Organisms (ATCC)	Growth	Colony Appearance
Bifidobacterium longum (15707)	+++	red-brown (maroon)
Bifidobacterium infantis (15697)	+++	red-brown (maroon)
Streptococcus thermophilus (14486)	-	-
Lactobacillus acidophilus (314)	-	-
Lactobacillus bulgaricus (11842)	-	-

Table 1. *Bifidobacterium sp.* cultural characteristics on BSM Agar

Name	Brand	Cat. No.	Pack Size
BSM Agar	Fluka	**88517**	500 g
BSM Broth	Fluka	**90273**	500 g
BSM Supplement	Fluka	**83055**	5 g
Wilkins Chalgren Anaerobic Agar	Fluka	**W1761**	500 g
Mupirocin	Sigma	**M7694**	50 mg 100 mg
Lithium Mupirocin	Sigma	**07188**	1 g 100 mg

Table 2. Selective medium and supplements for *Bifidobacterium*

Bifidobacterium grow very well on this medium, while *Lactobacillus* and *Streptococcus* strains are inhibited. *Bifidobacterium* colonies grow within 24-48 hours (occasionally up to three days because of the highly selective conditions). The *Bifidobacterium* colonies are purple-brown and therefore are easy to differentiate from other organisms.

In a Swiss governmental evaluation study for the enumeration of bifidobacteria in sour milk products, the traditional method was compared to Wilkins-Chalgren Agar with 100 mg/L mupirocine and BSM Agar. The traditional method produced statistically significant differences, while Wilkins-Chalgren Agar and BSM Agar showed similar results without any significant variances. The study concluded, "On the BSM Agar, the bifidobacteria forms purple-brown colonies which made the enumeration easy" [3].

Lactobacillus Species

Lactobacilli are rod-shaped, Gram-positive, fermentative, facultative anaerobic or microaerophilic organotrophs. Normally they form straight rods, but under certain conditions spiral or coccobacillary forms have been observed. In most cases, they form chains of varying length. Lactobacilli belong to the lactic acid bacteria and comprise the major part of this group. As their name implies, they produce lactic acid and derive energy from the fermentation of lactose, glucose and other sugars to lactate via homofermentative metabolism. About 85-90% of the sugar utilized in the fermentative process is converted to lactic acid. However, there are some heterofermentative lactobacilli that produce alcohol in addition to lactic acid from sugars. This acid-producing mechanism inhibits growth of other organisms and favors the growth of lactobacilli that thrive in low pH environments. ATP is generated during the process by non-oxidative substrate-level phosphorylation.

Some strains of lactobacilli were shown to produce, like bifidobacteria, a bacteriocin-like substance and are able to inhibit a broad range of pathogens [8]. Lactobacilli also produce adhesins (proteins), which perform a vital role in recognizing specific host components (extracellular matrix) important for the bacterial adhesion and colonization at host surfaces, as well as in bacterial interaction with physiological and immunological processes [8].

In the last several years, several new *Lactobacillus* species have been introduced as probiotics, including *Lactobacillus rhamnosus, Lactobacillus casei,* and *Lactobacillus johnsonii*. As of this time, probiotics have not been used in the pharmaceutical industry due to the many open questions that remain to be answered [7].

Figure 1. Yogurt sample cultured on BSM Agar. Bifidobacteria appears as purple-brown colonies.

Medium for Lactobacilli

Since lactobacilli prefer acidic conditions, natural extracts and juices from tomatoes and oranges, as well as other single metabolic acids (e.g. malic acid), are often used as media ingredients. Casein and yeast extract provide rich amino acid sources, and the maltose is used as a carbohydrate source for lactobacilli, which cannot utilize glucose as fermentable sugar. Fructose is the carbohydrate source of *Lactobacillus fructivorans*. Polysorbate, sorbitan mono-oleate and other related compounds act as a source of fatty acids and stimulate the lactic acid bacteria.

Today, it is standard practice to differentiate lactobacilli based on their phenotype using selective media. Classical phenotypic tests for identification of lactobacilli are based on physiological characteristics, like motility, growth temperature, respiratory type, and growth in sodium chloride, as well as on diverse biochemical characteristics, such as fermentation type, metabolism of carbohydrate substrates, production of lactic acid isomers, coagulation of milk, and presence of specific enzymes like arginine dihydrolase. In Bergey's Manual, *Lactobacillus* is described as a Gram-positive rod, non-spore forming, acid fast negative and catalase negative. The colony morphology on certain media is taken for the presumptive identification.

Figure 2. 3D rendering of isolated *Lactobacillus*

Cat. No.	Name	Description
Media for Differentiation		
17153	LS Differential Agar Antibiotic free skim milk powder (Fluka 70166) TTC Solution (Fluka 17779)	For the maximum growth and differentiation of lactobacilli and streptococci on the basis of colonial morphology, T.T.C. reduction and casein reaction.
17158	Litmus Milk	For maintenance of lactobacilli and for determining the action of bacteria on milk.
17222	WL Nutrient Agar	For the examination of materials encountered in brewing and for industrial fermentations containing mixed flora of yeasts and bacteria.
17215	WL Differential Agar	For selective isolation and enumeration of bacteria encountered in breweries and industrial fermentations.
Nonselective Media		
Y3127	Yeast Malt Agar	Used for the isolation and cultivation of yeasts, molds and other aciduric microorganisms.
17216	Tomato Juice Agar	For the cultivation and enumeration of lactobacilli.
80957	Plate Count Skim Milk Agar	For the enumeration of bacteria in milk and dairy products.
17218	Tomato Juice Broth	For cultivation of yeasts and other aciduric microorganisms.
T2188	Tryptone Glucose Yeast Extract Agar	Recommended for enumeration of bacteria in water, air, milk and dairy products.
17123	Elliker Broth	For culturing streptococci and lactobacilli of importance in the dairy industry.
Selective Media (by low pH and selective agents)		
R1148	Rogosa SL Agar	Used as a selective medium for cultivation of lactobacilli.
85515	Sorbic Acid Agar (Base)	For the isolation and differentiation of lactobacilli from food, faeces etc. according to Reuter.
17154	Lactobacillus Bulgaricus Agar (Base)	Used with acetate buffer for isolation and identification of *Lactobacillus bulgaricus.*
69966	MRS Broth	For the enrichment and isolation of all species of lactobacilli from all types of material.
41782	MRS Agar, Vegitone	This MRS Agar is free of animal derived material. It is recommended for the isolation and cultivation of *Lactobacillus* species.
69964	MRS Agar	For the enrichment, cultivation and isolation of all species of *Lactobacillus* from all types of material according to De Man, Rogosa and Sharpe.
38944	MRS Broth, modified, Vegitone	This MRS Broth contains plant peptone instead of animal peptone. It is recommended for the isolation and cultivation of *Lactobacillus* species.
30912	MRS Agar, original acc. DeMan-Rogosa-Sharpe	For the enrichment, cultivation and isolation of all species of *Lactobacillus* from all types of materials. Recommended by the "Schweizerisches Lebensmittelbuch" 5th ed., chapter 56A.
75405	Orange-serum Agar	For the isolation, cultivation and enumeration of acid-tolerant spoilage microorganisms in fruit juice and fruit juice concentrates, in particular from citrus fruit, according to Hays, Troy and Beisel.
17226	Universal Beer Agar	For culturing microorganisms of significance in the brewing industry.
W2261	WL Nutrient Broth	Recommended for the cultivation of bacteria encountered in breweries and industrial fermentations.
02538	Raka Ray Agar Lactic Acid Supplement, modified (Fluka 14121) Cycloheximide solution (Fluka 18079)	A medium for selective isolation of lactic acid bacteria from beer and brewing processes.
83920*	Rogosa Agar	Selective agar for the isolation and enumeration of lactobacilli belonging to the buccal and intestinal flora, as well as from food such as milk and meat.
64198	NBB Agar Base, modified	Selective medium used for the detection of contaminating/spoilage microorganisms in brewery.
50725	NBB Broth Base, modified	Selective medium used for the detection of contaminating/spoilage microorganisms in brewery.

Table 3. Media for lactobacilli detection, differentiation, isolation, enumeration and cultivation

*Available in Europe only.

Modern Methods to Detect Lactobacilli

As a modern alternative, molecular biology-based methods, like PCR, can be consulted. However, they are often quite expensive. Under our Fluka Brand, Sigma-Aldrich provides a revolutionary molecular biology method that is rapid, easy and cost effective. Based on the detection of rRNA, this method completely avoids the need for PCR amplification. The sandwich hybridization test, called HybriScan, is performed on a microtiter plate. The range of lactobacilli detected by HybriScan tests is listed in Table 4.

More information about the test and the technical principles behind it are found on our web site ***sigma-aldrich.com/hybriscan***

Cat. No.	Brand	Description	Specifity	Reactions
62533	Fluka	HybriScan®***D*** Beer	Detects all beer spoiling bacteria of the genera *Lactobacillus, Pediococcus, Pectinatus* and *Megasphaera*	96
68301	Fluka	HybriScan®***D*** Drinks	Detects yeast of the genera *Saccharomyces, Zygosacchromyces, Brettanomyces, Torulaspora, Pichia, Candida* and bacteria of the genera *Lactobacillus, Acetobacteraceae* and *Alicyclobacillus*	96
59744	Fluka	HybriScan®***D*** Lactobac	Detects bacteria of the genera *Lactobacillus* and *Pediococcus* in fruit juices and non-alcoholic beverage.	96
75724	Fluka	HybriScan®***I*** *Lactobacillus brevis*	Identification of *Lactobacillus brevis*	48
80065	Fluka	HybriScan®***I*** *Lactobacillus buchneri*	Identification of *Lactobacillus buchneri*	48
86827	Fluka	HybriScan®***I*** *Lactobacillus lindneri*	Identification of *Lactobacillus lindneri*	48

Table 4. HybriScan products (HybriScan®D = detection kit; HybriScan®I = identification kit)

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Genus *Yersinia*

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Detection, identification, differentiation and cultivation of *Yersinia* species

Yersinia is a rod shaped and facultative anaerobic Gram-negative bacteria belonging to the family of Enterobacteriaceae. It has a fermentative metabolism, is oxidase-negative, mannitol-positive, glucose-positive and lactose negative. On the Bismuth sulfite Agar (Fluka 95388) *Yersinia* can be differentiated from *Salmonella* because it is not able to produce hydrogen sulphide. It is a psychrophilic organism, surviving and proliferating at low temperatures of 0-4 °C (e.g., on food products in a refrigerator). Some *Yersinia* species are also relatively highly heat resistant. However, they can be quite easily inactivated by oxidizing agents such as hydrogen peroxide and potassium permanganate.

Pigs, rodents, rabbits, sheep, cattle, horses, dogs, and cats are the natural sources of *Yersinia*. At the moment, most human illness cases caused by *Yersinia* originate from *Y. enterocolitica*. This organism is the cause of yersiniosis, an infectious disease with symptoms like fever, abdominal pain, and diarrhea. Other clinically important species of this genus are *Y. pseudotuberculosis* (symptoms similar to *Y. enterocolitica* except in most cases no diarrhea is seen) and *Y. pestis* (organism responsible for the bubonic plague). Most infections are acquired through contaminated food, like raw or undercooked pork products, seafood, vegetables, unpasteurized milk or untreated water. However, infections may also occur after contact with infected animals or faeces, or through transmission by fleas.

The bacteria received its name from A. E. J. Yersin, a Swiss microbiologist, who discovered the *Yersinia pestis* bacterium in 1894 in Hong Kong.

Species	Differentiation Properties
Y. enterocolitica	catalase-positive, most strains are ornithine-positive, motile at 22-26 °C, non-motile at 37 °C, urea-positive, sorbitol- and cellobiose- positive, most strains are sucrose-positive
Y. pseudotuberculosis	motile at 22-26 °C, non-motile at 37 °C, urea-positive, rhamnose-positive, esculin-positive, ornithine decarboxylase negative, indole negative
Y. pestis	produces two antiphagocytic components (antiphagocytic slime), catalase positive non-motile at 22-26 °C and 37 °C, esculin-positive, indole negative, ornithine decarboxylase negative

Table 1. Some differentiation of the important *Yersinia* species C

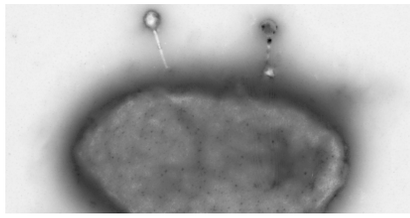

Figure 2. Bacteriophage *øR1-37* attached to *Y. enterocolitica* YeO3-R1 cell (length of cell = 1,7 µm; Source Mikael Skurnik, University of Turku, Finland)

Cat. No.	Description
Nonselective Broths	
53286	Brain Heart Broth
40893	Peptone Water, phosphate-buffered, Vegitone
77187	Peptone Water, phosphate-buffered
Selective Enrichment Broths	
17156	ITC Broth (Base)
69965	Mossel Broth
17192	Peptone Sorbitol Bile Broth
Basal Media for Carbohydrates Utilization	
A0715	Andrade Peptone Water
28943	Andrade Peptone Water, Vegitone
Medium for Autoagglutination	
39484	Methyl Red Voges Proskauer Broth
Nonselective Agars for Differentiation & Confirmation	
27048*	Christensen's Urea Agar
60787	Kligler Agar
M1428	Motility Indole Ornithine Medium
75315	OF Test Nutrient Agar
O5386	Ornithine Decarboxylase Broth
51463	Urea Broth
Selective Agars for Detection and Isolation	
95760*	Yersinia Selective Agar (CIN Agar)
Selective Agars with Differential System for Differentiation, Detection and Isolation	
95388	Bismuth sulfite Agar
70135	DCLS Agar
90035	DCLS Agar No. 2
17213	Violet Red Bile Glucose Agar without Lactose
53605	Violet Red Bile Glucose Agar without Lactose, Vegitone
95273	VRB MUG Agar

Table 2. Media for *Yersinia*

*Available in Europe only.

Cat. No.	Supplements
38266	Ferrioxamine E
75258	Yersinia selective Supplement
17778	Ticarcillin Supplement
17777	Potassium chlorate Supplement

Table 3. Selective and growth supplement for *Yersinia*

Cat. No.	Test for *Yersinia* Diagnostics
80507	Bile Esculin Disks
88597	Catalase Test
56481	Cellobiose Disks
63367	Dextrose (Glucose) Disks
05686	DMACA Indole Disks
49825	DMACA Reagent
67309	Kovac's Reagent for Indoles
78719	Kovac's Reagent Strips
28816	Lactose Disks
94438	Mannitol Disks
08714	Methyl Red Solution
07345	Oxidase Reagent acc. Gaby-Hadley A
07817	Oxidase Reagent acc. Gaby-Hadley B
18502	Oxidase Reagent acc. Gordon-McLeod
40560	Oxidase Strips
70439	Oxidase Test
93999	Rhamnose Disks
92971	Salicin Disks
93998	Sorbitol Disks
94309	Sucrose Disks

Table 4. Test for identification and differentiation of *Yersinia*

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More details about the media or tests can be found on our website ***sigma-aldrich.com/microbiology.***