+	+	(+/-)	(+/-)	+	+	+
pH 8.0	+	-	-	+	-	-	+	+	+
pH 8.5	(+/-)	-	-	+	-	-	(+/-)	(+/-)	+
Catalase activity	-	-	-	-	-	-	(+/-)	(+/-)	(+/-)
Gas from gluconate	-	-	+	-	-	-	-	-	-
Arginine hydrolysis	+	-	-	-	-	-	+	+	-
Hippurate hydrolysis	-	-	-	-	-	-	-	-	+
Production of acetoin	(+/-)	(+/-)	(+/-)	-	(+/-)	-	(+/-)	(+/-)	-
Lactate configuration	DL	DL	L(+)	L(+) (3%D(-))	DL	DL	DL	DL	L(+)
Litmus milk reaction									
Acid	(+/-)	-	-	-	(+/-)	-	+	+	ND
Reduction	(+/-)	-	-	-	(+/-)	-	+	+	ND
Clotting	(+/-)	-	-	-	-	-	(+/-)	+	ND
Acid produced from or splitting of									
Arabinose	(+/-)	-	-	+	-	-	+	-	(+/-)
Ribose	+	-	-	+	-	-	+	+	ND
Xylose	+	-	-	-	-	-	(+/-)	-	(+/-)
Fructose	+	+	+	+	+	+	+	+	+
Rhamnose	(+/-)	-	-	-	-	-	(+/-)	-	ND
Glucose	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+
Galactose	+	(+/-)	+	(+/-)	+	(+/-)	+	+	+
Maltose	-	(+/-)	+	+	+	(+/-)	+	+	+
Trehalose	(+/-)	(+/-)	(+/-)	+	+	+	+	(+/-)	(+/-)
Cellobiose	+	+	+	+	+	+	+	+	ND
Sucrose	(+/-)	(+/-)	(+/-)	+	-	-	-	(+/-)	+
Lactose	(+/-)	-	(+/-)	-	+	-	+	+	(+/-)

+, >90% strains positive; +/-, 10-90% strains positive; -, <10% strains positive; ND, not determined.

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TABLE 10 (continued)

Character	*P. acidilactici*	*P. damnosus*	*P. dextrinicus*	*P. halophilus (syn. 'Tetra-togenococcus halophilus')*	*P. inopinatus*	*P. parvulus*	*P. pentosaceus*	*P. pento-saceus subsp. intermedius'*	*P. urinae-equi*
Acid produced from or splitting of									
Melibiose	-	-	-	-	-	-	(+/-)	(+/-)	ND
Melezitose	-	(+/-)	-	+	-	-	-	-	ND
Raffinose	(+/-)	-	-	(+/-)	-	-	(+/-)	(+/-)	+
Maltotriose	-	(+/-)	+	+	(+/-)	(+/-)	(+/-)	(+/-)	ND
Dextrin	(+/-)	-	+	(+/-)	(+/-)	(+/-)	-	-	(+/-)
Starch	-	-	+	-	-	-	-	-	-
Inulin	-	-	(+/-)	-	-	-	(+/-)	(+/-)	-
Glycerol	(+/-)	-	-	(+/-)	-	-	(+/-)	(+/-)	-
Mannitol	(+/-)	-	-	-	-	-	-	-	(+/-)
Sorbitol	-	-	-	-	-	-	-	-	(+/-)
alpha-Methyl glucoside	-	(+/-)	(+/-)	+	-	-	-	-	ND
Salicin	(+/-)	(+/-)	+	+	+	+	+	(+/-)	+
Amygdalin	(+/-)	(+/-)	+	+	(+/-)	(+/-)	+	(+/-)	ND

+, >90% strains positive; +/-, 10-90% strains positive; -, <10% strains positive; ND, not determined.

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TABLE 11
Frequently Occurring Gram-Positive Cocci

	Van	PYR	LAD	gas from Glucose	6.5% NaCl	Growth at 10°C	45°C	Bile esculin
Streptococcus	S[g,h]	(-)[g]	(+)[g]	(-)[g]	V[g]	(-)[g]	V[g]	V[g]
Lactococcus	S[i,j,g]	(-)[j,g]	(+)[j,g]	(-)[j,g]	V[j,g]	(+)[j,g]	V[j,g]	(+)[j,g]
Pediococcus	R[g,i]	(-)[g]	(-)[g]	(-)[g]	V[g]	(-)[g]	V[g]	(+)[g]
Leuconostoc	R[g,i]	(-)[d,g]	(-)[g]	(+)[g]	(+)[g]	V[g]	V[g]	V[g]
Lactobacillus	Rse[,i]	(-)[e]	V[e]	V[e]	V[e]	V[e]	V[e]	V[e]
Enterococcus	S[e,h,i]	(+)[e]	(+)[e]	(-)[g]	(+)[g]	(+)[g]	(+)[g]	(+)[g]
Aerococcus	S[g]	(+)[f,g]	(-)[f,g]	(-)[g]	(+)[g]	(-)[g]	(-)[g]	V[g]
Globicatellae	S[g]	(+)[g]	(-)[f,g]	(-)[g]	(+)[g]	(-)[g]	V[g]	V[g]
Heliococcus	S[g]	(+)[f,g]	(-)[f,g]	(-)[g]	(+)[g]	(-)[g]	(-)[g]	(-)[g]

Abbreviations: V, variable reaction, S, susceptible (presence of zone of inhibition), or (absence of zone of inhibition). Van, Vancomycin testing (S; susceptible or R, resistant), PRY = pyrrolidonylarylamidase, LAP = leucine aminopeptidase (+), positive reaction, (-), negative reaction, [a]gas from glucose in MRS broth, [b]NaCl, growth in broth, containing 6.5% NaCl, [c]hydrolysis of bile esculin.

Data from [d]Cai et al. 1998 (12), [e]Facklam et al. 1989 (264), [f]Facklam et al. 1995 (265), [g]Facklam et. al. 1989 (40), [h]Hartman 1992 (48), [i]Rodriquez 1996 (267), [j]Ruoff 1993 (233), [k]Schelifer 1985 (43), [l]Simpson et al. 1988 (261).

TABLE 12
Lactic Acid Bacteria
The Genus Lactobacillus
(Streptobacteria Betabacteria and Thermobacteria)

General Media

Acidic Tomato Broth	APT Agar	Arginine Agar	Arginine Broth	Arginine Tetrazolium Agar
Bif Agar	Elliker Agar	Glucose Lemco Broth	Homofermentative Heterofermentative Differential Medium	Manganese dioxide Agar or Modified MRS Agar + Manganese 0.75% Xanthan gum 0.50%
Methylene Blue Milk Agar	Modified APT + Tween	Modified MRS Broth or Agar	Moeller decarboxylase	M17 Agar
MRS Broth or Agar	MRS + 0.5% Cysteine	MRS Broth + 5% glucose	MRS + 4% Sodium gluconate	MRS + 5% Sucrose
MRS + 0.4% Teepol	Reddy's Broth or Agar	Rogosa Agar	Semisolid Citrate Milk Agar	SL Agar
Tetramethyl-Benzidine Medium	Thallous Acetate Tetrazolium Agar	Trypticase Soy Agar + 5% Sucrose	Yeast Glucose Agar	Yeast Glucose Phosphte Broth
Yeast Lactose Agar	Yogurt Lactic Agar			

The Genus Lactobacillus

Streptobacteria & Related Bacteria

Acetate Agar	All-Purpose Agar + Tween	Apple Juice Medium	Blood Agar	Brain Heart Infusion Yeast Extract Agar
CaSO-Agar	Cresol Red Thallous Acetate Sucrose Agar	Fructose Tween 80 Medium	Homofermentative Heterofermentative Differential Medium	Lactobacillus-Agar + 0.04 sorbate

TABLE 12 (continued)
Lactic Acid Bacteria
The Genus Lactobacillus
Streptobacteria and Related Bacteria

(continued)

Lactic Bacteria Broth	Lactobacillus Heteroferm Screen Agar	Lactobacillus Heteroferm Screen Broth MRS Modified	Lactobacillus Selection Corn Starch Agar	Lactobacillus Selective Agar
Liver Infusion Sake Medium	Malate Decarboxylating Medium	Modified Homohiochii Medium	Modified Lactobacillus Selection Medium	Modified MRS Broth or Agar
MRS Agar (deman Rogosa Sharpe Agar)	MRS 5.5	MRS+Beer	MRS Broth or Agar	MRS + 0.1% Sorbate
MRS +20 % Tomato Juice	NBB Medium	Nitrite Actidione-Polymyxin Agar	Nutrient Agar	Raka Ray NO. 3 Medium
Rogosa Agar	Rogosa SL broth (Acetate Medium)	Rogosa SL Broth + DL mevalonic acid & 40ml/L ethanol	Sanfranciso Medium	Selective SL Medium
Standard 1 Nutrient Agar Sucrose Substituted for glucose	Sucrose Agar	Tomato Juice Medium	Tomato Juice Medium	Tryptone Glucose Yeast extract Agar

The Genus Lactobacillus

Betabacteria

AOAC Lactobacillus Broth or Agar	Apple Juice Yeast extract Agar	Betabacterium Medium	Fresh Extract of Yeast	Lactic Bacteria Broth

TABLE 12 (continued)

		Betabacteria		
		(continued)		
Lactobacillus sake Medium	**MRS Agar**	**MRS Agar or Broth**	**MRS Agar or Broth +** mevalonic acid	**MRS +Maltose 1.5% FYE**
Tryptone Yeast extract Glucose Agar	**Xyl-YP Broth + CaC03**			
		The Genus Lactobacillus		
		Thermobacteria		
Blood Agar	**Briggs Tomato Juice Medium**	**Lactobacillus bulgaricus agar**	**Lactic Bacteria Broth**	**Lactobacillus Broth AOAC**
Lactobacillus Medium 169 ATCC Medium	**Lactobacillus Medium 416 ATCC 78**	**Lactobacillus Medium 1006**	**Lactobacillus MRS Broth**	**Low Glucose Agar**
MRS Agar	**MRS Broth**	**MRS + 0.5% cysteine**	**MRSS 5.7 +** 0.6-1.0 % sorbic sorbic acid	**Panthothenate Culture Agar**
Rogosa Agar	**SL-Medium + 0.5% Meat**	**Tomato Juice Agar ATCC Medium 33**	**Tomato Juice Broth ATCC Medium 433**	**Tomato Juice extract milk Medium ATCC Medium17**
Tomato Juice Medium				

TABLE 12 (continued)

The Genus Lactococcus

Alsan	**Arginine Tetrazolium Agar**	**Blood Agar**	**Elliker's Broth**	**Glucose Lemco Broth at or + $40^{\circ}C$ pH 9.2 4% NaCl**
KM Agar Selective Agar	**Lactococcus Streptococcus Differential Medium**	**Litmus Milk**	**M17 Agar**	**0.1% Methylene Blue Milk Agar**
Nutrient Agar	**Reddy's Differential Agar**	**Reddy's Differential Broth**	**Semi-Solid Citrate Milk Agar**	**Thallous acetate Tetrazolium Glucose Agar**
WACCA	**Yogurt Lactic Agar**			

The Genus Leuconostoc

Acetate Agar	**Acidic Grape Broth**	**Acid Tomato Agar +** Cycloheximide 20 µg /ml	**Acidic Tomato Broth**	**Acidic Tomato Broth +** 10% triammonium citrate
Citrate Malate Broth	**Fructose Tween 80**	**HP Medium**	**Leuconostoc mesenteroides-Medium**	**Leuconostoc oenos Medium**
Litmus milk	**LUSM Agar**	**Modified MRS Agar**	**Modified Rogosa Medium**	**MRS Agar** (wine)
MRS Agar + arginine	**MRS Agar +** Cycloheximide 50 µg/ml	**MRS + 0.05% Cysteine**	**MRS Agar +** glucose	**MRS Agar +** trehalose
MRS Broth or **Agar**	**Nakagawa Agar**	**Orange Serum Agar**	**Sucrose Agar** (austrialian wines)	**5% Sucrose Agar**

TABLE 12 (continued)

The Genus Leuconostoc

(continued)

Tetrazolium Sucrose Agar	**Thallous-Acetate Tetrazolium Sucrose**	**Tomato Juice Agar ATCC 33**	**Tomato Juice Agar** Cycloheximide 50 µg/ml	**Tomato Juice Agar** Cycloheximide (100 mg/L)
Tomato Juice Broth	**WACCA**	**Yeast Glucose Citrate Agar** Cycloheximide 20 µg/ml	**Yeast Glucose Citrate Broth**	**Yeast Glucose Citrate Broth**
Yeast Glucose Phosphate Broth	**Yeast Glucose Peptone Broth + Thallous Acetate 0.1% Crystal violet 0.0005%**			

The Genus Pediococcus

Acetate Agar	**Glucose Yeast Broth + 4-5 % Sodium Chloride**	**Glucose Yeast Extract Agar**	**GYP-CaCO3 Medium**	**Kirin Ohkochi Taguchi Agar**
Lee's Multidifferential Agar	**Low Glucose Agar**	**MRS Agar**	**MRS Broth or Agar**	**Modified MRS Agar without glucose**
NBB Medium	**Pediococcus Medium**	**Pediococcus Medium +** 0.003% mevalonic acid	**Raka Ray No. 3**	**Sucrose Agar**
Tomato Juice extract	**Tomato Juice Agar**	**Wallerstein Laboratory Nutrient Broth**	**WL Differential Agar**	**Universal Beer Agar**

TABLE 12 (continued)

The Genus Pediococcus

(continued)

Acetate Agar	**Acidic Tomato Broth**	**Aesculin Bile Azide Agar**	**Alsan**	**Arginine Tetrazolium Agar**
BIF Medium	**Briggs Agar**	**Citrate Azide Tween Carbonate Agar**	**deman Rogosa Sharpe Agar**	**Elliker Agar**
Fructose & Tween 80 Medium	**Homofermentative Heterofermentative Differential Medium**	**Isolation Medium**	**Kanamycin Aesculin Azide Agar**	**Lactobacillus Agar + 0.04% Sorbate**
LAMVAB	**Low Glucose Agar**	**M-Enterococcus Agar**	**0.1%Methylene Blue Milk Medium**	**Modified MRS Agar** Antimicrobials 5mg/l MRS +Ampicillin MRS +Clindamycin MRS + X-gal & 1% Glucose
Modified Rogosa Broth or Agar	**Moretros DML Medium**	**MRS Agar**	**MRS Agar**	**MRS broth or Agar +** Atmospheric Conditions
MRS Broth + Cysteine 0.05%	**MRS + 4% Sodium gluconate**	**MRS + 0.1% Sorbate**	**MRS Agar + Sorbate 0.06%-0.2%** pH < 5.7	**MRS + 5% Sucrose**
Nitrite Actidione Polymyxin Agar	**Oxoid MRS Agar** Tomato Serum or 20% apple juice Cycloheximide (100 mg/L) pimaricin--(50-100 mg/l)	**Sucrose Agar**	**Tomato Juice Agar or Broth**	**Thallous Acetate Tetrazolium Glucose Agar**
Tomato Juice Arginine Glucose Medium	**Yeast Glucose Phosphte Broth**			

TABLE 13

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Acetate Agar	**5.4**	**AA**	Garvie 1984 (51) Whittenbury 1965 (68) Holzapfel 1992 (59) Holzapfel 1992 (52) Cavett 1963 (108)	Vegetables Psychrophilic Meats	Enumeration Isolation Cutivation	Lactobacillus Leuconostoc Pediococcus Meat LAB
Acetate Agar	**5.6 - 5.8**	**SL Agar**	Sharpe 1981 (4) Raccah 1987 (227) Hammes 1992 (55)	Milk & Dairy Intestinal Sources	Isolation	Lactobacillus Pediococcus Leuconostoc
Acid Tomato Agar + Cycloheximide 20μg /ml	**4.8**	**ATA**	Priest & Pleasant 1988 (212) Atlas 1993 (56)	Wort & Barley Malt	Cultivation	Leuconostoc *Leuc. oenos* Lactobacillus
Acidic Tomato Broth	**4.8**	**ATB**	Holzapfel 1992 (52) Garvie 1967 (94) Garvie 1984 (51) Holzapfel 1992 (59) Priest et al. 1988 (212)	Wine	Cultivation	Lactobacillus Leuconostoc Pediococcus *Leuc. oenos*

Symbols & Abbreviations

Symbols & Abbreviations that are used to represent commonly used terms, species or subspecies names.

Bif. = *Bifidobacterium*, *Cb.* = *Carnobacterium*, Cult. = Cultivation, *E.*=*Enterococcus*, Enum.= Enumeration, Heteroferm = Heterofermenter, Homoferm = Homofermenter, *L.* = *Lactobacillus*, *Lac.* = *Lactococcus lactis* *Lc.* = *Lactococcus*, *Leuc.* = *Leuconostoc*, Mesophilic Streptococci = *Lactococci*, *Lactics* = *Lactic Acid Bacteria*, ND. =No Data available, *P.* = *Pediococcus*, *Ped.* = *Pediococcus*, Str. = *Streptococcus*, *ssp.* = subspecies, VP = Voges Proskauer.

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Acidic Tomato Broth + 10% triammonium citrate	**4.8**	**ATB**	Garvie 1967 (94)	Wine	Isolation Citrate fermentation	Leuconostoc Pediococcus
Acidic Grape Broth	**4.8**	**AGB**	Holzapfel 1992 (59)	Wine	Isolation	*Leuc. oenos*
Aesculin Bile Azide Agar	**7.1**	**ABA**	Mossel et al. 1978 (260) Reuter 1992 (259) Difco Catalog 1995 (130) Atlas 1993 (56) Hartman 1992 (48)	Meat & Milk Chicken Liver Minced beef & Pork	Isolation	Enterococcus
All-Purpose Agar + Tween	**6.7**	**APT**	Holzapfel 1992 (52) Sharpe 1981 (4)	Cured Meats general	Isolation	Lactobacillus Leuconostoc Pediococcus Carnobacteria
Alsan	**5.9**		Al-Zoreky & Sandine 1991 (244)	Fermented Food products milk, cheeses & pickles & sausage	Selective & Differential Agar	*Lac.* ssp. *lactis* (yellow or white colonies) *Lac.* ssp. *diacetylactis* (blue-green to green) colonies
AOAC Lactobacillus Broth or Agar	**6.8**		Rogosa 1974 (30) Atlas 1993 (56)	Vitamin materials	Cultivation of stock cultures for vitamin assay	*L. viridescens ATCC 12706* *L. casei* ATCC 7469 *L. fermentum* 9338 *L. leichmannii* ATCC 4797

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
APT Agar	**6.7**	**APT**	Hammes 1992 (55) Sharpe 1981 (4) Holzapfel 1992 (52) Difco 1995 (130)	Cured meats	General purpose	LAB especially *L. viridescens*
Apple Juice Yeast extract Agar	**4.8**		Hammes 1995 (145) Carr 1972 (155)	Apple Juice	Cultivation	*L. collinoides*
Arginine Broth	**7.0**		Schillinger 1987 (1) Harrigan et al. 1976 (39)		Detection of arginine hydrolysis	LAB
Arginine Tetrazolium Agar	**6.0**		Harrigan 1976 (39) Sandine 1988 (100)	Various Cheeses Dairy Products Starter Cultures & Fermented Foods	Detection of Arginine hydrolysis	*Lc. lactis* *Lc. cremoris*
Betabacterium Medium	**7.2**		Gherna ATCC 1989 (87) Medium 84 Atlas 1993 (56)		Cultivation Enumeration	Betabacteria
BIF Medium	**7.4**	**BIF**	Pacher 1996 (254)	Fermented Milk Products	Enumeration	Lactococcus Streptococcus Bifidobacteria Lactobacillus
Brain Heart Infusion Yeast Extract Agar	**6.8**	**BHIYE**	Holzapfel 1992 (52)	Meats	Selective isolation	Most LAB

TABLE 13 (continued)
Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Briggs Tomato Juice Medium	**6.8**	**BRIGGS**	Holzapfel 1992 (52) Baird 1987 (71)	Milk & Dairy products	Cultivation	LAB Some Streptococcus & Enterococcus
Blood Agar	**6.8**	**BAP**	Schleifer 1985 (43) Teuber 1995 (86) Difco 1995 (130) Rogosa 1974 (30) Collins 1976 (7)	Dairy Products Fermented Vegetables Intestinal tract	Cultivation Enumeration	Fastidious LAB
CaSO-Agar sucrose substituted for glucose	**7.5-8.0**		Holzapfel 1992 (52)	Refrigerated prepacked meat products	Differentiation Enumeration	Carnobacterium
Citrate Azide Tween Carbonate Agar	**7.0**	**CATC**	Reuter 1992 (259) Baird 1987 (71) Mossel et al. 1978 (260) Hartman 1992 (48)	Milk & Meat Human & animal feces	Isolation Cultivation Differentiation	*E. faecalis* & *E. faecium*
Citrate Malate Broth	**4.2 & 4.7**	**CMB**	Garvie 1967 (94) Garvie 1984 (51) Dicks 1990 (210)	Wine	Differentiate *Leuconostoc oenos* from other Leuconostoc spp.	*Oenococcus oenos* Leuconostoc

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Cresol Red Thallous Acetate Sucrose Agar	**8.5-9.0**	**CTAS**	Holzapfel 1992 (52)	Fresh water fish prepacked meat and poultry	Enumeration Isolation	Carnobacteria
deman Rogosa Sharpe Agar	**6.2**	**MRS Agar**	Baird 1987 (71) Holzapfel 1992 (52)	General Purpose	Isolation	Most LAB
Elliker's Broth	**6.8**	**Lactic Agar**	Elliker 1956 (81) Teuber 1995 (86) Difco technical manual (196)	Dairy products	Isolation Enumeration Differentiate *S. cremoris* & *S. lactis*	Fastidious Lactococcus
Fructose Tween 80 Medium	**5.2**	**FT**	Holzapfel 1992 (52) Holzapfel 1992 (59) Cavin et al. 1989 (255)	Wine	Culture	*Leuconostoc oenos*
Fresh Extract of Yeast	**5.5**	**FYE**	Weiss et al. 1984 (162)	Yeast	Supplement	*L. sanfrancisco*
GYP-CaCO3 Medium	**6.8**	**GYP-CaCO3**	Tanasupawat 1983 (235) Simpson 1995 (42)	Beer Fermented Beverages	Detection of Spoilage bacteria	*P. urinae-equi* & *P. halophilus*
**Glucose Lemco Broth at or + **	**9.2 40^{o}C 4% NaCl**		Harrigan 1976 (39)	Dairy products	Base medium for Differential tests	*Lac.* ssp. *lactis* *Lac.* ssp. *cremoris* *Lac.* ssp. *diacetylactis*
Glucose Lemco Broth	**9.2**		Harrigan 1976 (39)	Dairy products	Detection of growth at pH 9.2 Detection of acetoin from *Lc. diacetylactis*	Lactic Streptococci

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media Medium	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Glucose Yeast Broth + 4-5 % Sodium Chloride	**7.0**		Atlas 1993 (56) Simpson 1995 (42) Gherna 1989 (87)		Isolation Enumeration	*P. halophilus*
Glucose Yeast Extract Agar	**6.5**	**GYEA**	Atlas 1993 (56) Holzapfel 1992 (59)	Plant materials	Isolation Culture & Enumeration	Leuconostoc Pediococcus
HP Medium	**5.4**		Holzapfel 1992 (59) Holzapfel 1992 (52)	Milk & dairy products salt anchovies	Isolation & Enumeration	Leuconostoc
Homofermentative Heterofermentative Differential Medium	**7.0**	**HHD**	Holzapfel 1992 (52) Fleming 1992 (54) McDonald 1987 (58)	Fermented Vegetables	Differentiate between Homofermenters & Heterofermenters	Streptococcus Lactobacillus Pediococcus Leuconostoc
KM Agar	**6.6**	**KM**	Kempler & McKay	Dairy	Differentiate	*Lac.* ssp. *lactis* (white)
Selective Agar			1980 (195)	Products	Citrate fermenters	*Lac.* ssp. *diacetylactis* (blue)
Kanamycin Aesculin Azide Agar	**7.2**	**KAA**	Reuter 1992 (259) Baird 1987 (71) Mossel et al. 1978 (260) Atlas 1993 (56) Hartman 1992 (48)	Milk & Water Packaged Sliced Cooked Meat Products	Isolation	Group D Streptococcus

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media Medium	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Kirin Ohkochi Taguchi Agar	**5.4**	**KOT**	Taguchi 1990 (237) Simpson 1995 (42)	Beer	Detection of Beer Spoilage Bacteria	Pediococcus LAB
Lactic Bacteria Broth	**5.1-5.3**		Atlas 1993 (56) Hammes 1995 (145) Kandler 1986 (3) Simpson 1995 (42) Gherna 1989 (87) ATCC Medium 80	Milk & Cheese Brewery Grain Mashes	Cultivation of Thermobacteria	*L. buchneri* *L. delbrueckii* *Ped. damnosus*
Lactobacillus Agar + 0.04% Sorbate	**5.0**	**LaS**	Holzapfel 1992 (52) Baird 1997 (71)	Meats	General Purpose	Lactobacillus Lactococcus Leuconostocs Pediococcus
Lactobacillus Broth AOAC	**6.8**		Atlas 1993 (56) Kandler 1986 (3)	Beer Wine Cheese	Cultivation of Thermobacteria	*L. fermentum ATCC 9338* *L. leichmanni ATCC 4797* *L. casei ATCC 7469* *L. viridescens ATCC 12706*
Lactobacillus bulgaricus agar	**6.8**	**LBS**	Atlas 1993 (56)	Hard cheeses	Cultivation of Thermobacteria	*L. bulgaricus*
Lactobacillus Heteroferm Screen Agar	**5.5**	**MRS Modified**	Atlas 1993 (56)	Salad dressings	Isolation & Enumeration Lactobacilli	Heterofermentative Lactobacillus
Lactobacillus Heteroferm Screen Broth MRS Modified	**4.3**	**Modified Heteroferm Screen**	Atlas 1993 (56) Smittle 1992 (158)	Salad dressings	Differentiate between Homofermentative vs Heterofermentative Lactobacillus	Fastidious Lactobacilli e.g. *L. fructivorans* *L. brevis*

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Lactobacillus Medium 169 ATCC Medium	**7.0**		Atlas 1993 (56) Hammes 1995 (145)	Saliva & vaginal secretions	Cultivation	*Lactobacillus casei* Thermobacteria
Lactobacillus Medium 416 ATCC 78	**6.5**		Atlas 1993 (56) Gherna 1989 (87) Hammes 1995 (145) Kandler 1986 (3)	Mouth Intestinal tract	Cultivation	*L. fermentum* *L. salivarius* Thermobacteria
Lactobacillus Medium 1006	**6.3**		Atlas 1993 (56) Hammes 1995 (145)	Fermented plant materials Vaginal secretions	Cultivation	*L. delbreuckii* *L. jensenii* Thermobacteria
Lactobacillus MRS Broth	**6.5**		Atlas 1993 (56)	Dairy Products	Cultivation	*Aerococcus viridans* *Bif. coryneforme* *Lactococcus plantarum* Leuconostoc Pediococcus
Lactobacillus sake Medium			Atlas 1993 (56) Gherna 1989 1989 (87)	Sake	Cultivation	*L. fructivorans* *L. homohiochii*
Lactobacillus Selective Agar	**5.4**	**LBS**	Macdonald 1992 (159) Fleming 1992 (54) Harrigan 1976 (39)	Fermented vegetables & cheeses	Isolation & Cultivation	Lactobacillus Pediococcus

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media. Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Lactobacillus Selection Corn Starch Agar	**5.4**	**LBS Corn Starch Agar**	Nakamura 1979 (38) deman Rogosa Sharpe 1960 (247)	Waste Corn Fermentation	Culture & Enumeration	Starch hydrolysing Lactobacilli &. *L. amylophilus*
Lactobacillus Streptococcus Medium	**6.1**	**LS Differential Medium**	Atlas 1993 (56) Holzapfel 1992 (52)	Yogurt	Differentiation & Enumeration	Lactobacilli spp. (Irregular Red colonies with an Opaque zone) Streptococci spp. (Red colonies with a Clear zone)
LAMVAB	**6.5**		Hartemink et al. 1997 (293)	Feces	Isolation	Common Lactobacillus Bifidobacteria & Enterococcus
Lee's Multidifferential Agar	**5.5**	**LMDA**	Lee & Jangaard 1975 (232) Raccah 1987 (227) Simpson 1995 (42)	Beer & Fermentation Process	Isolation	Pediococcus
Leuconostoc mesenteroides- Medium	**7.8**	**LMM**	Holzapfel 1992 (52)	Plant Foods	Isolation	*Leuc. mesenteroides* spp. *mesenteroides*
Leuconostoc oenos Medium	**4.8**		Atlas 1993 (56)	Wine	Cultivation	*Leuc. oenos*
Litmus Milk	**6.8**		Harrigan 1976 (39) MacFaddin 1980 (34) Sharpe 1972 (63)	Dairy Products	Differentiate organisms by acid formation reduction or gas clot formation	LAB

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Liver Infusion Sake Medium			Atlas 1993 (56) Gherna 1989 (87) Hammes 1995 (145)	Spoiled sake	Culture	*L. fructivorans* *L. homohiochii*
Low Glucose Agar	**7.0**		Sharpe 1962 (26) Frasier 168 (176)	Dairy Products	Benzidine test for Iron Porphorins	LAB
LUSM Agar	**5.3-5.8**		Benkerroum 1993 (202)	Dairy Products Vegetables	Isolation Enumeration	Leuconostoc
M17 Agar	**7.15**	**M17 Agar**	Law & Sharpe 1978 (186) Sharpe 1978 (83) Terzaghi & Sandine 1975 (84) Teuber 1995 (86) Difco Technical (85)	Yogurt	Selective Isolation Lactococcus as mesophilic starter cultures	*Lac.* ssp. *cremoris* *Lac.* ssp. *lactis* *Lac.* ssp. *diacetylactis* *Str. thermophilus*
M17 Agar	**6.8**		Sharpe 1978 (83) Terzaghi et al. 1975 (84) Teuber 1995 (86) Harrigan & McCance 1976 (39) Difco Technical 1995 (130)	Yogurt	Selective Isolation	*Str. thermophilus* *Lactobacillus bulgaricus*

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
M-Enterococcus Agar	**7.2**	**ME**	Baird 1987 (71) Atlas 1993 (56) Reuter 1992 (259) Hartman 1992 (48)	Milk & Vegetation Water Sewage Intestinal sources	Isolation	Enterococcus Group D Streptococci
Malate Decarboxylating Medium	**7.0**	**MD**	Fleming 1992 (54) Acuff 1992 (117)	Pickles Cucumbers	Differentiation of malate decarboxyling Lactics	*Lactobacillus plantarum* Decarboxylating Lactics
Manganese dioxide Agar or **Modified MRS Agar + Manganese 0.75% Xanthan gum 0.50%**	**5.4**	**MDO Agar**	Schillinger 1987 (1) Whittenbury 1964 (70) Whittenbury 1965 (68)	Meat products	Detection of H_2O_2	LAB H_2O_2 producers
0.1% Methylene Blue Milk Agar, Broth	**6.4**	**MBM**	Mac Faddin 1980 (34) Deibel & Seeley 1978 (245) Sharpe 1965 (27) Sharpe 1966 (239) Atlas 1993 (56) Mundt 1986 (44)	Milk products	Detects Methylene blue Reduction	LAB

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media. Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
MRS Agar	**6.2 - 6.5**	**MRS**	Holzapfel 1992 (59) Garvie 1984 (51) Reuter 1983 (146) Difco 1995 (49) Holzapfel 1992 (52) Hammes 1992 (55) Wibowo 1985 (156)	Dairy oral intestinal Silage & Vegetable fermentation processes Meat wine	Isolation Cultivation	LAB
MRS Agar deman Rogosa Sharpe Agar	**6.2-6.5**	**MRS Agar**	Hiu 1984 (126)	Diseased Rainbow trout	Enumeration with acetate	*Carnobacterium piscicola*
		Modified MRS Agar without acetate	Holzapfel 1983 (123) Holzapfel 1992 (52) Collins 1976 (7) Rogosa 1974 (30) deman Rogosa & Sharpe 1960 (247)	Refrigerated prepacked meat	without acetate	*Carnobacterium divergens*
			Kandler 1983 (3) Kandler 1983 1983 (170)	Kefir	Cultivation majority LAB	*Lactobacillus kefir* LAB
			Schillinger 1987 (1) fig.3 Holzapfel 1982 (168) Hammes 1992 (55)	Namib Spring South West Africa	General purpose	*Lactobacillus kandleri* LAB

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
MRS 5.5	**5.5**	**MRSS**	Holzapfel 1992 (52)	Meats	Cultivation	Lactobacillus Leuconostoc
MRS Broth	**6.5**	**MRS-B**	Garvie 1984 (51) Difco 1995 (49)	Oral & Dairy fecal sources	Enrichment	Leuconostocs Pediococcus Lactobacillus
Modified	**6.2**	**MRS**	Simpson	Brewery	Cultivation	*P. pentosaceus*
MRS Agar without glucose		Replace with 1% mannose cellobiose or salicin	1995 (42)	Industry		*P. pentocaeeus* ssp. *intermedius* Lactobacillus
Modified MRS Agar	**6.0-**	**MRS**	Kneifel et al.	Silage	With antimicrobial	Cultivation of
Antimicrobials 5mg/l	**6.5**	serves	1994 (253)		Ampicillin	Various LAB
MRS +Ampicillin		as	deman Rogosa &		Recover Only---	Pediococci
MRS +Clindamycin		Basal	Sharpe		Recover only----	Enterococci
MRS + X-gal &		Medium	1960 (247)		Lactobacilli form--	(blue colonies)
1% Glucose					Gram + Cocci form- -	(white colonies)
Modified MRS Agar	**4.5**	**MRS**	Garvie 1984 (51) Holzapfel 1992 (59)	Silage & Vegetable fermentation processes	Enrichment	Pediococcus Lactococcus Leuconostoc Lactobacillus
Modified MRS	**8.5**	**D-MRS**	Holzapfel	Vacuum packaged	Cultivation	Carnobacteria
Broth or Agar		**Agar**	1992 (52)	refrigerated meats diseased fresh water fish		
Modified MRS	**4.0-4.3**	**MRS**	Garvie	Wine	Selective differential	Lactobacillus
Broth or Agar			1984 (51) Atlas 1993 (56) Raccah 1987 (227)	Beer	of Lactobacillus & Pediococcus from Leuconostocs	Pediococcus

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Modified Rogosa Medium	**4.5**	**RA Modified**	Beelman 1982 (154) Edwards 1997 (151)	Wine Grape juices	Enrichment Enumeration	Leuconostoc Wine LAB
Modified APT + Tween	**6.7**	**APT**	Baird 1987 (71)	Meat products	Detection of H_2O_2	LAB H_2O_2 producers
Modified Broth or Agar	**4.3**	**MRS**	Garvie 1984 (51) Difco 1993 (49)	Dairy, fecal vaginal	Selective differential of Lactobacillus & Pediococcus from Leuconostocs	Lactobacillus Pediococcus
Modified Homohiochii Medium	**5.4**		Kleynmans 1989 (150) Hammes 1992 (55) Kandler 1986 (3)	Fruit mashes	Cultivation	Obligate Heterofermenters fastidious Lactobacillus
Modified Lactobacillus Selection Medium	**5.4**	**LBS + 0.5% fructose Rogosa Medium**	Macdonald 1992 (159) Harrigan 1976 (39) Smittle 1992 (158)	Dressings sauces	Cultivation	Lactobacillus Streptococcus Leuconostoc Pediococcus
Moeller decarboxylase Medium	**6.0**		Harrigan et al. 1976 (39) King 1948 (76) Facklam 1995 (40)	Food isolates	Detection of decarboxylase activity	*Lactococcus lactis* *Lc. garveiae* *Lc. hordniae* *Lc. plantarum* *Lc. raffinolactis* *Lc. xyloses*
Moretros DML Medium	**4.7**	**DML**	Moretro et al. 1998 (252)	Meat	Enumeration of Extremely Fastidious Lactobacillus	Lactococcus *L. curvatus* *L. plantarum &* *L. pentosus*

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
MRS Agar + arginine	**6.2**		Sharpe 1972 (63)		Arginine hydrolysis	Leuconostoc
MRS+Beer	**4.7**	**MRS -B**	Holzapfel 1992 (52) Simpson et al. 1995 (42)	Beer	Detection at 22°C	beer Pediococci
MRS Agar + Cycloheximide 50 µg/ml	**5.5**	**MRS Agar**	Holzapfel 1992 (59) Wibowo 1985 (156)	Wine	Isolation	*Leuconostoc oenos*
MRS + 0.5% Cysteine	**6.5**	**MRS + cysteine**	Garvie 1984 (51) Garvie 1986 (98) Kandler 1986 (3) Gherna 1989 (87) Atlas 1993 (56) Hammes 1992 (55)	Intestinal sources	Cultivation Maintenance	*Leuc. cremoris* *Leuc. oenos* Anaerobic Lactobacilli *L. acidophilus*
MRS Agar or Broth + mevalonic acid	**5.2**		Hammes 1995 (145) Weiss 1983 (167) Difco 1995 (49) Tl0882 L-Tl0882-1 Kandler 1986 (3)	Alcoholic beverages	Cultivation	*L. hilgardii &* *L. fructivorans* Alcohol tolerant LAB

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
MRS Agar + glucose	**6.2**		Schillinger 1987 (1)	Milk & Dairy	Detect gas (CO_2) from glucose (heterofermenter)	Leuconostoc form D(-) lactate and CO_2 (+) Betabacteria form DL lactate and CO_2 (+)
MRS broth or Agar + Atmospheric Conditions	**6.2**	**MRS +** H_2 CO_2	Kandler 1986 (3) Garvie 1986 (99) Garvie 1984 (51)	Wines & Various Juices Environmental Sources	Microaerophilic conditions carbon dioxide tension	Leuconostoc Pediococcus Lactobacillus
MRS Broth + Cysteine 0.05 - 0.5%	**6.2**	**MRS**	Garvie 1984 (51) Garvie 1986 (98) Atlas 1993 (56) Hammes 1992 (55)	Wines Intestinal sources	Enhanced growth for *L. cremoris* & *Leuc. oenos* For Cultivation & Maintanence	Leuconostoc Pediococcus Anaerobic lactobacilli Lactobacillus
MRS Broth + 5% glucose	**6.4**		Schillinger 1987 (1) Harrigan et al. 1976 (39)	Dairy products	Detection of gas from glucose	Homofermentative vs Heterofermentative LAB
MRS Modified Lactobacillus Heteroferm Screen Broth	**4.3**	**Modified Heteroferm Screen**	Atlas 1993 (56) Smittle 1992 (158)	Salad dressings	Differentiate between Homofermentative Heterofermentative Lactics	Fastidious Lactobacilli such as *L. fructivorans* *L. brevis*
MRS +Maltose 1.5% FYE	**5.5**	**Modified MRS**	Weiss et al. 1984 (162) Kandler 1986 (3)	Sour dough	Growth	*L. sanfrancisco*

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
MRS + 4% Sodium gluconate	**7.0**	**Modified MRS Broth**	Facklam 1989 (199) Harrigan 1976 (39) Sharpe 1972 (63) MacFaddin 1980 (34) Topley 1982 (37) Teuber 1995 (86)	Milk products	Determine ability to form gas from gluconate as sole Carbon Source	Betabacteria Streptobacteria
MRS Agar + Sorbate 0.06%-0.2% Selective pH < 5.7	**5.7**	**MRSS Agar**	Reuter 1985 (160) Baird 1987 (71) Holzapfel 1992 (52)	Various Sources	Selective Isolation of All Lactics	Most LAB
MRS Agar + 5% Sucrose	**7.0**	**MRS + sucrose**	Harrigan et al. 1976 (39) Garvie 1984 (51) Sharpe 1979 (219) Holzapfel 1992 (52) Garvie 1967 (94) Dellaglio et al. 1995 (95)	Sugar products meats	Detection of slime formation	*Leuc. mesenteroides* ssp. *mesenteroides* *Leuc. mesenteroides* ssp. *dextranicum*
MRS + 0.4% Teepol			Sharpe 1962 (26) Makanjuola 1984 (64)	Isolates	Test resistance or susceptiblity to MRS +Tergitol	*Lc. plantarum*

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
MRS + 20% Tomato juice	**4.5-5.5**	**MRS Agar**	Wibowo 1985 (156)	Australian wines	Cultivation isolation	Wine Leuconostocs
MRS Agar + trehalose	**6.2**		Schillinger 1987 (1) Sharpe 1972 (63)		Trehalose fermentation	Leuconostoc
Nachweismedium für bierschädliche Baketerien		**NBB**	Simpson et al. 1992 (42) Hammes 1992 (55) Holzapfel 1992 (52)	Beer	Isolation & Enumeration	Pediococcus
Nakagawa Agar			Wibowo 1985 (156) Simpson 1995 (42)	Australian Wines & Brewery Materials	Isolation Cultivation Enumeration	Lactococcus Leuconostoc Pediococcus
Nitrite Actidione Polymyxin Agar	**5.5**	**NAP**	Davidson & Cronin 1973 (250) Holzapfel 1992 (52)	Meat	General purpose	Lactobacillus Lactococcus Leuconostoc
Nutrient Agar	**7.2**	**NA**	Rogosa 1974 (30) Schleifer 1985 (43) Harrigan 1976 (39) Difco 1995 (130) Teuber 1995 (86)	Dairy products fermented vegetables intestinal tract	Culture & enumeration	Lc. plantarum *Lactococcus garviae* Nonfastidious LAB

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Orange Serum Agar	**5.5**	**OSA**	Parish & Huggins 1988 (217) Hammes 1992 (55) Holzapfel 1992 (52)	Fruit Juices Citrus fruit	Isolation	Lactobacillus Leuconostoc Pediococcus
Oxoid MRS Agar Tomato Serum or 20% apple juice Cycloheximide (100 mg/L) pimaricin--(50-100 mg/L)	**5.5**	**Oxoid MRS Agar**	Pilone 1996 (152) Pimaricin 50-100 mg/L Prevent Yeast/mold Growth	Wines	Enrichment	Wine Lactics
Panthothenate Culture Agar	**6.8**		Atlas 1993 (56)	Microbiological Assay for Pantothenic acid	Cultivation	*L. plantarum*
Pediococcus Medium	**5.2**	**Lactobacillus MRS Broth**	Atlas 1993 (56) Garvie 1984 (51) Gherna 1989 (87)	Wine	Isolation Enumeration & Cultivation	*P. damnosus* Pediococcus Leuconostoc
Pediococcus Medium + 0.003% Mevalonic acid	**5.2**	**Lactobacillus MRS Broth**	Gherna 1989 (87) Atlas 1993 (56) Simpson et al. 1995 (42)	Beer	Enumeration of Beer Spoilage Pediococci requiring Mevalonic acid	*P. damnosus*
Raka Ray No. 3 Medium	**5.4**		Saha et al. 1974 (157) Difco Technical (215) Simpson et al. 1995 (42) Atlas 1993 (56)	Beer	Isolation	Lactobacillus Pediococcus spoilage microbes

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Reddy's Differential Agar or Broth	**5.9 - 6.2**		Reddy 1969 (193) Vedamuth 1992 (50) Reddy 1969 (89) Reddy et al. 1971 (194) Acuff 1992 (117)	Dairy Products	Isolation & Differentiation	*Lac.* ssp. *lactis* *Lac.* ssp. *cremoris* *Lac.* ssp.*diacetylactis*
Rogosa Agar	**5.4 - 5.5**	**RA**	Reuter 1985 (160) Baird 1987 (71) Holzapfel 1992 (52) Atlas 1993 (56) Sharpe 1981 (4) Macdonald 1992 (159) Hammes 1992 (55)	All	General purpose	LAB *L. viridescens* except species sensitive to acetate
Rogosa SL Broth + DL mevalonic acid & 40ml/L ethanol	**5.4**	**SL Broth**	Atlas 1993 (56) Hammes 1995 (145) Hammes 1992 (55) Kandler 1986 (3)	Spoiled rice	Cultivation	*L. homohiochii*

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Rogosa SL Broth Acetate Medium	**5.4**	**Modified SL Broth**	Schillinger 1987 (1) Sharpe 1981 (4) Collins 1987 (121)	All	Cultivation	Lactobacillus Bifidobacteria Streptococcus
SL-Medium + 0.5% Meat	**5.6**	**SL**	Hammes 1992 (55) Hammes 1995 (145)	Dairy products	Selective Isolation	*L. bulgaricus* Thermophilic Lactobacilli
Sanfranciso Medium	**6.3**		Vogel 1994 (163)	Sour dough	Cultivation	*L. sanfrancisco* *L. brevis* *L. fermentum* *L. fructivorans*
Selective SL Medium	**5.4**	**SL Medium**	Sharpe 1981 (4) Hammes 1992 (55)	Meats dairy fermented vegetables	Selective isolation	Lactobacillus Pediococcus Leuconostoc
Semi-Solid Citrate Milk Agar			Harrigan 1976 (39)	Dairy products	Citrate fermentation	*Lac. lactis* ssp. *lactis* *Lac. lactis* ssp. *diacetylactis*
Semisolid Tomato Juice skim milk agar	**6.5**		Sharpe 1965 (27) Harrigan 1976 (39)	Dairy products	Detection of gas from glucose	Homofermentative vs Heterofermentative LAB
Standard 1 Nutrient Agar Sucrose Substituted for glucose	**8.0**		Holzapfel 1983 (123) Hammes 1992 (55) Holzapfel 1992 (52)	Refrigerated prepacked meat	Isolation and enumeration	*Carnobacterium divergens*

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Sucrose Agar	**6.2**	**SAB**	Wibowo 1995 (156) Holzapfel 1992 (52) Hammes 1992 (55)	Wines Beer	Isolation Cultivation & Enumeration	Lactobacillus Leuconostoc Pediococcus
5% Sucrose Agar	**7.0**	**SA**	Garvie 1984 (51) Dellaglio 1995 (95) Holzapfel 1992 (52)	Dairy products Sugar cane	Detection of Dextran formation	Leuc. lactis *Leuc. mesenteroides* ssp. *paramesenteroides* *Leuc. mesenteroides* ssp. *mesenteroides*
Sucrose Agar	**6.2**	**SAB**	Hammes 1992 (55) Holzapfel 1992 (52) Raccah 1987 (227)	Beer	Isolation Cultivation & Enumeration	Lactobacillus Leuconostoc Pediococcus
Tetramethyl-Benzidine Medium		**TMB**	Eschenbach et al. 1989 (69)	Vaginal Secretions	Detection of H_2O_2	Lactobacilli producing H_2O_2
Tetrazolium Sucrose Agar	**6.0**	**TS**	Holzapfel 1992 (59) Holzapfel 1992 (52)	Frozen Peas & Vegetables	Isolation & Enumeration	Leuconostoc Lactococcus Enterococcus
Thallous Acetate Tetrazolium Glucose Agar	**6.0 - 6.1**	**TLTG**	Baird 1987 (71) Schillinger 1987 (1) Harrigan 1976 (39)	Bacon	Selective Isolation Differentiation of Enterococcus ammonia due to arginine hydrolysis	Enterococci Streptococci but not Group D *E. faecalis* & *E. faecium* Lactococcus Lactobacillus

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Thallous Acetate Tetrazolium Sucrose		**TATS**	Holzapfel 1992 (52) Holzapfel 1992 (59) Garvie 1984 (51)	Plant materials	Isolation Differentiate *Lc. lactis* & *E. faecalis*	Leuconostoc Lactococcus Enterococcus
Tomato Juice Agar	**7.2**	**TJA**	Holzapfel 1992 (59) Difco Technical 1987 (215) Atlas 1993 (56)	Beer	Cultivation	Lactobacillus Leuconostoc Pediococcus Propionobacteria
Tomato Juice Agar ATCC 33			Saha 1974 (157) Difco Technical (215) Atlas 1993 (56) Keer 1983 (214) Gherna 1989 (87)	Beer	Isolation & Enumeration	Pediococcus Lactobacillus
Tomato Juice Agar + Cycloheximide 50 µg/ml	**5.5**	**TJA**	Holzapfel 1992 (52) Holzapfel 1992 (59) Wibowo 1985 (156)	Wines	Isolation	LAB

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Tomato Juice Agar Cycloheximide (100 mg/L)	**5.0**	**TJA**	Difco Technical (215) Holzapfel 1992 (52) Hammes 1992 (55) Atlas 1993 (56)	Wine	Isolation	LAB (slow growing)
Tomato Juice Agar ATCC Medium 33	**7.2**	**TJA**	Atlas 1993 (56) Gherna 1989 (87) TJA 33 & TJB 433	Raw Milk Fermenting Apple Juice Wine Feces	Cultivation	Lactobacillus Leuconostoc Pediococcus
Tomato Juice Agar or Broth	**5.0**	**TJA**	Hammes 1992 (55) Holzapfel 1992 (52)	Wines	Isolation	Slow growing LAB
Tomato Juice Arginine Glucose Medium	**5.5**	**TJAG**	Liu et.al 1995 (246)	Wine	Detection of Carcinogen Ethyl carbamate	LAB
Tomato Juice Broth	**4.8**	**TJB**	Tracey 1987 (213)	Wine	Isolation Enumeration	Leuconostoc
Tomato Juice Broth ATCC Medium 433	**7.2**		Atlas 1995 (175) Atlas 1993 (56) Gherna 1989 (87)	Corn Steep Liquor Grass Silage and Feces	Cultivation	Lactobacillus Aerococcus Pediococcus Bifidobacteria Fastidious bacteria
Tomato Juice extract			Simpson 1995 (42)	Beer unhopped wort	Cultivation	Pediococcus Slow-growing Pediococci

TABLE 13 (continued)
Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Tomato Juice Extract Milk Medium ATCC Medium17	**7.0**		Atlas 1993 (56) Gherna 1989 (87) Kandler 1986 (3)	Milk Dairy Products	Cultivation	Fastidious LAB
Tomato Juice Medium	**6.1**	**TJM**	Atlas 1993 (56) Difco Technical 1987 (215) Wibowo 1985 (156)		Cultivation	*L. acidophilus*
Tomato Juice Medium	**5.0**	**TJM**	Holzapfel 1992 (52) Hammes 1992 (55) Atlas 1993 (56) Kandler 1986 (3)	Wines	Isolation	Slow growing LAB
Trypticase Soy Agar + 5% Sucrose		**TSB + sucrose**	Garvie 1984 (51) Sharpe 1979 (219)	Sugar products	Detection of slime formation	*Leuc. mesenteroides* ssp. *mesenteroides* *Leuc. mesenteroides* ssp. *dextranicum*
Tryptone Glucose Yeast extract Agar	**7.0**	**TGYEA**	Holzapfel 1992 (52) Hammes 1992 (55)	Refrigerated prepacked meat products	Recovery and enumeration	Carnobacteria
Tryptone Yeast extract Glucose Agar		**TYEGA**	Rogosa 1974 (30) Difco 1995 (130) Harrigan 1976 (39)	Wine	Cultivation	*L. hilgardii*

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Universal Beer Agar	**6.3**	**UBA**	Difco 1987 (215) Atlas 1993 (56) Atlas 1995 (175)	Beer	Cultivation	Pediococcus Lactobacillus
WACCA	**7.3**	**WACCA**	Holzapfel 1992 (59) Teuber 1995 (86)	Fermented Dairy Products	Culture & Enumeration	Leuconostoc Lactococcus *Lc. lactis* ssp. *diacetylactis*
Wallerstein Laboratory Nutrient Broth	**5.5**	**WL Nutrient Broth**	Atlas 1993 (56) Difco Technical (215) Difco 1995 (130)	Beer Brewery materials	Isolation & Enumeration	Beer Bacteria & Yeasts
Xyl-YP Broth + CaCO3	**6.8**		Okada 1979 (172) Atlas 1993 (56)	Cow dung	Isolation	*L. vaccinostrericus*
Yeast Glucose Agar	**6.8-7.0**	**YGA**	Sharpe et al. 1978 (83)	Yogurt Cheese starters Dairy products	General purpose medium	Mesophilic Streptococci (Lactococci)
Yeast Glucose Citrate Agar Cycloheximide 20 μg/ml	**6.5**	**YGC Medium**	Priest & Pleasant 1988 (212) Holzapfel 1992 (59) Atlas 1995 (175)	Malt & grain whiskey	Isolation Enumeration & Cultivation	Leuconostoc Lactobacillus

TABLE 13 (continued)

Summary Culture Media Section

Cuture Media	pH	Media Abbrev.	Reference Cited	Food Source	Purpose of Medium	Microorganisms Recovered
Yeast Glucose Citrate Broth	**6.7**	**YGCB**	Garvie 1967 (94) Priest 1988 (212) Holzapfel 1992 (59)	Non-wine Leuconostocs	Cultivation from a Variety of sources	Leuconostoc
Yeast Glucose Peptone Broth + Thallous Acetate 0.1% Crystal violet 0.0005%	**6.8**	**YGPB**	Holzapfel 1992 (59) Garvie 1984 (51)	Vegetable Matter Vegetables	Enrichment	Leuconostoc Lactobacillus
Yeast Lactose Agar	**7.0**	**YLA**	Sharpe 1978 (83)	Yogurt	Isolation	*Str. thermophilus*
Yeast Glucose Phosphte Broth	**6.7**	**YGPB**	Garvie 1967 (94) Garvie 1984 (51) Holzapfel 1992 (59)	Vegetable & Wines	Enrichment	Leuconostoc *Leuc. oenos*
Yogurt Lactic Agar	**6.8**	**YLA**	Matalon & Sandine 1986 (91) Holzapfel 1992 (52)	Yogurt	Differentiation of Rods & Cocci	*Str. thermophilus* (Red colonies) *L. bulgaricus* (White colonies)

tion and hydrolysis of arginine hydrolysis. Those that are ribose negative only are considered Group I; ribose and arginine negative are Group II; Group III Pediococci are ribose positive and arginine negative.[99,228] The differential characteristics of the Pediococci in flow diagram format Figure 7.

P. halophilus, *P. pentososaceus*, *P. pentosaceus* ssp. *intermedius,* and *P. acidilactici* may be separated by ribose and arginine tests. The Pediococci are unable to anaerobically ferment glycerol or sorbitol, with the exception of *P. pentosaceus,* which ferments both glycerol and sorbitol.[39,227] *P. pentosaceus* also produces what is referred to as a pseudocatalase, and thus produces a false-positive catalase test when grown on media with a low glucose concentration.[22,39,99,176,227] *P. halophilus* may be distinguished from other Pediococci by its growth in the presence of 15% NaCl broth, and fermentation of melezitose. *P. pentosaceus* ssp. *intermedius* can be easily differentiated from *P. pentosaceous* and *P. acidilactici* by its inability to ferment arabinose and xylose. Both *P. pentosaceus* and *P. acidilactici* ferment either arabinose or xylose or both. *P. acidilactici* grows at 50°C, whereas *P. pentosaceus* does not. *Pediococcus dexrinus* is identified by the production of acid and gas from sodium gluconate, growth at pH 8.0, fermentation of dextrin, failure to ferment pentoses or hydrolyze arginine, and is the only Pediococcus to hydrolyze starch. *P. damnosus* is the only Pediococci unable to grow at 35°C. *Pediococcus inopinatus* ferments lactose, while *P. parvulus* does not.[99,227,228,234,238]

XXVII. SUMMARY

Of Lactic Acid Bacteria Characteristics

The Lactic Acid Bacteria (LAB) represent a diverse group of microorganisms widely distributed in nature and associated with dairy, vegetable, and meat products.[1,2,3] The LAB consist of Gram-positive rods or cocci and are comprised mainly of four genera: Lactobacillus, Leuconostoc, Pediococccus, and includes the newly proposed genus Lactococcus.

The LAB are oxidase negative, lack a cytochrome system, and therefore are benzidine test negative.[3,28,32,33,34] They lack iron porphorins and are catalase negative with the exception of some Pediococci and the Aerococci, which produce a pseudocatalase that, unlike catalase, is not inhibited by azide, cyanide, or acid pH.[239] The presence of catalase or pseudocatalase is detected by growing microorganisms on a low glucose medium, and then testing with hydrogen peroxide.[2,22,28,30,99,176,219,240,241]

The LAB may be grouped according to the isomer of lactic acid produced and whether lactic acid is produced as the major product of the fermentation of glucose.[2,25,27,28,30] The LAB produce lactic acid as their major product and are referred to as Homofermentative, whereas the Heterofermentative LAB produce additional products such as carbon dioxide, ethanol, and acetic acid.[1,2,22,25,27,28,30,39,242]

The Homofermentative Lactics include Pediococcus and Streptococcus; the Leuconostoc and certain Lactobacillus are Heterofermentative.[1,22,28,99,176]

Lactobacillus

The genus *Lactobacillus* may be subdivided into three groups: Betabacteria, Streptobacteria, and Thermobacteria.[1,2,22] The thermobacteria are capable of growth at 45°C or greater but are unable to grow at 15°C. The Streptobacteria grow at 15°C, but most do not grow at 45°C. The Betabacteria also grow at 15°C. The Streptobacteria are able to ferment ribose and other pentoses and form CO_2 from gluconate, but do not form CO_2 from the fermentation of glucose.[1,4,22,37]

The Betabacteria are considered heterofermentative because they produce CO_2 from the fermentation of glucose. In contrast to both the Streptobacteria and Thermobacteria, most Betabacteria form CO_2 from glucose and hydrolyze arginine with the formation of ammonia. Almost all the Streptobacteria and Betabacteria ferment ribose, whereas none of the Thermobacteria do. Both the Streptobacteria and Thermobacteria are Homofermentative.[1,4,22,37]

The Streptobacteria may be divided into two main groups based on their ability to ferment mannitol. They may be further differentiated by other biochemical tests as well as by the type of lactic acid produced during fermentation of glucose.[1,22]

The Betabacteria may be differentiated further by their pattern of arginine hydrolysis and ribose fermentation. Those that hydrolyze arginine and ferment both ribose and cellobiose are differentiated further by trehalose and xylose, salicin and type of lactate formed, and inulin and mannitol fermentation. Betabacteria that fail to both hydrolyze arginine and ferment ribose are divided further into two groups, based on galactose and maltose fermentation. The final step of identification is based primarily on sugar fermentation pattern, the type of lactic acid formed from glucose, and the presence of meso-diaminopimelic acid in the cell wall.[1,22]

Betabacteria that hydrolyze arginine and ferment ribose but are cellobiose negative are tested with melibiose. Those isolates that are melibiose positive are tested with melezitose. Isolates fermenting melezitose are considered *L. buchneri,* whereas those negative for melezitose are considered members of the *L. brevis-buchneri* group (see Figure 3).[1] Other identification schema have been employed.[1,39,243]

Leuconostoc

The Leuconostoc are Gram-positive cocci that occur in pairs or chains. Their metabolism is heterofermentative, resulting in the formation of CO_2 from the fermentation of glucose. The Leuconostocs are unable to hydrolyze arginine.[1,39] The Leuconostoc require a more alkaline environment, pH >= 4.5, than the Lactobacilli and Pediococci, which are more acid tolerant and that survive a pH of 3.6 to 4.0.[1,22,39,51,94,95,98]

Although MRS agar is suitable for the Leuconostocs, Yeast Glucose Phosphate Peptone Broth is recommended.[1,49,51,94] MRS Broth, with the addition of 0.05% cysteine and a reduced oxygen atmosphere, enhances the growth of both *Leuconostoc cremoris* and *Leuconostoc oenos*.[51,98] Both *L. mesenteroides* ssp. *mesenteroides* and *L. mesenteroides* ssp. *dextranicum* may be recognized when grown on MRS containing 5% sucrose because they produce mucoid capsules.[51,94] *L. paramesenteroides* is biochemically similar to *L. mesenteroides* and *L. dextranicum* but does not form a capsule. *L. paramesenteroides* also differs from the other two species in that it fails to hydrolyze gluconate.[34,51,63,98,199] *L. cremoris* is one of the few Leuconostocs capable of producing diacetyl from citrate. This process is important in the dairy industry because diacetyl is an important dairy flavor.[95] *L. cremoris* is unable to ferment trehalose and sucrose but ferments galactose.[51,199] *L. cremoris* also produces carbon dioxide from citrate, is unable to grow in 2% NaCl, or hydrolyze arginine.[39,81,83,90,186] *Leuconostoc oenos* is important in the wine process and ferments trehalose but not sucrose.[41,51,94,95,98]

Lactococcus

The Lactococci are commonly found in dairy products and may be selectively isolated on Elliker's lactic agar, Arginine Tetrazolium Agar, or Alsan Medium.[39,44,81,86,100,244] The Lactococci may be distinguished by their temperature tolerance, presence of Group N Antigen, action in litmus milk, production of carbon dioxide, formation of diacetyl from citrate, fermentation pattern of a variety of carbohydrates (Table 8), as well as their salt tolerance.[39,81,82,86,90,100] Both *Lactococcus lactis* ssp. *lactis* and *L. lactis* ssp. *diacetylactis* may be differentiated from other Lactococci by their hydrolysis of arginine and by citrate utilization. *Lactococcus lactis* ssp. *diacetylactis* is used as a starter culture in the dairy industry. It produces carbon dioxide from citrate, diacetyl, and acetoin.[75,90]

Many of the Lactococci produce the Lancefield Group N antigen grow at 10°C, but not at 45°C, and grow in broth with 4% NaCl (except *L. lactis* ssp. *cremoris*). The Lactococci differ with the Streptococci by growing at 10°C whereas the Streptococci do not. The Lactococci differ from the Enterococci by not growing at 45°C.[1,43,86,187,245] Both the Lactococci and Streptococci are often screened by tests commonly used for Enterococcal identification and can be differentiated from the Enterococci by growth at both 10°C and 45°C, in 6.5% NaCl, at pH 9.6, and reduction of 0.1% Methylene Blue Milk Medium.[34,39,40,43,44,47,56,86,90,187,245] In 1985, *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus hordiae*, *Lactobacillus garviae*, *Lactobacillus plantarum*, and *Lactobacillus raffinolactis* were reclassified as members of the genus Lactococcus.[39,43,47,86,90,187] These species may be differentiated by their fermentation patterns for galactose, lactose, maltose, melibiose, melezitose, raffinose, ribose, and arginine hydrolysis.[39,40,43,187]

Lactococcus lactis ssp. *lactis* and *Lactococcus lactis* ssp. *diacetylactis* differ from other Lactococci by fermenting maltose; however, only *Lactococcus lactis* ssp. *diacetylactis* forms diacetyl from citrate.[90] *Streptococcus thermophilus* is distinguished by its growth at 50°C; neither *Lactococcus cremoris* nor *Leuconostoc cremoris* grow at 40°C.[90] Both *Lactococcus lactis* and *Leuconostoc cremoris* may be separated by salt tolerance; *Lactococcus cremoris* is inhibited above 4.0% NaCl and *Leuconostoc cremoris* is inhibited by 2% NaCl.[39,81,83,90,186]

Pediococcus

The Pediococcus, unlike the other Lactics, have a tetrad arrangement. The Pediococci grow on MRS media, and growth may be enhanced, as with the Leuconostocs, by culturing them under reduced atmospheric conditions.[3,40,51,99] The Pediococci are particularly useful as starter cultures for various types of sausages;[42,99] however, in the pickle industry and in the wine and beer industry they are unwanted contaminants when they produce off flavors.[2,8,39,42,246]

Culture Media for Lactics from Various Food Sources

There are a number of sources of information on culture media for the culture and isolation of Lactics; much of this has been reviewed by Holzapfel.[52] Media for growth and enumeration of Lactics from meat are Acetate Agar.[52] MRS Agar,[52,247] MRS with 0.1% sorbate,[52,71,160,248,249] Lactobacillus Sorbic Acid Agar,[52,71] Nitrite Actidione-Polymyxin Agar.[52,250] Moretro's DML Medium provides vitamins for fastidious meat Lactobacilli.[251,252]

For vegetables and other plants, Homofermentative Heterofermentative Differential Medium (HHD) may be used.[52,58] For Silage, Modified MRS Agar with antimicrobics at 5 mg/L are used to either inhibit or select for Pediococci, Enterococci, and Lactobacilli. MRS with ampicillin 5 mg/L selects for Pediococci but suppresses Lactobacilli and Enterococci. With clindamycin at 5 mg/L, only Enterococci are recovered.[253] MRS Agar with 50 mg/L of X-gal and 1% glucose separates Lactobacilli (blue colonies due to beta

D-galactosidase activity) from cocci colonies (white, lacking Beta-galactosidase activity).[253]

BIF Medium provides excellent recovery and maintainance for Bifidobacteria from fermented milk and dairy products.[254] Lactic Acid Bacteria cultivated in Acidic Tomato Broth pH 4.8,[52,94] Tomato Juice Agar,[52,55,203,255] and Fructose and Tween 80 Medium FT pH 5.2 are the best choices of media for cultivating lactic acid bacteria from wines.[52,255,256] For Beer, Sucrose Agar pH 6.2 is used.[52,55,203] Pediococcus *halophilus* from salted anchovies have been recovered on Soy Sauce Isolation Medium.[52,55,248]

On both MRS Agar, and MRSS Agar with 0.2% sorbate, *Lactobacillus* spp. appear as small greyish-white colonies, flat or raised, and smooth, rough, or intermediate.[52,71,160,247] Deman, Rogosa, and Sharpe Agar (sorbate 0.14%, pH 5.7) enables a selective enumeration of Lactobacillus; however, the International Standardization Organization (ISO) is in the process of evaluating the effectiveness of this formulation.[52,71,160,248,249,251,257]

Modified Oxoid MRS Agar at pH 5.5 is used to isolate LAB from wine. It is supplemented with 20% tomato juice serum with or without cycloheximide (100 mg/L) to suppress fungi. With resistant yeasts and fungi e.g. Bretannomyces, Pimaricin is recommended (50 mg/L to 100 mg/L). These antimicrobics are added after autoclaving. The acetic acid bacteria (Acetobacter species) oxidize alcohol to acetic acid.[9,258] Gram-negative bacteria may be suppressed in this way with the addition of kanamycin 94 mg/L.[152,9] A Modified Rogosa Broth pH 4.5 is used to isolate Lactic Acid Bacteria from grape juice and wines.[154,195]

In Lactobacillus Sorbic acid agar (LaS), 0.04% sorbate pH 5.0 enables a selective enumeration of the *Lactobacilli*.[251,257] On LaS Agar, colonies are white or greyish, flat or raised, smooth or rough.[52,71] This medium is ideal for isolating *Lactobacilli* from yogurt with mixed flora acetate, citrate, sorbate, and low pH help suppress the Enterococci, *Streptococcus thermophilus*, and to a slight degree *Lactobacillus bulgaricus*.[52,59,71,152,160,248]

Homofermentative Heterofermentative Differential medium (HHD) is used primarily for the isolation of Lactics from fermented vegetables. It also provides separation between Homofermentative and Heterofermentative LAB.[52,54,58] Heterofermentative Lactobacilli lower the pH to 4.6 by reducing fructose to mannitol to fructose with colonies appearing white, whereas Homofermentative Lactobacilli colonies appear blue to green in color (>5.5 pH).[52,54,58]

Briggs Agar is a nonselective, tomato base medium and is used primarily for the isolation of Lactobacilli from milk and dairy products. It may be better than MRS Agar for some strains of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Streptococcus lactis*.[71] *Lactobacillus* spp. appear as small, grayish-white colonies, 1 to 3 mm in diameter, and may be flat or raised, smooth or rough or intermediate on Briggs Agar.[52,71]

Both the Enterococci and the Group D Streptococci may be cultivated on a variety of culture media, Thallous Acetate Tetrazolium Glucose Agar (TLTG), Aesculin Bile Azide Agar (ABA), Kanamycin Aesculin Azide Agar (KAA), Citrate Azide Tween Carbonate (CATC), and M-Enterococcus Agar (ME) Table 13.[56,71,108,130,259,260]

No truly selective medium exists for the Enterococci.[259] For example, with TLTG a incubation at 30°C for 24 h results in the appearance of other Gram-positive organisms, including some LAB. A similiar situation occurs with KAA, CATC, and ME Agar, where contaminants may make it difficult to distinguish the Enterococci.[259]

TLTG is selective for the Enterococci, but recommended times and temperatures have varied from 37°C for 24 h or 42°C for 18 h.[39,71,259] One recommendation is for incubation at 37°C for 24 h or 37°C for 4 h, followed by 44 to 45°C for 20 h.[39,71,259] A more recent report recommends that 42°C for 18 h is sufficient to provide selectivity for the Group D Streptococci. Colonies of *Enterococcus faecalis* on TLTG appear deep red in the center with a narrow, outer white periphery. The red color is due to a reduction of the tetrazolium to a maroon formazan; *Enteroccocus faecium* forms white colonies. Some Streptococcus species are capable of growing on the medium but are very tiny and are white or red in color.[71]

Aesculin Bile Azide Agar and TLTG allow the growth of *Staphylococcus*, *Pseudomonas*, and Enterobacteriaceae. Kanamycin Aesculin Azide Agar allows the growth of Corynebacteria, partial growth of mesophilic Lactobacilli, and other Lactobacilli resistant to bile. Colonies that are presumptively Group D Streptococci are white or grey with black halos on KAA.[71]

Citrate Azide Tween Carbonate medium provides greater selectivity for the Enteroccocci than the other selective media mentioned above. *Enterococcus faecalis* forms distinct, red colonies on CATC Medium.[71,259] Colonies that are pink or with a red center are considered *Enterococcus faecium*. Enterococci and Streptococci form pink colonies <0.5 mm in diameter and grow more slowly than *E. faecalis* and *E. faecium*.[71] M-Enterococcus Agar reduces the colony size of the Lactococci, Leuconostocs, and Pediococci and lacks full supression of non-lactics. Both Group D Streptococci and the Enterococci appear as pink to dark maroon on ME Table 13.[71,259,260]

XXVIII. CONCLUSION

Lactics And Their Scope

The Lactic acid bacteria have been particularly useful as starter cultures in the manufacture of dairy products.[2,24] The lactics have also been, in recent years, implicated in a number of clinical infections ranging from mild infections such as urethritis to life-threating infections such as endocarditis, endometritis, pneumonia, septic arthritis, and meningitis.[10,16,19,96,233] Meningitis in a 16-year-old girl with no underlining disease was the first reported case of meningitis ascribed to a Leuconostoc.[15] Aquirre and Collins[10] list the variety of infections in which LAB have been implicated. Vancomycin-resistant Gram-positive strains involved in serious infections require a definitive identification along with a prompt antibiotic sensitivity and treatment.[10,15,19,96]

One study found that all brewery isolates of Pediococcus, Leuconostoc, Lactococcus, and Heterofermentative Lactobacilli were resistant to Vancomycin (30 μg) and avoparcin (20 μg) per disk; however, *Lactobacillus delbrueckii* ssp. *delbrueckii*, *L. delbrueckii* ssp. *lactis*, *L. delbrueckii* ssp. *leichmannii*, *L. delbrueckii* ssp. *helveticus*, *L. delbrueckii* ssp. *bulgaricus* were sensitive to these two antimicrobial agents.[261]

The use of antibiotic susceptibility patterns might be helpful for the identification of the LAB, particularly with the greater incidence in the number of case reports of involvement of the lactic acid bacterial involvement in clinical infections. The use of Vancomycin discs has been recommended as a aid in the identification of Gram-positive cocci with foods.[10,17,18,19,50,96,261,262,263]

Facklum[265] proposed a disk screening method to presumptively identify clinical strains of Gram-positive, catalase negative cocci using leucine-aminopeptidase (LAP), pyrrolidonylarylamidase (PYRase), and Vancomycin resistance. The Enterococci, Pediococci, Leuconostocs, and some Lactobacillus spp. are resistant to Vancomycin (VRB) 30 μg.[261,264] Many Enterococci of medical importance are Vancomycin resistant, PYRase (+), and LAPase (+), while the Pediococci are Vancomycin susceptible, LAP(+), and PYRase (–). The Leuconostoc are Vancomycin resistant but lack Pyrase and LAPase activity. The Viridans Streptococci, *Streptococcus bovis* strains, Leuconostoc, and the Pediococci are PYRase negative. LAPase is also useful because all Aerococci, Globicatellae, Helcococci, and Leuconostocs are negative. Vancomycin resistance, PYRase (+), and LAPase (+) presumptively identifies an isolate as an Enterococcus, while Vancomycin resistance, PYRase (–), and LAPase (–) indicates a Leuconostoc, whereas Vancomycin resistance and LAPase (+) indicates a presumptive Pediococcus.[261,264,265]

Both conventional biochemical methods and nucleic acid probes for identification are needed when vancomycin-resistant Enterococci are suspected. In one study, Microscan Walkaway 40 by Baxter Scientific misidentified Vancomycin-resistant isolates of Lactobacilli as Enterococci, which could lead to improper treatment.[40,264,266,267]

Recent developments in biochemical identification include the API Rapid CH-50; it has been approved for the identification of the Lactobacilli by the Compendium of Microbiological Methods.[61,268] Today, many clinical and industrial laboratories in the U.S. use this kit for the identication of LAB.[43,61,222,226,241,264,269,270,271]

Biolog Inc. has developed a 96 well microtiter plate method for identifying Bifidobacterium, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, and Weissella. The system is intended to identify 57 LAB species. Each microtiter plate consists of 95 carbon substrate utilization tests.[43,61,222,226,269,270,271,272]

Ribotyping provides DNA fragments by enzymes, the subsequent fragments are separated by electrophoresis and compared to known sequences of ribosomal RNA. Ribotyping is a method that is both accurate and applicable for the identification of all strains of Lactobacillus spp. and is used for epidemiology and taxonomy.[271]

XXIX. RECENT SPECIES

Leuconostoc oenos is distinct enough to warrant its inclusion as a new distinct genus, Oenococcus, with a new species, *Oenococcus oeni*.[273] The transfer of *Leuconostoc paramesenteroides* and related species *Lactobacillus kandleri, L. viridescens, L. halotolerans*, *L. confusus,* and a new species from sausage, *W. hellenica,* to a new genus, *Weissella*, has been proposed.[57]

L. parabuchneri is related to *L. buchneri* (30% homology). This microorganism has been isolated from beer, cheese, and human saliva. It is a heterofermentative, Gram-positive rod, is facultative anaerobic, catalase negative, and grows at 10°C and 40°C. This organism occurs singly, in pairs, and in chains. It ferments L-arabinose, D-fructose, galactose, gluconate, D-glucose, maltose, melezitose, melibiose, D-raffinose, ribose, and sucrose. It hydrolyzes arginine but not esculin and is Voges-Proskauer negative. It differs from *L. buchneri* by not fermenting D-xylose [274].

Lactobacillus malefermentans is associated with spoiled beer. It is nonmotile, and grows at both 15°C and 30°C but not 37°C. As a Heterofermenter it forms gas from glucose.[275] It also ferments *N*-acetyl-glucosamine, D-glucose, maltose, alpha-methyl-D-glucoside, and ribose.[55,134,274,275]

Lactobacillus graminis is a relatively new species found in grass silage. *L. graminis* cells are slightly curved and form a flocculent sediment after 3 days of incubation in broth. *L. graminis* forms colonies on MRS Agar that are nonpigmented, smooth, and round. This species is differentiated from *L. sake* and *L. curvatus* by the fermentation of maltose, ribose, and xylose. *L. graminis* ferments xylose, but not ribose or maltose; both *L. sake* and *L. curvatus* ferment maltose and ribose but not xylose.[276]

Lactobacillus suebicus is a new, obligate Heterofermenter found in fruit mashes.

The species name refers to the area, Swabia, in southern Germany. *L. suebicus* is a microaerophile and forms cells with rounded ends. It can grow at 10°C and at a pH of 2.8. It is capable of growing in up to 14% ethanol.[150] *L. suebicus* ferments arabinose, galactose, maltose, ribose, and xylose. It forms 2-mm colonies on Homohiochii agar that are white, round, convex, smooth, slimy, in an anaerobic atmosphere of 95% N_2 and 5% CO_2 (Table 6).[55,145,150,276]

Lactobacillus oris is from the human oral cavity. It resembles *L. brevis*. Cells are rod shaped, occur singly, in pairs, or in small chains. It grows at 30°C and 40°C but not at 28°C or 48°C and forms colonies that are small, raised, semirough, and rough. It is nonmotile, produces D- and L-lactic acid from glucose. *L. oris* ferments amygdalin, L-arabinose, D-arabitol, D-fructose, D-galactose, beta-gentiobiose, D-glucose, lactose, maltose, melibiose, raffinose, D-ribose, sucrose, and D-xylose. *L. oris* may be differentiated from *L. brevis* in that the former ferments amygdalin, D-arabitol, beta-gentiobiose, lactose, raffinose, and sucrose. It differs from *L. fermentum* by producing acid from amygdalin, D-arabitol, and beta-gentiobiose.[277]

Lactobacillus vaginalis was found in association with patients suffering from vaginitis due to trichomonas. *L. vaginalis* is nonmotile, and cells occur singly, in pairs, and short chains. It grows at 30°C and 45°C but not at 22°C or 48°C. It is a facultative anaerobe, a Heterofermenter, catalase negative, and produces both D- and L-lactic acid. It forms white to grey colonies that vary in size from 1 to 5 mm, and are semirough with raised areas. It is catalase negative and ferments D-galactose, D-glucose, lactose, D-levulose, D-mannose, maltose, melibiose, D-raffinose, and sucrose. Most strains ferment ribose.[73,277]

Lactobacillus piscium was isolated from diseased Rainbow yearling fish. It appears to be related to *Lactococcus raffinolactis* based on rRNA sequencing. It is cultured in Yeast Glucose Phosphate Broth. *L. piscium* differs from *L. raffinolactis* by producing acid from ribose and gluconate. It also differs from the similiar Vagococci by producing different unsaturated cellular fatty acids isomers.[191]

Lactobacillus paraplantarum has been isolated from beer and human feces. Although similar to *Lactobacillus plantarum*, it does not ferment alpha-methyl-D-mannoside. The four strains of *L. paraplantarum* may be distinguished from *L. plantarum* and *L. pentosus* by the fermentation of glycerol, xylose, and not melezitose.[278] *L. plantarum* ferments only melezitose, while *L. pentosus* ferments both xylose and glycerol but not melezitose. *L. paraplantarum* does not ferment xylose, glycerol, or alpha-methyl-D-mannoside. Only *L. plantarum* ferments alpha-methyl-D-mannoside. *L. paraplantarum* forms smooth, round, cream colored colonies that are domed shaped. This microorganism occurs singularly, in pairs, and in short chains. It is nonmotile, Heterofermentative, and ferments ribose, galactose, D-glucose, D-fructose, D-mannose, mannitol, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, beta-gentiobiose, and gluconate. It hydrolyses esculin and fails to deaminate arginine.[191,278,279,280]

Lactobacillus panis was isolated from rye sourdough. *L. panis* is rod shaped and forms cells that are chains or clusters. On agar the colonies are white to grey, smooth, convex, and up to 2 mm in diameter. A similar organism, *L. oris,* grows at both 30°C and 45°C but not 15°C. It forms both D- and L-lactic acid. Arginine is not hydrolyzed, but esculin is hydrolyzed. Acid is formed from L-arabinose, fructose, galactose, glucose, lactose, maltose, D-mannose, melibiose, alpha-methyl-D-glucoside, beta-methyl-D-xyloside, raffinose, D-ribose, salicin, sucrose, and D-xylose.[281]

Lactobacillus amylolyticus is a Gram-positive rod with rounded ends and occurs in pairs or short chains. It is a microaerophile and forms colonies on MRS Agar that are rough with a dull surface and may be beige to dirty white in color. It is an obligate Homofermenter and forms DL-lactic acid from the fermentation of glucose. It produces acid from dextrin, fructose, galactose, glucose, maltose, mannose, and sucrose, hydrolyzes esculin but not arginine. It is associated with germinated barley, green malt, cured malt, mash, and unhopped beer wort.[282]

Emendments

Both *L. curvatus* and *L. sake* grow at 2°C and at pH 3.9.[102] *L. curvatus* is negative for both melibiose fermentation and arginine hydrolysis, whereas *L. sake* is positive for both substrates. Previous studies disagreed on the discriminatory power of these two tests.[102,283]

Trends

LAB are being studied not only for their fermentative properties but also for their ability to produce compounds that possess antibacterial and other beneficial properties that are not entirely elucidated. Many of the genera produce a class of unique compounds called bacteriocins. The major bacteriocin used in the U.S. for preservation of food products is Nisin.[284]

As of 1989, Nisin has been granted GRAS (Generally Recognized as Safe) status by the FDA. This FDA ruling allows the use of Nisin in pasteurized cheese spreads to prevent the outgrowth of *Clostridium botulinum* and with fruits, vegetables, or meats at 500 to 10,000 IU Nisin/g of food product.[114,284,285,286]

Nisin can improve the self-life of pasteurized processed cheese, canned fruit, vegetables, nonfermented milk, dairy products, meat and fish, as well as beer.[284] Nisin has been used to preserve flavor and prevent off-flavors.[119] It has also been used to prevent spontaneous malolactic fermentation in wine that may occur after alcoholic fermentation is completed.[119,284] The use of Nisin-resistant strains in conjunction with Nisin can eliminate contaminating LAB that may affect the aesthetic properties of wine as well as interupt the delicate balance of the malolactic fermentation process.[119,120,284] The production of Nisin or bacteriocins by LAB in foods can extend shelf life of food products by preventing contaminates from growing and subsequently producing off-flavors, pellicles, turbidity, and or diacetyl formation.[20,119,284] Nisin acts by inactivating sulfydryl groups of the cytoplasmic membrane, causing loss of cell contents due to leakage. It also prevents endospore germination by preventing the exosporium from swelling, which is essential for endospore release.[114] The effectiveness of Nisin can be expanded to include Gram-negative bacteria by the inclusion of chelating agents.[284,287,288]

Probiotic therapy is the ingestion of viable microorganisms.[113] It is postulated that probiotics may be effective against food allergies by enhancing how antigens are processed into "tolarogens".[112,284,289,290] Probiotic strains have been used to reduce the severity of Atopic Dermatitis, Crohn's disease, Rheumatoid Arthritis, Ulcerative Colitis, antibiotic associated diarrhea, and Acute Gastroenteritis due to rotavirus.[289,291] The patented Lactobacillus GG strain has been found associated with an increased improvement in the level of immune response toward Rotavirus, an increase in the total number of antibody secreting cells, as well a corresponding increase in the level of IgA antibody.[289]

LAB presence is believed to help prevent viral intestinal infections by preventing pathogens from becoming established.[112,113,114,284,289,290,292,293] Placebo-controlled double-blinded studies support the idea that LAB promote health by augmenting the gut mucosal barrier to pathogen invasion. LAB have been shown to be useful when colon intestinal levels of 10^6 cfu are maintained.[263,284,289,285,290]

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