Monitoring the Microbial Population Dynamics during Thick Juice Storage in the Sugar Industry

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Despite use of generally accepted good storage practices, thick juice degradation caused by microbiological contamination occasionally occurs, causing considerable financial loss. For this reason, thick juice samples from a pilot scale reactor were analyzed using conventional culturing techniques as well as culture-independent approaches to identify the present taxa. A DNA macroarray was developed, confirming the predominance of the halophilic bacterium *Tetragenococcus halophilus*. Based on its abundance (10^6-10^7 cfu/ml) and persistence, *T. halophilus* is believed to play an important role in thick juice degradation. Additionally, the species *Bacillus* spp. and *Staphylococcus* spp. are present in smaller concentrations varying from 10^3-10^4 cfu/ml, but are also capable of fermenting sucrose. The developed macroarray was considered reliable and has potential for monitoring the bacterial thick juice population during industrial thick juice storage in a single assay.

Introduction

Storing sugar extracts as thick juice, a concentrated sucrose syrup, is commonly practiced by the sugar refining industry. Even when good storage practices are followed, microbiologically induced thick juice degradation occasionally occurs. The most prominent symptoms of this degradation are a pronounced drop in pH (from pH 9 to pH 5 or 6) and a marked rise in reducing sugars. Improving control of this problem requires greater understanding of the microbial dynamics during thick juice storage. Therefore, the objectives of this research were to identify the bacterial taxa present in degrading thick juice and to develop and evaluate a DNA macroarray protocol for specific detection of these thick juice bacteria in a single assay.

Development of a thick juice DNA array

T-RFLP
-PCR on 16S ribosomal RNA (rRNA) gene
-Universal bacterial primers 27F-1387R (1)
-27F-FAM labeled
-Multiple single digestion with HhaI, RsaI, MspI (2)
-Analysis with TAP T-RFLP (3)

Clone Libraries
-PCR on 16S rRNA gene
-Universal bacterial primers 27F-1492R(2)
-Sequencing
-Analysis with ARB and PAUP

Classical plating
-Columbia Blood Agar (CBA)
-Five days aerobic incubation at 30°C
-Sequencing 16S rRNA gene of selected colonies
-Blast analysis (4)

Characterization bacterial thick juice population

- *Tetragenococcus* spp.
- *Staphylococcus* spp.
- *Bacillus* spp.
- *Kocuria* spp.
- *Tetragenococcus halophilus*
- *Leuconostoc mesenteroides*
- *Aerococcus viridans*

Development of a DNA macroarray (5)

-Nylon-membrane

-20-mer specific oligonucleotides, printed in duplo at different locations

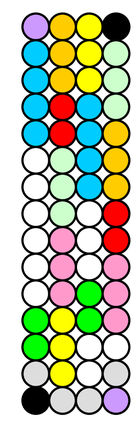
-PCR on 16S rRNA gene

-Universal bacterial primers 27F-1492R

-Simultaneous digoxigenin labelling during PCR

-Hybridization

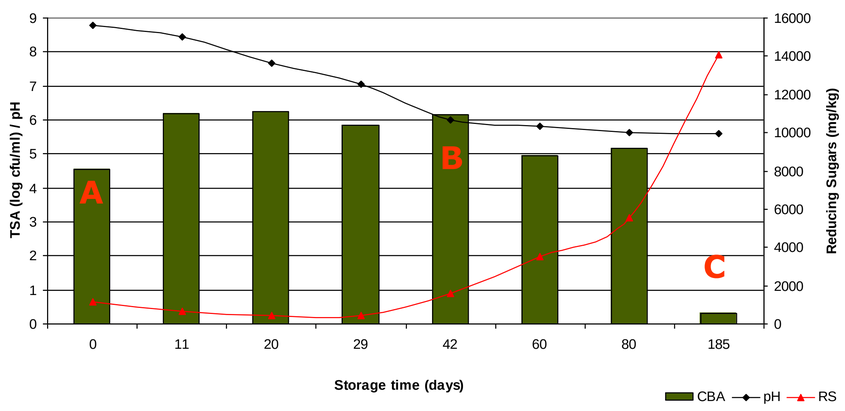
-Detection with anti-digoxigenin alkaline phosphatase conjugate and CDP-Star substrate



- Control for hybridization
- Control for detection
- Universal Bacterial Oligonucleotides

Analysis of thick juice samples

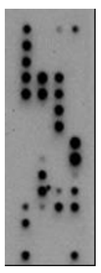
Both CLA and T-RFLP demonstrated that *Tetragenococcus halophilus* was the most consistent and predominant taxon present in both degraded and non-degraded samples. Classical plating on rich medium confirmed the dominance of this halophilic *Tetragenococcus* species, but revealed also other osmophilic bacteria possibly pernicious during sugar production including *Bacillus* spp., *Staphylococcus* spp., *Kocuria* spp., *Aerococcus viridans*, *Leuconostoc mesenteroides* and other *Tetragenococcus* species. In order to monitor the bacterial population dynamics during thick juice storage (Fig. 1), a specific thick juice macroarray was developed covering these bacteria. The sensitivity of the oligonucleotides varied between 0,1 pg and 10 pg target DNA and should allow detection of these contaminants at concentrations relevant for sucrose loss. Using the array, a diverse microbial population was found at the beginning of storage, which evolved to dominance of *Tetragenococcus* species, often accompanied by *Staphylococcus* spp. or *Bacillus* spp.



A. Initial Thick juice

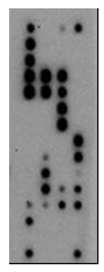
Detection of
-Aerococcus viridans
-Bacillus spp.
-Leuconostoc mesenteroides
-Staphylococcus spp.
-Tetragenococcus spp.
-Tetragenococcus halophilus

B. Thick juice after 42 days of storage



Detection of
-Staphylococcus spp.
-Tetragenococcus spp.
-Tetragenococcus halophilus

C. Thick juice after 185 days of storage



Detection of
-Staphylococcus spp.
-Tetragenococcus spp.
-Tetragenococcus halophilus

Fig. 1. Dynamics of pH, Fastidious Bacteria (FB) and Reducing Sugars (RS) concentration during thick juice storage. Thick juice samples A, B and C after respectively 0, 42 and 185 days of storage were analysed with the developed thick juice macroarray presenting the predominant bacterial thick juice flora.

The macroarray results were evaluated and confirmed by (selective) plating methods. The developed macroarray was considered reliable and has potential for monitoring thick juice during storage. Based on its high density (10^6–10^7 cfu/ml), we believe that *Tetragenococcus halophilus* potentially plays a role in thick juice degradation. However, although present in smaller concentrations, (10^3–10^4 cfu/ml) the species *Bacillus* spp. and *Staphylococcus* spp. are also capable of fermenting sucrose.

References

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