



Fruit juice analysis

Alicyclobacillus spp. detection with real-time PCR

- *Alicyclobacillus* spp.
- Multiplex for *Alicyclobacillus* spp./
A. acidoterrestris/*A. acidocaldarius*
- Optimized DNA preparation
for concentrates



Alicyclobacillus spp. – a fruit juice spoiler group

The problem for the fruit juice and beverage industry: the typical taste and flavor is changed adversely. Thus, an early identification of *Alicyclobacillus* is therefore of great importance, as spores of that bacteria cannot be deactivated by a usual pasteurization process. Consequently, a contamination with *Alicyclobacillus* can cause a significant reduction in quality and economic losses.

Background information

Fruit juices are usually concentrated, the pulp pasteurized and afterwards the concentrate is sold as a semi-finished product. The pasteurization avoids a microbiological contamination or growth. Since 1982, however, the soil bacterium genogroup *Alicyclobacillus* spp. has been of growing interest. This bacterium is aerobic and acidophilic and grows at pH 2 - 6 and a temperature between about 40 - 60 °C. This rising spore-forming bacterium finds ideal conditions in fruit juice concentrates and fruit juices (orange, apple, tomatoes and other fruits). Spores can be activated by short heating process as by pasteurization processes.

This bacterium is not pathogenic, some of the approximately 20 species, in particular *A. acidoterristris* and *A. acidocaldarius* form the substance guaiacol.

Application

Juices or juice concentrates will be preincubated 1:10 in BAT medium for two to three days. In case of nega-

thereby it affects the smell and taste of the juice or product. Classical analysis is based on the culture in the medium BAT or other media with different temperature and takes long time.

The classical microbiologic analysis is described in the IFU 12 method and consists of cultivation in BAT medium and other media. The procedure takes around 2 weeks. Especially for export/import of fruit concentrates the time to result and therefore the quarantine time is quite long.

The real-time PCR enables fast and highly specific results after three days pre-enrichment. The DNA preparation has been optimized for detection of bacteria and spores out of difficult matrices, as pulp being rich of fibers.

tive PCR results a prolongation for up to five days is recommended to detect damaged spores or bacteria.

GEN-IAL® *Alicyclobacillus* multiplex, Art. No. Q721 - Q724



Sampling

- 1 mL of representative sample
- Centrifugation and removal of fibers and debris
- Thermal and lysozyme mediated lysis
- Binding of DNA to spin filter, twofold purification

The DNA preparation kit **Q701** enables the use of difficult matrices as pulp and fruit juice concentrates.

Analytic procedure

A simple screening of *Alicyclobacillus* spp. may be performed on all commercial qPCR thermocycler devices using the kit **Q721 - Q724**.

While *Alicyclobacillus* spp. is detected in the FAM

channel, the Internal Amplification Control (IAC) in the ROX or HEX channel excludes wrong negative results.

Multiplex screening and differentiation

Using the kit **Q721 - Q724** the parameters

- *Alicyclobacillus* spp.
- *A. acidoterrestris*
- *A. acidocaldarius*
- Internal Amplification Control

can be tested in one sample. Beside the genogroup of *Alicyclobacillus*, the most important Gujacol forming species will be identified.

For this, a qPCR cycler with the detection channels

for FAM, ROX, Cy5, and HEX is required.

The time for analysis will be around 2 - 3 hours post pre-enrichment.

Real-time PCR

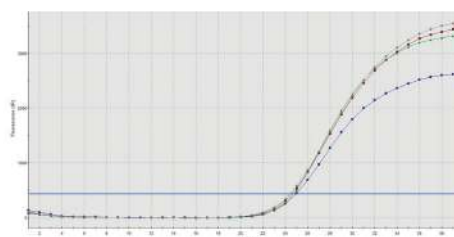
- Fast results
- Highly specific
- Easy to use
- Reduced time to result

GEN-IAL® First *Alicyclobacillus* multiplex run



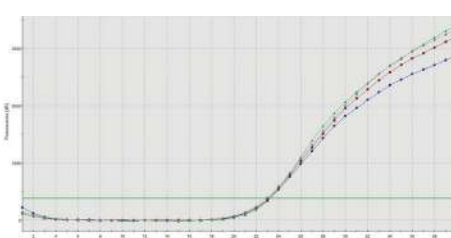
Alicyclobacillus acidocaldarius

CY5



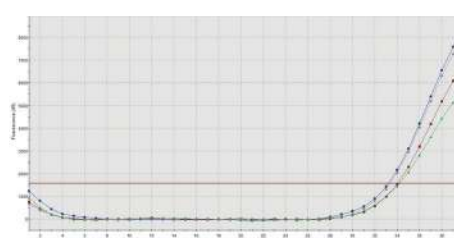
Alicyclobacillus acidoterrestris

FAM



Alicyclobacillus spp.

HEX



Internal Control (IC)

ROX

Sensitivity/specificity

Inclusivity – 17 *Alicyclobacillus* species: 100 % positive

Exclusivity – 50 non *Alicyclobacillus* species: 100 % negative

Examples of testes bacteria species	
Species	<i>Alicyclobacillus</i>
<i>Alicyclobacillus acidocaldarius</i> subsp. <i>acidocaldarius</i>	positive
<i>Alicyclobacillus acidocaldarius</i> subsp. <i>rittmannii</i>	positive
<i>Alicyclobacillus acidoterrestris</i>	positive
<i>Alicyclobacillus acidiphilus</i>	positive
<i>Alicyclobacillus contaminans</i>	positive
<i>Alicyclobacillus cycloheptanicus</i>	positive
<i>Alicyclobacillus disulfidooxidans</i>	positive
<i>Alicyclobacillus fastidiosus</i>	positive
<i>Alicyclobacillus ferrooxydans</i>	positive
<i>Alicyclobacillus herbarius</i>	positive
<i>Alicyclobacillus hesperidum</i>	positive
<i>Alicyclobacillus macrosporangioides</i>	positive
<i>Alicyclobacillus pohliae</i>	positive
<i>Alicyclobacillus pomorum</i>	positive
<i>Alicyclobacillus sacchari</i>	positive
<i>Alicyclobacillus sendaiensis</i>	positive
<i>Alicyclobacillus vulcanalis</i>	positive
<i>Lactobacillus</i> spp.	negative
Acetic acid bacteria	negative
<i>Pediococcus</i> spp.	negative
<i>Oenococcus oeni</i>	negative
Enterobacteriaceae	negative
<i>Leuconostoc</i> spp.	negative
<i>Schizosaccharomyces pombe</i>	negative

Examples of tested yeasts	
Species	<i>Alicyclobacillus</i>
<i>Saccharomyces</i> spp.	negative
<i>Zygosaccharomyces</i> spp.	negative
<i>Dekkera</i> spp.	negative
<i>Candida</i> spp.	negative
<i>Debaromyces hansenii</i>	negative
<i>Kluyveromyces marxianus</i>	negative
<i>Pichia anomala</i>	negative
<i>Pichia membranaefaciens</i>	negative

GEN-IAL® products for fruit juice analysis

Product	Description	Tests	Art. No.
GEN-IAL® DNA preparation			
Simplex® Easy DNA Kit	DNA preparation of beverages	100 preparations	Q001
Simplex® Easy Spin DNA Kit	<i>Alicyclobacillus</i> DNA preparation e.g. from fruit or vegetable juices or concentrates	50 preparations	Q701
GEN-IAL® Qualitative real-time PCR			
First <i>Alicyclobacillus</i> multiplex PCR Kit	Qualitative identification of <i>Alicyclobacillus</i> spp., <i>Alicyclobacillus acidocaldarius</i> and <i>Alicyclobacillus acidoterrestris</i>	48 reactions	Q721 – high profile* Q722 – low profile*** Q723 – white strips** Q724 – low profile*** MyGo Pro



High*

- Agilent MX3005P
- Applied Bioscience ABI 7500 or higher
- ThermoFisher QuantStudio®5 or higher



White**

- Roche Lightcycler® 480 II and LC96
- BioRad CFX96™
- Analytik Jena qTOWER³
- ThermoFischer PikoReal 24



Low***

- IT-IS MyGo Pro
- ABI 7500 FAST or higher
- BioRad CFX96™