MMM Beverage Partner Kft.

Identification of *Alicyclobacillus* bacteria using MMM Beverage Partner's ACB LC RT-PCR Workflow System

Summary

MMM Beverage Partner's ACB LC RT-PCR Workflow System detects viable thermo-, acidophilic bacteria in beverage samples belonging to the genus of *Alicyclobacillus*.

Detection of *Alicyclobacillus* bacteria is very important because some of these thermo-acidophilic, spore-forming bacteria can produce guaiacol which causes off-flavour, spoilage of the acidic beverage and food.

Alicyclobacillus acidoterrestris was reported by Yamazaki et al. (1) as guaiacol caused off-flavour producer of fruit juices. Other *Alicyclobacilli* had been not reported to be able to produce guaiacol for years. In 2002 Matsubara et al. proved that *A. acidiphilus* has the same character (2).

MMM Beverage Partner's ACB LC RT-PCR Workflow System detects and at identifies the *Alicyclobacillus* bacteria in one step. Identification is carried out by melting peak analysis. The melting peak analysis discovers the differences of the target nucleotide sequences of the different *Alicyclobacillus* bacteria. The different target nucleotide sequences result in different melting temperatures while the identical target nucleotide sequences result in the same melting peak.

MMM Beverage Partner's ACB LC RT-PCR Workflow System detects all bacteria belonging to *Alicyclobacillus* genus and identifies automatically the following *Alicyclobacillus* bacteria at species level: *A. acidiphilus, A. acidocaldarius, A. acidoterrestris, A. cycloheptanicus, A. herbarius, A. hesperidum, A. pomorum.*

Background of the melting temperature analysis

The temperature at which a DNA strand separates or melts when heated can vary over a wide range, depending on the sequence, the length of the strand, and the GC content of the strand. Based on these, melting temperature profiles can be used to identify and genotype DNA products.

To analyze sample melting temperature profiles, the fluorescence of the samples must be monitored while the temperature is steadily increased. As the temperature increases, sample fluorescence decreases. For HybProbe probes, this is due to the separation of target-probe hybrids resulting in the spatial separation of the dye molecules and a consequent drop in fluorescence. The presence of a mismatch in the sequence of the probe binding site lowers the temperature at which the probe melts off the sequence. The difference in melting temperature depends on the type of mismatch, the mismatch position within the probe sequence, and the base pairs immediately adjacent to the mismatch.

The same melting temperature achieved from different samples indicates the same *Alicyclobacillus* species and if the melting peaks are different the presence of different *Alicyclobacillus* species are confirmed. The *Alicyclobacillus* species names are defined automatically by the specific kit macro.

Rapid Detection and Identification of Viable Spoilage Microorganisms

Type strains used

A. acidiphilus DSM 14558,
A. acidocaldarius DSM 446,
A. acidoterrestris DSM 3922,
A. cycloheptanicus DSM 4006,
A. herbarius DSM 13609,
A. hesperidum DSM 12489,
A. pomorum DSM 14955

Materials and methods

MMM Beverage Partner's ACB LC RT-PCR Workflow System kit was used according to the kit instruction manual.

Results

The melting peaks of the different *Alicyclobacillus* bacteria were achieved from the melting peak analysis. The melting peak data are demonstrated in Table 1.

Table 1. Melting temperature data of different *Alicyclobacillus* bacteria

Name of micro-organisms	Melting Temperature data, °C
Alicyclobacillus acidiphilus	61 + 71,1
Alicyclobacillus acidocaldarius	63 + 71,7
Alicyclobacillus acidoterrestris	73<
Alicyclobacillus cycloheptanicus	60
Alicyclobacillus herbarius	48,9
Alicyclobacillus hesperidum	69,9
Alicyclobacillus pomorum	70,4

The melting peaks of the *Alicyclobacillus* bacteria are shown in Fig. 1.

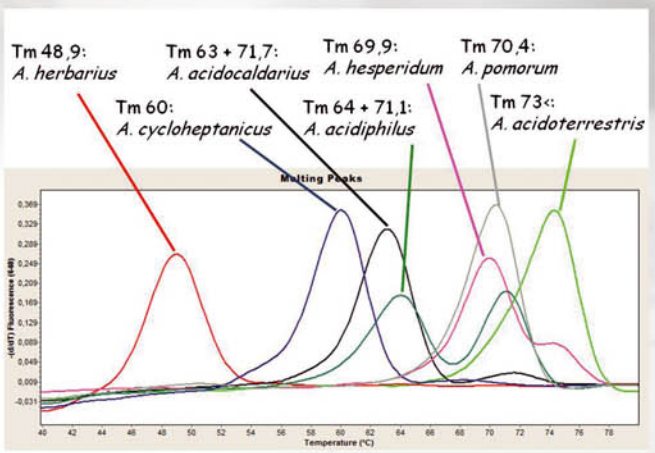

Fig. 1. Melting peaks of different *Alicyclobacillus* bacteria

Reference

1. Yamazaki, K., Teduka, H. & Shinano, H. (1996). Isolation and identification of *Alicyclobacillus acidoterrestris* from acidic beverages; Biosci Biotechnol Biochem 60, 543-545.

2. Hiroshige Matsubara, Keiichi Goto, Terumi Matsumura,Kaoru Mochida, Masaharu Iwaki, Motohiro Niwa, and Kazuhide Yamasato (2002) *Alicyclobacillus acidiphilus* sp. nov., a novel thermo-acidophilic, ω-alicyclic fatty acid containing bacterium isolated from acidic beverages; International Journal of Systematic and Evolutionary Microbiology, 52, 1681–1685

Contact information...

Mailing address
MMM Beverage Partner Ltd.
Otthon u. 28/b.
1118 Budapest
Hungary

Laboratory
MMM Beverage Partner Ltd.
Építők útja 2-4.
2040 Budaörs
Hungary
Phone: +36 23 428896
E-mail: info@mmmbeverage.hu
web: www.mmmbeverage.hu