Xylose-Lysine Deoxycholate Agar (XLD)

Intended Use

XLD Agar is a selective medium recommended for the isolation, identification and enumeration of Salmonella typhi and other Salmonella species in accordance with FDA BAM 1998.

Typical Composition (g/litre)

Yeast extract 3.0; L-Lysine 5.0; Xylose 3.750; Lactose 7.50; Sucrose 7.50; Sodium deoxycholate 2.50; Ferric ammonium citrate 0.800; Sodium thiosulphate 6.800; Sodium chloride 5.0; Phenol red 0.080; Agar 15.0

Mode of Action

XLD Agar is both selective and differential medium. It utilizes sodium deoxycholate as the selective agent and therefore it is inhibitory to gram-positive microorganisms The medium contains yeast extract, which provides nitrogen and vitamins required for growth. Sodium chloride maintains the osmotic balance of the medium. Lysine is included to differentiate the Salmonella group from the non-pathogens. Subsequently lysine is decarboxylated by the enzyme lysine decarboxylase to form amines with reversion to an alkaline pH. However, to prevent this reaction by lysine-positive coliforms, lactose and sucrose are added to produce acid in excess. Degradation of xylose, lactose and sucrose to acid causes phenol red indicator to change its colour to yellow. Bacteria that decarboxylate lysine to cadaverine can be recognized by the appearance of a red colouration around the colonies due to an increase in pH. These reactions can proceed simultaneously or successively, and this may cause the pH indicator to exhibit various shades of colour or it may change its colour from yellow to red on prolonged incubation. An H2S indicator system, consisting of sodium thiosulphate and ferric ammonium citrate, is included for the visualization of hydrogen sulphide produced, resulting in the formation of colonies with black centres. The non-pathogenic H2S producers do not decarboxylate lysine therefore, the acid reaction produced by them prevents the blackening of the colonies. XLD Agar is both selective and differential medium. It utilizes sodium deoxycholate as the selective agent and therefore it is inhibitory to gram-positive microorganisms.

Preparation

Suspend 56.93 grams in 1000 ml distilled water. Heat to boiling with frequent agitation. Do not autoclave or overheat. Transfer immediately to a water bath at 50°C. After cooling, pour into sterile Petri plates. It is advisable not to prepare large volumes that will require prolonged heating, thereby producing precipitate. Note: Slight precipitation in the medium may occur, which is inherit ant property of the medium, and does not affect the performance of the medium.

Final pH (at 25°C) 7.4±0.2

Storage

Store below 30°C in a tightly closed container and use freshly prepared medium. Use before expiry date on the label.

Experimental Procedure and Evaluation

According to the BAM procedure, 25g of the food sample is pre-treated with suitable diluents such as Lactose Broth or Buffered Peptone Broth or Universal Pre-Enrichment Broth depending upon the type and nature of the sample. Typically, for specimens with low microbial load, sample to broth ratio has been recommended to be 1:9. Inoculated broth is further incubated at 35 ± 0.2°C for 24 ± 2 hrs. In case of food samples with high microbial load, 0.1 ml of sample mixture is mixed with 10 ml of Tetrathionate broth and incubated at 43 ± 0.2°C for 24 ± 2hrs. After incubation, 10 µl of the corresponding broth is inoculated on Xylose Lysine Deoxycholate Agar. After incubation period of 24 ± 2 hrs at 35°C, plates are checked for Salmonella colonies. Typical Salmonella colonies appear as pink to red colored with or without black centres. Many cultures of Salmonella may produce colonies with large, glossy black centres or may appear as almost completely black colonies. Atypically a few Salmonella cultures produce yellow colonies with or without black centres. Cultures identified using XLD agar are further confirmed through biochemical tests.

Quality Control

Organism	Inoculum (CFU)	Growth	Recovery	Color of Colony
Salmonella Typhimurium ATCC 14028	50 - 100	Luxuriant	≥ 50%	Pink-red with black centres
Salmonella Abony NCTC 6017	50 - 100	Luxuriant	≥ 50%	Pink-red with black centres
Salmonella Paratyphi A ATCC 9150	50 - 100	Good-Luxuriant	≥ 50%	Pink
Salmonella Paratyphi B ATCC 8759	50 - 100	Good-Luxuriant	≥ 50%	Pink-red with black centres
Salmonella Enteritidis ATCC 13076	50 - 100	Good-Luxuriant	≥ 50%	Red with black centres
Salmonella Typhi ATCC 6539	50 - 100	Good-Luxuriant	≥ 50%	Pink-red with black centres

Reference

1. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins.
2. Chadwick, P., Delisle, G. H.. and Byer, M. 1974. Can. J. Microbiol., 20: 1653-1664
3. Wehr, H.M. and Frank, J.H. 2004. Standard Methods for the Examination of Dairy Products. 17 ed.
4. FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
5. Taylor, W. L. and Schelhart, B. 1969. Appl. Microbiol., 18: 393-395