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Assignment name : isolation and

And identification of

Lactobacillus

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Haq

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Lactobacillus :

Lactobacillus is a genus of Gram-positive, facultative anaerobic or microaerophilic, rod-shaped, non-spore-forming bacteria. They are a major part of the lactic acid bacteria group. Lactobacillus is the most

common probiotic found in food such as yogurt, and it is diverse in its application to maintain human well-being, as it can help treat diarrhea, vaginal infections, and skin disorders such as eczema.

. They are normal inhabitant of healthy vagina .

. Lactobacillus are easily available in market as probiotic.

Culture media :

De Man, Rogosa and Sharpe agar, often abbreviated to MRS, is a selective culture medium designed to favour the luxuriant growth of Lactobacilli for lab study.

Developed in 1960, this medium was named for its inventors.



LMRS Agar Plate

Composition of MRS agar :

Ingredients	Gms / Litre
Proteose peptone	10.000
HM Peptone B #	10.000
Yeast extract	5.000
Dextrose (Glucose)	20.000

Tween 80 (Polysorbate 80)	1.000
Ammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate	0.100
Manganese sulphate	0.050
Dipotassium hydrogen phosphate	2.5
Agar	12.000
Final pH (at 25°C)	6.5±0.2

Directions :

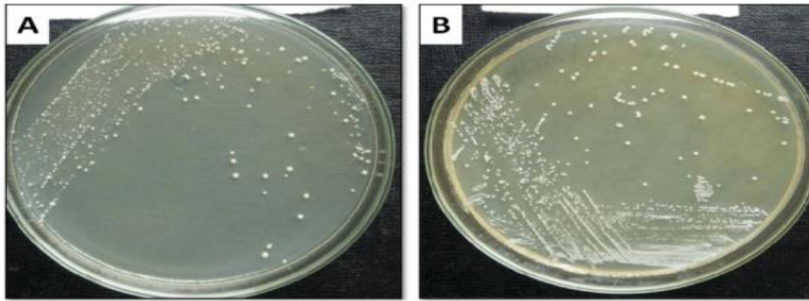
Suspend 67.15 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Procedure :

.After inoculation of lactobacillus on MRS agar Lactobacilli MRS Agar plates are incubated at 35°C. for 24-72 hours in a CO₂ incubator or under microaerophilic conditions (5% carbon dioxide, 5-10% oxygen).

The isolated colonies in the MRS agar growth medium were hemispherically

round with white or yellow color.



Typical characteristics of the Lactobacillus isolates grown on MRS agar medium. (A) Isolated Lactobacillus colonies and (B) Single screened colonies on MRS media

Identification of lactobacillus :

the single colony of Lactobacillus was isolated by observing their colony morphology and some biochemical tests such as gram staining, catalase and oxidase test. Well isolated colonies were picked up and transferred to MRS broth for enrichment of Lactobacillus at 37°C

Gram staining for identification :

.Applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture. ...

.The addition of iodide, which binds to crystal violet and traps it in the cell.

.Rapid decolorization with ethanol or acetone.

.Counterstaining with safranin.

Results :

Under microscope gram + stained lactobacillus look rod shap ,non spore

forming bacteria

