

Screening of Thermotolerant Acidophilic Bacilli by PCR-ELISA Method

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Summary

Alicyclobacillus acidoterrestris is found in soil and on fruit, and as its spores are heat-resistant it can be detected at various stage of food manufacturing. It is known that the spore forming and acidothermophilic bacteria, *A. acidoterrestris* is able to spoil fruit juice by producing the malodorous substance guaiacol. It has been reported that this bacteria has been the cause of spoilage in fruit juice in Japan. To develop countermeasure against such food spoilage; an easy and speedy detection method for *A. acidoterrestris* at various stages of the manufacturing process is needed.

We have developed a new detection method, based on PCR-ELISA (*Roche Molecular Biochemicals*) for *A. acidoterrestris*. We designed PCR primers to target a bacterial 16S rRNA and a biotin-labeled capture probe that specifically recognizes an internal sequence in the PCR amplified target DNA. In the first step, the *Taq* DNA polymerase incorporates digoxigenin-11-dUTP (DIG) into the target DNA. In the second step, the biotin-labeled capture probe is hybridized to the DIG labeled PCR product. The third step involves detection of hybrid, DIG-labeled PCR product and biotin-labeled capture probe on a streptavidin-coated microtiter plate.

As a result of testing this method on a number of bacterial species, we were able to detect *A. acidoterrestris* specifically. These result shows that this method has the potential to permit the specific detection of *A. acidoterrestris* from a wide range of bacteria, and can be used at various stages in the manufacturing process.