



Alicyclobacillus Medium, Modified (BAT Medium, Modified) M1561A

Intended Use:

Recommended for enrichment of *Alicyclobacillus* species in fruit juice. The composition and performance of this medium is as per the specifications laid down in IFU Method No.12

Composition**

IFU Method No.12 specifications

Ingredients	g / L
Yeast extract	2.000
Dextrose (Glucose)	5.000
Calcium chloride, dihydrate	0.25066
Magnesium sulphate, heptahydrate	0.500
Ammonium sulphate	0.200
Potassium dihydrogen phosphate	3.000
Zinc sulphate, monohydrate	0.00018
Copper sulphate, pentahydrate	0.00016
Manganese sulphate, hydrate	0.00015
Sodium molybdate, dihydrate	0.0003
Final pH (at 25°C)	4.0±0.2

BAT Medium (Alicyclobacillus Medium)

Ingredients	g / L
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Dextrose (Glucose)	5.000
Calcium chloride, dihydrate	0.25066
Magnesium sulphate, heptahydrate	0.500
Ammonium sulphate	0.200
Potassium dihydrogen phosphate	3.000
Zinc sulphate, monohydrate	0.00018
Copper sulphate, pentahydrate	0.00016
Manganese sulphate, hydrate	0.00015
Sodium molybdate, dihydrate	0.0003
Final pH (at 25°C)	4.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 10.63 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Note: Adjust the pH of the medium to 4.0 ± 0.2 (after sterilization) using 1N H_2SO_4 or 1N NaOH.

Principle And Interpretation

Alicyclobacillus species are gram positive, aerobic thermophilic, acidophilic bacteria. 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 in the beverages, damaging the brand (1,2). These bacteria are able to grow at pH values as low as 2.5 and also at elevated temperatures as high as 45°C. BAT (Bacillus AcidoTerrestris) medium has a pH of 4.0 ± 0.2 which supports growth of *Alicyclobacillus* and inhibits most of the microbial flora (3). Rest of the microbial flora is inhibited at 45°C, which is the optimum growth temperature for *Alicyclobacillus* species.

Yeast extract provides vitamins especially group b vitamins. Dextrose (Glucose) serves as energy source. Trace elements in the medium provide other growth requirements.

Type of specimen

Beverage samples- Fruit juices

Specimen Collection and Handling:

For sample processing, please refer IFU Method 12 (4).

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

Colour and Clarity of prepared medium

Light amber coloured clear solution in tubes

Reaction

Reaction of 1.1% 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 45± 1°C for upto 5 days.

Productivity

Organism	Inoculum	Growth
<i>Alicyclobacillus acidoterrestris</i> ATCC 49025	50-100	good-luxuriant
<i>Alicyclobacillus acidocaldarius</i> DSM 4006	50-100	good-luxuriant
<i>Alicyclobacillus cycloheptanicus</i>	50-100	good-luxuriant
<i>Alicyclobacillus hesperidium</i>	50-100	good-luxuriant

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°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 (5,6).

Reference

- Ceny G., W. Hennlich and K Rocallia-Furchtsaftwerb durch Baciilen. Isobioerung and Charakteriseeuing des Verdebserregens-Z hebers Utres Forsch 179: 224-227, 1984.
- 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.
- Baumgart and Merve S. The Impact of *Alicyclobacillus acidoterstris* on the quality of Juices and Soft Drinks Fruit processing 7 : 251-254 (2000).
- IFU International Fruit and Veetable Juice Association. Method on the detection and enumeration of spore forming thermo-acidophilic spoilage bacteria (*Alicyclobacillus* spp.). Method of Analysis No.12:2019.
- Isenberg, (Ed.), 1992, Clinical Microbiology Procedures Handbook, Vol. I, American Society for Microbiology, Washington, D.C.
- 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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