

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	Zinc sulfate.....	0,00018
Dextrose.....	5,00000	Copper sulfate	0,00016
Potassium hydrogen phosphate.....	3,00000	Manganese sulfate.....	0,00015
Calcium chloride	0,25000	Sodium molybdate.....	0,00030
Magnesium sulfate	0,50000		
Ammonium sulfate.....	0,20000	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 ± 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 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 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 heat shock and enrichment is recommended.

 In all the methodologies incubation for 2-4 days à 45 ± 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) 10⁴-10⁶ CFU (selectivity) according to ISO 11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
<i>Escherichia coli</i> ATCC® 25922	Inhibited	-
<i>Bacillus cereus</i> ATCC® 11778	Inhibited	-
<i>Alicyclobacillus acidoterrestris</i> 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) Fruchtsaftverdeb 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).