

Also known as BAT Medium

Specification

Liquid medium used for the enrichment of *Alicyclobacillus*, from fruit juices and other acidic foods, according to IFU Standard Method No. 12.

Formula * in g/L

Yeast extract	2,00000
Dextrose	5,00000
Potassium hydrogen	
phosphate	3,00000
Calcium chloride	0,25000
Magnesium sulfate	0,50000
Ammonium sulfate	0,20000

Zinc sulfate	0,00018
Copper sulfate	0,00016
Manganese sulfate	0,00015
Sodium molybdate	0,00030

Final pH 4,0 ±0,2 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 10.95 g in 1 L of distilled water and distribute in suitable containers. Sterilize in the autoclave at 121°C for 15 minutes. Cool to 45-50°C and adjust the pH to 4,0 \pm 0,2 by adding 1N H2SO4 , or 1M HCl. Mix well to homogenize and aseptically distribute into sterile tubes. Avoid overheating the medium after the pH adjustment.

Description

Since the early 1980s, when spoilage of fruit juices by acid dependent thermotolerant spore-forming bacteria was recognized Cerny *et al.*, 1984) members of the genus *Alicyclobacillus* have been identified as food spoilage organisms of major significance to the fruit juice industry (Baumgart & Menje, 2000). Spoilage is generally manifested as the formation of off flavours and odours from compounds such as guaiacol and the halogenated phenols. The economic impact of such incidents can be very high, nevertheless, to date, no human risk is known to be associated with the consumption of juices and other food products containing *Alicyclobacillus* bacteria.

An acidic environment is required to grow alicyclobacilli and BAT (*Bacillus AcidoTerrestris*) media supports the growth of all current known species of *Alicyclobacillus* (*A. acidocaldarius*, *A. acidoterrestris*, *A. cycloheptanicus* and *A. hesperidium*). These media comply with the Standard IFU Method for the detection of organisms that taint fruit juices.

The low pH-value of the media, in combination with the high incubation temperature inhibits the growth of contaminating microbiota.

K Agar (Art. No. 01-674) when incubated at 45°C supports the growth of predominantly *A. acidoterrestris* and the limited growth of other species of the genus. Therefore, K Agar (Art. No. 01-674) can be used to detect predominantly *A. acidoterrestris* strains.

Technique

The IFU Standard describes three methods of detection depending on the sample composition and the time elapsed since processing:

1. Raw materials (including processed water): A heat shock treatment is prescribed followed by direct plating (optional), filtration or enrichment in liquid medium of the heated material.

2. final products: sampled directly after (heat) processing where an additional heat shock is unnecessary: Pre-incubation of the sample in liquid medium is required.

3. final products taken from the market: Pre-incubation of the sample, and heat shock treatment are optional. However if spoilage is suspected and no alicyclobacilli detected after direct plating, a heath shock and enrichment is recommended.

In all the methodologies incubation for 2-4 days à 45 \pm 1°C is recommended for the enrichment Alicyclobacillus spp. except for A. acidocaldarius That require incubations above 50 ° C.

Quality control

Incubation temperature:	45°C ±1,0	Incubation time: 3 - 5 days	
Inoculum: ≤100 CFU. Min. 50 CFU (Productivity) 10 ⁴ -10 ⁶ CFU (selectivity) according to ISO 11133:2014/Amd 1:2018.			
Microorganism	Growth	Remarks	
Escherichia coli ATCC [®] 25922	Inhibited	-	
Bacillus cereus ATCC [®] 11778	Inhibited	-	
Alicyclobacillus acidoterrestris ATCC	[®] 49025 Good	-	

References

- 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, J. & S. MENJE (2000) The impact of Alicyclobacillus acidoterrestris on the quality of juices and soft drinks. Fruit Processing 7:251-254.
- CERNY, G., W. HENNLICH & K. PORALLA (1984) Fruchsaftverdeb durch Bazillen: Isolierung und Charakterisierung des Verdebserregers. Z. Lebens. Unter Forsch. 179:224-227.
- · IFU STANDARDS (2004) Method No. 12 on the detection of taint producing Alicyclobacillus in fruit juices. Revision march 2007.
- . IFU STANDARDS (2019) Method No. 12 on the detection and enumeration of spore-forming thermo-acidophilic spoilage bacteria (*Alicyclobacillus* spp.).
- . SMITH, Y., M. CAMERON, P. VENTER & R.C. WITTHUHN (2011) *Alicyclobacillus* spoilage and isolation A review. Food Microbiol. 28(3):331-349.
- . ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).