

Also known as

BAT Medium

Specification

Solid medium for the detection and isolation of *Alicyclobacillus*, in fruit juices and other acidic food, according to IFU standard Method No. 12.

Formula * in g/L

Yeast extract	2,00000	
Dextrose.....	5,00000	Copper sulfate.....
Potassium phosphate.....	3,00000	Manganese sulfate.....
Calcium chloride.....	0,25000	Sodium molybdate.....
Magnesium sulfate	0,50000	Agar.....
Ammonium sulfate.....	0,20000	
Zinc sulfate.....	0,00018	Final pH 4,0 ±0,2 at 25 °C

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

Directions

Suspend 30.95 g of BAT Agar in 1 L of distilled water and bring to the boil to dissolve. Distribute in suitable containers and sterilise 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 homogenise and pour into sterile Petri dishes. Avoid heating or remelting the medium after the pH adjustment.

Description

Since the early 1980's, 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 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 currently 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 (No. 12).

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 limited growth of other species of the genus. Therefore, K Agar can be used to detect predominantly *A. acidoterrestris* strains.

In the latest revision of the IFU standard n. 12: 2019, the components cobalt chloride and boric acid have been removed from the formulation due to toxicity.

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 required 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 methodology incubation for 3-5 days at 45 ± 1°C is recommended. Count all colonies growing on the BAT Agar as presumptive alicyclobacilli. Confirm these colonies by further testing.

Note: *A. acidocaldarius* require incubations above 50 ° C.

Quality control

Incubation temperature: 45°C ±1,0

Incubation time: 3 - 5 days

Inoculum: Practical range 100 ± 20 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	Productivity > 0.70	-

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).