



BAT Agar (Alicyclobacillus Agar)

M1650

Intended use

Recommended for isolation of *Alicyclobacillus* species in fruit juice.

Composition**

Ingredients	Gms / Litre
Yeast extract	2.000
Dextrose (Glucose)	5.000
Calcium chloride	0.25066
Magnesium sulphate	0.500
Ammonium sulphate	0.200
Potassium dihydrogen phosphate	3.000
Zinc sulphate	0.00018
Copper sulphate	0.00016
Manganese sulphate	0.00015
Cobalt chloride	0.00018
Boric acid	0.00010
Sodium molybdate	0.00030
Agar	18.000
Final pH (at 25°C)	4.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 28.95 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.

Note: Adjust the pH of the medium to 4.0 ± 0.2 (after sterilization) using 1N H₂SO₄ or 1N NaOH

Principle And Interpretation

Alicyclobacillus species are gram positive aerobic thermophilic, and spore forming acidophilic bacteria. *Alicyclobacillus* are sometimes called Acidophilic Thermophilic Bacteria (ATB). These spore forming organisms are able to survive the relatively mild pasteurization temperatures used for fruit juices and drinks and some are able to grow out and cause spoilage of the beverage. Even very low numbers of *Alicyclobacillus* are able to cause spoilage and produce objectionable flavours and odours specially affecting the quality of fruit juice (2,3) and in the beverages, damaging the brand. These bacteria are able to grow at pH values as low as 2.5 and also at elevated temperatures as high as 60°C. BAT (*Bacillus AcidoTerrestris*) Agar has a pH of 4.0 ± 0.2 which supports growth of *Alicyclobacillus* species and inhibits most of the microbial flora (1). Rest of the microbial flora is inhibited at 60°C, which is the optimum growth temperature for *Alicyclobacillus* species.

Type of specimen

Beverage samples- Fruit juices

Specimen Collection and Handling

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further recovery from this enriched broth onto selective media is required.
2. Biochemical characterization is carried out from pure isolates for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.8% Agar gel

Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 2.89% w/v aqueous solution at 25°C. pH : 4.0±0.2

pH

3.80-4.20

Cultural Response

Cultural characteristics observed after an incubation at 60°C for 48-72 hours.

Organism	Growth
<i>Alicyclobacillus acidoterrestris</i> ATCC 49025	good to luxuriant
<i>Alicyclobacillus acidocaldarius</i> ATCC 27009	good to luxuriant
<i>Escherichia coli</i> ATCC 25922 (00013*)	inhibited
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	inhibited
<i>Candida albicans</i> ATCC 10231 (00054*)	inhibited
<i>Saccharomyces cerevisiae</i> ATCC 19615	inhibited

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. BAUMGART, J. (2003) Media for detection and enumeration of *Alicyclobacillus acidoterrestris* and *Alicyclobacillus acidocaldarius* in foods. In handbook of culture Media for Food Microbiology, J.E.L. Corry et al, (Eds.) Elsevier Sci B.V. Amsterdam.
2. Baumgart and Merve S. The Impact of *Alicyclobacillus acidoterrestris* on the quality of Juices and Soft Drinks Fruit processing 7 : 251-254 (2000)
3. Ceny G., W. Hennlich and K Rocallia-Furchtsaftwerb durch Bacillen. Isobioerung and Charakterisierung des Verderberregens-Z hebers Utres Forsch 179: 224-227, 1984
4. Isenberg, (Ed.), 1992, Clinical Microbiology Procedures Handbook, Vol. I, American Society for Microbiology, Washington, D.C.

5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W.(2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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