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Altering the flavour profile of cocoa using aromatic and pectinolytic yeasts in a mixed starter culture for cocoa fermentation

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Introduction

Cocoa beans, the principal raw material of chocolate, originate as seeds in fruit pods of the tree *Theobroma cacao*. Raw cocoa has an astringent, unpleasant taste and flavour and has to be fermented, dried and roasted to obtain the characteristic cocoa flavour and taste. Here, we report for the first time the use of a mixed starter culture encompassing yeast with high flavour production potential with the aim of directly influencing the flavour profile of Forastero cocoa by enhanced production of flavour active secondary metabolites. Two defined mixed starter cultures were used as inoculants in cocoa fermentations performed at the Cocoa Research Institute of Ghana. The cultures consisted of *Lactobacillus fermentum* and *Acetobacter pasteurianus*, previously isolated from spontaneous cocoa fermentations, in combination with a pectinolytic and highly aromatic yeast strain (Y1) or a pectinolytic strain isolated from spontaneous cocoa fermentations (Y2). Culture dependent molecular techniques were used to identify and quantify the yeast populations at different time points during 120 hours of fermentation, while DGGE was performed to confirm culture dependent findings.

Results

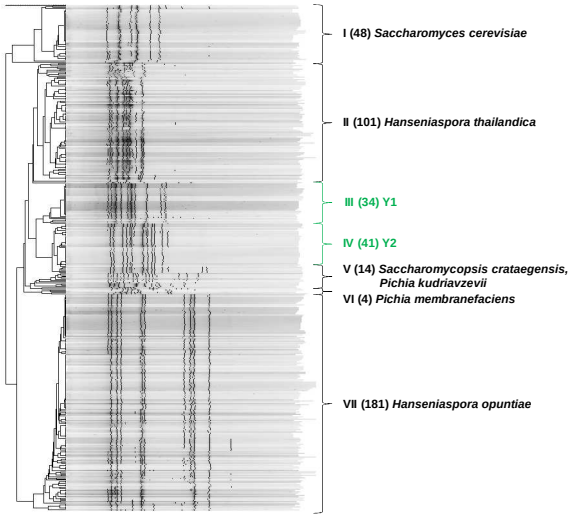

Fig. 1: Dendrogram derived from $(GTG)_5$ based rep-PCR fingerprints of 417 yeast isolates from cocoa fermentations. Value in brackets indicate number of isolates within respective clusters.

Identification

$(GTG)_5$ based rep-PCR fingerprinting was used to group the 417 yeast isolates investigated in this study followed by 26S rRNA (D1/D2 region) and actin gene sequencing. 7 major groups were identified by cluster analysis (Fig.1) with the predominant species being *Hanseniaspora opuntiae* and *Hanseniaspora thailandica* representing ~68% of all isolates identified. Isolates identified as Y1 or Y2 species represented ~8% and ~10% of the total yeast population, respectively.

Growth and survival

Both inoculation strains were able to grow during the first 12 hours of fermentation and were present in cell numbers around 10^6 cfu/g for 24 hours (Fig. 2). Although initially present in lower numbers than the indigenous yeasts, the inoculated strains dominate fermentation after 24 hours whereafter they rapidly die out. Yeasts isolated after 48 and 60 hours all belong to the species *Saccharomyces cerevisiae*.

Typing to strain level

Since both Y1 and Y2 species are frequently isolated from spontaneous cocoa fermentations [1,2], PFGE was used to confirm isolate identity at strain level (Fig. 3). The inoculated strains represented 88% and 98% of the isolates in the Y1 and Y2 populations.

Verification of culture dependent findings

DGGE was performed on DNA extracted directly from cocoa pulp at different stages of fermentation to confirm culture dependent results. Indeed, the species revealed by DGGE profiling supports the sequencing results very well as shown in Fig.4.

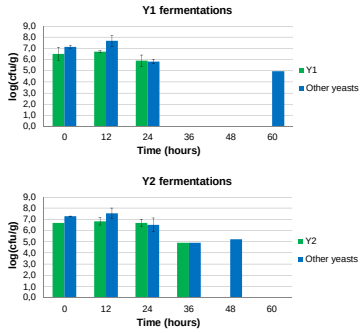

Fig. 2: Cell densities (cfu/g) of inoculation strains and wild yeasts at different timepoints during fermentation. Values are means of two fermentations whereever standard deviations are shown as vertical bars.

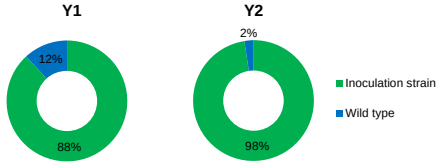

Fig. 3: Diagram showing the distribution of inoculation and wild type isolates of Y1 and Y2 yeast strains based on PFGE karyotyping.

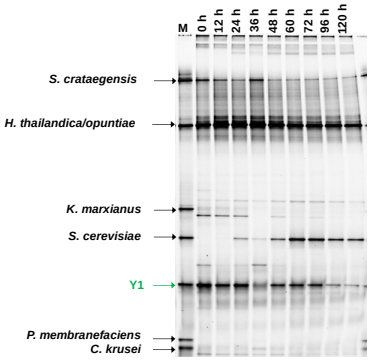

Fig. 4: PCR-DGGE profiles (30-65% denaturant) representing 26S rRNA gene fragments of cocoa pulp sampled at different timepoints during a Y1 inoculated fermentation.

Acknowledgements

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References

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[2] Nielsen, D.S., Teniola, O.D., Ban-Koffi, L., Owusu, M., Andersson, T.S. and Holzapfel, W.H. (2007) The microbiology of Ghanaian cocoa fermentations analysed using culture-dependent and culture-independent methods. Int. J. Food Microbiol. 114, 168-186.

Conclusions

- **Both yeast strains were able to grow during the first 12 hours of fermentation and were present in sufficiently high numbers to influence metabolite production during the initial phase of fermentation.**
- **PFGE karyotyping revealed that 88% and 98% of the isolates respectively identified as Y1 and Y2 were identical to the inoculation strains.**
- **Preliminary sensory evaluation of the cocoa beans inoculated with Y1 showed a change in flavour profile towards more floral notes.**