Lecture one: Lactic acid bacteria isolation

- Lactic acid bacteria (LAB) are an order of gram-positive, low-GC, acid-tolerant, generally nonsporulating, nonrespiring, either rod-shaped (bacilli) or spherical (cocci) bacteria that share common metabolic and physiological characteristics. These bacteria, usually found in decomposing plants and milk products, produce lactic acid as the major metabolic end product of carbohydrate fermentation.
- Linked LAB with food fermentations, as acidification inhibits the growth of spoilage agents. Proteinaceous bacteriocins are produced by several LAB strains and provide an additional hurdle for spoilage and pathogenic microorganisms. Furthermore, lactic acid and other metabolic products contribute to the organoleptic and textural profile of a food item.
- The industrial importance of the LAB is further evidenced by their generally recognized as safe (GRAS) status, due to their ubiquitous appearance in food and their contribution to the healthy microbiota of animal and human mucosal surfaces.
- The genera that comprise the LAB are at its core *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Streptococcus*, as well as the more peripheral *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Sporolactobacillus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*; these belong to the order **Lactobacillales**.

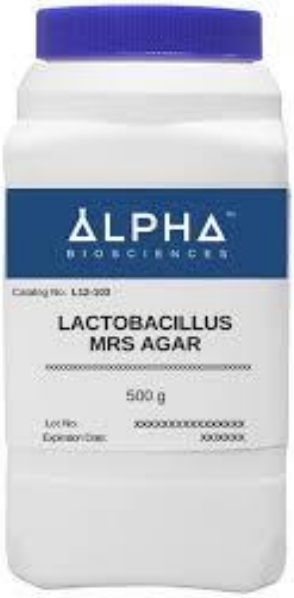
Culture media of Isolation

Many culture media used to lactic acid bacteria isolation, these media have characteristic for isolated one specie or genus from lactic acid bacteria

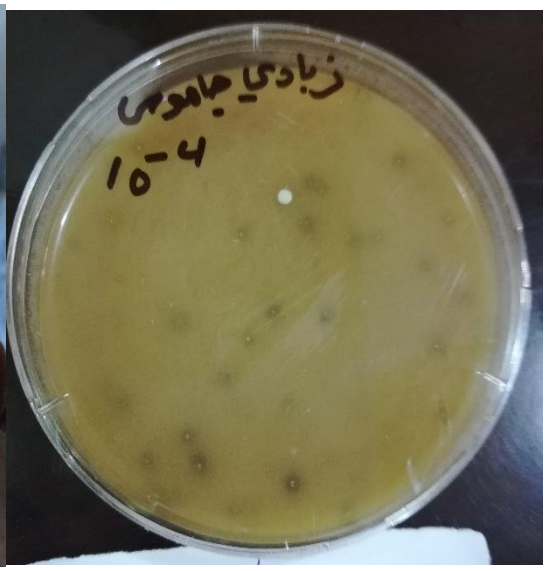
1- **MRS**: De Man, Rogosa and Sharpe agar, often abbreviated to MRS, is a selective culture medium designed to favor the luxuriant growth of *Lactobacilli* for lab study. Developed in 1960, this medium was named for its inventors. It contains sodium acetate, which suppresses the growth of many competing bacteria (although some other Lactobacillales, like *Leuconostoc* and *Pediococcus*, may grow). This medium has a clear brown color.

MRS agar typically contains(w/v): 1.0 % peptone,1.0 % beef extract, 0.4 % yeast extract, 2.0 % glucose, 0.5 % sodium acetate trihydrate, 0.1 % polysorbate 80 (also known as Tween

80), 0.2 % dipotassium hydrogen phosphate ,0.2 % triammonium citrate, 0.02 % magnesium sulfate heptahydrate ,0.005 % manganese sulfate tetrahydrate ,1.0 % agar, pH adjusted to 6.2 at 25°C.



In sometime, to increase the selectivity characteristics of MRS we add 0.1-0.5% of $CaCO_3$



2-**M17:** This bacterial growth medium was developed in 1971 for *Lactococcus* species and *Streptococcus thermophilus* isolated from milk products. It was originally called M16

medium, but in 1975 Terzaghi and Sandine added disodium-β-glycerophosphate to the medium as a buffer, and named the new growth medium M17 medium. It was later found that the addition of disodium-β-glycerophosphate inhibits the growth of many *Lactobacillus* species.

M17agar typically contains(w/v):5.0 g Pancreatic digest of casein, 5.0 g Soy Peptone, 5.0g Beef extract, 2.5 g Yeast extract, 0.5 g Ascorbic acid, 0.25g Magnesium sulfate, 10.0 g Disodium-β-glycerophosphate, 11.0 g Agar and 10% of lactose.

3-Ragosa agar: Rogosa Agar is primarily a selective medium for the cultivation of *Lactobacillus*. High acetate concentration and low pH effectively suppress other bacteria,but also many strains of other lactic acid bacteria. The modification of the pH to 6.2 instead of 5.5 alters the selectivity of the medium for the whole group of lactic acid bacteria. Casein enzymic hydrolysate, yeast extract provide nitrogenous compounds, sulphur, trace elements and vitamin B complex, essential for growth of Lactobacilli. Glucose acts as fermentable carbohydrate. Polysorbate 80 is the source of fatty acids. Ammonium citrate and sodium acetate inhibit moulds, Streptococci and many other organisms. Monopotassium phosphate provides buffering capability. Magnesium sulphate, manganese sulphate and ferrous sulphate are sources of inorganic ions. Low pH of the medium and addition of acetic acid makes the

medium selective for Lactobacilli, inhibiting other bacterial flora. It is recommended that the plates should be incubated at 30°C for 5 days or at 37°C for 3 days in an atmosphere of 95% hydrogen and 5% carbon dioxide . High acetate concentration and acidic pH suppress many strains of other lactic acid bacteria.

Rogosa agar typically contains: Tryptone 10g, Yeast extract 5g, Glucose 20g, Potassium dihydrogen orthophosphate 6g, Tween 80 1ml, Triammonium citrate 2g, Sodium acetate 15g, Magnesium sulphate,$7H_2O$ 0.575g, Manganese (II) sulphate,H_2O 0.11g, Iron (II) sulphate, $7H_2O$ 0.034, and Agar 15g. Final pH (at 25°C) 6.2±0.1.

Metabolism of Lactic acid bacteria

The lactic acid bacteria belong to two main groups – the homofermenters and the heterofermenters. The pathways of lactic acid production differ for the two. Homofermenters produce mainly lactic acid, via the glycolytic (Embden–Meyerhof) pathway). Heterofermenters produce lactic acid plus appreciable amounts of ethanol, acetate and carbon dioxide, via the 6-phosphoglucanate/phosphoketolase pathway. All lactic acid bacteria except Leuconostocs, group III lactobacilli, oenococci and weissellas, use the glycolytic pathway. Normal conditions required for this pathway are excess sugar and limited oxygen.

Homolactic fermentation

The fermentation of 1 mole of glucose yields two moles of lactic acid;

$C_6H_{12}O_6$ →	2 $CH_3CHOHCOOH$
Glucose	lactic acid

Heterolactic fermentation

The fermentation of 1 mole of glucose yields 1 mole each of lactic acid, ethanol and carbon dioxide;

$C_6H_{12}O_6$	→ $CH_3CHOHCOOH$+	C_2H_5OH+	CO_2
Glucose	lactic acid+	ethanol+	carbon dioxide

Lactic acid bacteria in fermented plant products.

Homofermenter	**Facultative homofermenter**	**Obligate heterofermenter**
Enterococcus faecium	*Lactobacillus bavaricus*	*Lactobacillus brevis*
Enterococcus faecalis	*Lactobacillus casei*	*Lactobacillus buchneri*
Lactobacillus acidophilus	*Lactobacillus coryniformis*	*Lactobacillus cellobiosus*
Lactobacillus lactis	*Lactobacillus curvatus*	*Lactobacillus confusus*
Lactobacillus delbrueckii	*Lactobacillus plantarum*	*Lactobacillus coprophilus*
Lactobacillusleichmannii	*Lactobacillus sake*	*Lactobacillus fermentatum*
Lactobacillus salivarius		*Lactobacillus sanfrancisco*
Streptococcus bovis		*Leuconostoc dextranicum*
Streptococcus thermophilus		*Leuconostoc mesenteroides*
Pediococcus acidilactici		*Leuconostoc paramesenteroides*
Pedicoccus damnosus		
Pediococcus pentocacus		