K Agar

M1752

K Agar is recommended for isolation and cultivation of *Alicyclobacillus* in fruit juices in accordance Official Method of IFU.

Composition**

Ingredients	Gms / Litre
Yeast extract	2.500
Peptone	5.000
Glucose	1.000
Tween	1.000
Agar	15.000
Final pH (at 25°C)	3.7±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to about 50°C and adjust pH 3.7 ± 0.1 with 25% L-malic acid.

Principle And Interpretation

Alicyclobacillus are aerobic thermophilic non-pathogenic, spore forming bacteria that can survive the relatively mild pasteurization temperatures used for fruit juices and concentrates. Even very low numbers of *Alicyclobacillus* cause spoilage and off flavors in the beverages, damaging the brand (3). These bacteria are able to grow at pH values as low as 2.5 and also at elevated temperatures above 200C.Their spores survive for long period in fruit concentrates and similar environments.

A. acidoterrestris is the most commonly occurring species that produce taints in juice and similar products, however other species may also produce taints.

Acidified environment are required to detect and isolate *A. acidoterrestris* (1, 2), therefore, K-Agar is recommended for detection of taint producing *Alicyclobacillus acidoterrestris* as per IFU (1) (standard IFU method No.12).

Peptone and yeast extract serve as a source of nitrogen, amino acids, vitamins, and other essential growth requirements. Glucose serves as a corbon source. Polysorbate 80 serves as an additional source of growth factor and fatty acid. The low acidic pH (3.7) of medium aobtained by addition of L-malic acid is inhibitory to several bacterial species.

K-Agar (when incubated at 45°C) supports the growth of predominant *A. acidoterrestris* and limited growth of other species. If the sample is filterable, filter it through 0.45µm membrane after pretreatment. Filter aseptically and transfer one membrane on to K- Agar and other membrane on YSG Agar (M1753). Simultaneously, streak standard cultures of *Alicyclobacillus acidoterrestris* and *Alicyclobacillus acidocaldarius* for comparison. Incubate at 45±1°C for 2-5 days. Examine daily, for detailed procedure refer standard IFU method N0.12 (1)

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Pale yellow coloured Clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 2.45% w/v aqueous solution at 25°C. pH : 3.7±0.1

pH
3.60-3.80

Cultural Response

M1752: Cultural characteristics observed after an incubation at 45- 46°C for 2 -5 days.

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
Alicyclobacillus acidocaldarius ATCC 27009	50-100	Poor-good	30-40%
Alicyclobacillus acidoterrestris ATCC 49028	50-100	luxuriant	>=50%
Alicyclobacillus acidocaldarius ATCC 43030	50-100	Poor-good	30-40%
Escherichia coli ATCC 25922	>=10^3	inhibited	0%
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. IFU (2004). Standard IFU method No.12- Microbiological detection of Alicyclobacillus in fruit juices. International Federation of fruit juice producer, Paris

2 Matsubara et al. (2002). Alicyclobacillus acidiphilus sp. nov., a novel thermo-acidophilic–alicyclic fatty acid-containing bacterium isolated from acidic beverages. Int. J. Syst. Environment. Microbiol. 52, 1681-1685.

3. Baungart and Merve S. The Impact of Alicyclobacillus acidoterstris on the quality of Juices and Soft Drinks Fruit processing 7 : 251-254 (2000)

Revision : 2 / 2015