

Guaiacol Detection Kit and Va-YSG Medium

Cat. No. KYO-08921 / KYO-08901

Last Updated: July 8th, 2016

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【I】 Background

Alicyclobacillus acidoterrestris is a gram-positive, spore-forming bacillus that occurs widely in nature. This specie has been studied substantially after the incident of turbidity of transparent apple juice in 1984. The *Alicyclobacillus* genus bacteria, which peculiarly grows under relatively high temperatures and acid conditions, is called Thermo-acidophilic Bacilli (TAB) [1, 2, 3].

TAB are not destroyed by pasteurization and may remain in final products of fabricated food and beverages. TAB are not known to be harmful to health but are known to degrade the quality of juices and other products by producing guaiacol, causing off-flavor. Even without the off-flavor problem, product values may be degraded by slight deterioration during distribution.

It is very difficult to avoid contamination of TAB, which are common in soil. Fortunately, *A. acidocaldarius* and other main species that cause contamination are known to be harmless, and damage caused by TAB can be minimized by monitoring the contamination of *A. acidoterrestris*, which is known to be harmful. These species, however, cannot be detected by usual isolation culture methods as they grow only under acidic and relatively high temperature conditions.

Because of frequent occurrence of fruit juice deterioration incidents in Europe and other regions, Japan Fruit Juice Association developed and publicized a unified test method for TAB in March 2003 [4]. To allow easy performance of this test, we developed a kit that differentiates *A. acidoterrestris* in terms of productivity of guaiacol, which causes off-flavor.

【II】 Kit Components

Va-YSG Medium (2mL) 100 tubes (Cat. No. KYO-08901)

Vanillic acid added YSG medium for guaiacol formation	2 mL x 100 tubes
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Guaiacol Detection Kit 100 tests (Cat. No. KYO-08921)

Reagent 1: 50mM Potassium Hydrogen Phthalate Buffer	60 mL x 2 bottles
Reagent 2: 1.3 % Hydrogen Peroxide Solution	2.5 mL x 1 tube
Reagent 3: Peroxidase-Phosphoric Acid Buffer	2.5 mL x 1 tube
Positive Control: Guaiacol (1050 ppm)	2.5 mL x 1 tube

【 III 】 Procedures

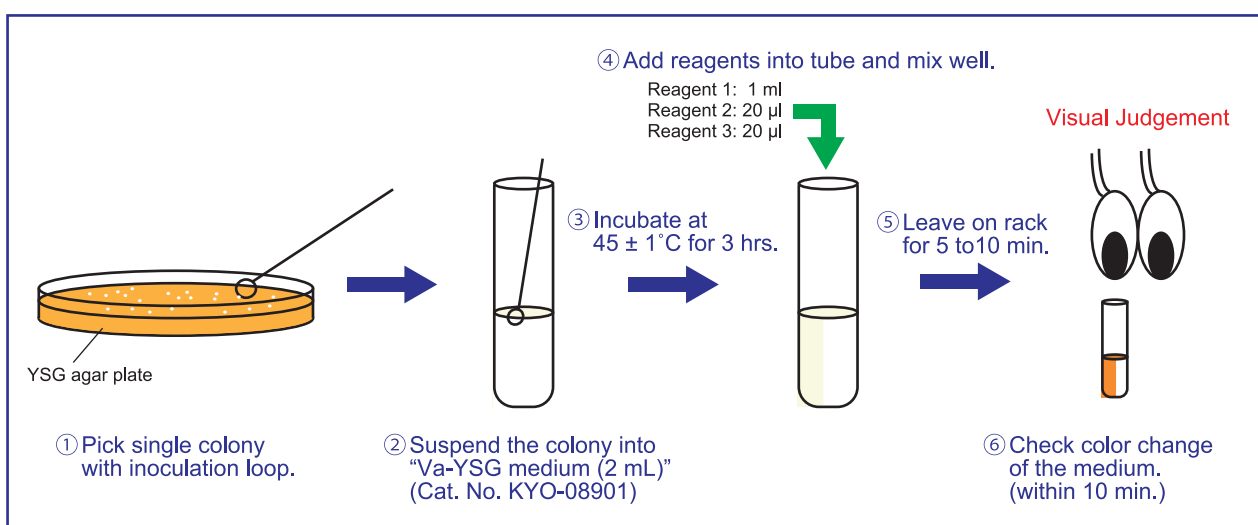
- 1 Take with single inoculation loop, 10 μ L, of young colony that has been isolated on YSG agar plate and that has not formed spores. Suspend the colony in a tube of "Va-YSG medium (2 mL)," ($\geq 1 \times 10^8$ cfu/mL).
- 2 Incubate at $45 \pm 1^\circ\text{C}$ for 3 hours.
- 3 Add 1 mL of Reagent 1 of "Guaiacol Detection Kit".
- 4 Add 20 μ L of Reagent 2 of "Guaiacol Detection Kit".
- 5 Add 20 μ L of Reagent 3 of "Guaiacol Detection Kit".
- 6 Mix well.
- 7 Leave on rack for 5-10 minutes.
- 8 Check the color change of the medium. Read the result within 10 minutes.

*Blank (Negative Control)

Use "Va-YSG medium (2 mL)" that has not been inoculated and follow steps (2)-(8).

*Positive Control

Mix well 2 mL of purified water and 100 μ L of Positive Control. Follow steps (3)-(8).



【 IV 】 Interpretation of Results

Darker brown than blank = Positive

*If the color change is not significant and difficult to determine, retest with 24 hours incubation instead of 3 hours, step(2)

Same color as blank = Negative

Verify the test results by checking the reading of positive control; positive control result should have significantly darker brown than blank.



- ① Negative Control
- ② *A. acidoterrestris* B2067
- ③ *A. acidoterrestris* ATCC49025T
- ④ *A. acidocaldarius* ATCC27009T
- ⑤ *A. acidocaldarius* HP2
- ⑥ *A. genomic* sp. P2
- ⑦ *A. genomic* sp. DSM11983

【 V 】 Storage

Store in dark and refrigerated (2-10 ° C)

【 VI 】 References

- 1) Niwa,M., A.Kawamoto, Development of a rapid detection method of *A. acidoterrestris*, hazardous bacteria to acidic beverage, Fruit Processing, 13,102-107(2003)
- 2) Niwa,M.(A course for “ Taxonomy and identification of microorganisms “ 1, Taxonomy and quality of new thermostable acidophile bacteria.) Technical data of soft drinks 2001,(1) 9-26(2001)
- 3) Niwa,M. et.al. (Development of a rapid detection of *A. acidoterrestris*, hazardous bacteria to acidic beverage) Report of Association for Fruit Juice (531) 23-30
- 4) Niwa, M. A. Kuriyama, *A. acidoterrestris* RAPID DETECTION KIT, Fruit Processing, 13,328-331(2003)
- 5) Niwa,M. et.al., Report of Association for Fruit Juice (541)(Sep. 2003), (Proposal for direct detection of *A. acidoterrestris* using peroxidase method)