SUMMARY

Functional role and development of a starter culture of lactic acid bacteria and acetic bacteria for controlled cocoa bean fermentation.

Fermented dry cocoa beans are the basic raw material for chocolate production. Cocoa beans are the seeds of the cocoa tree, *Theobroma cacao* L., which is cultivated within a narrow equatorial belt that crosses South and Central America, Africa, Asia, and Oceania. Unfortunately, cocoa cultivation faced many problems over the past years mainly due to unsustainable farming practices. Chocolate manufacturers and organisations invested in intensive training programs on sustainable cocoa farming practices to be able to supply enough fermented dry cocoa beans to accommodate the growing demand for high-quality cocoa. However, the influence of on-farm post-harvest processing of cocoa, namely cocoa bean fermentation and drying, on the quality of the fermented dry cocoa beans has been largely underestimated.

Recently, it has been shown by research group of Industrial Microbiology and Food Biotechnology (IMDO) of the Vrije Universiteit Brussel that the microbiota of cocoa bean fermentation processes is independent of the cocoa-producing region, cocoa population, and fermentation method, at least when good agricultural and fermentation practices are applied. Moreover, it turned out that a restricted yeast, lactic acid bacteria (LAB), and acetic acid bacteria (AAB) species diversity is crucial for successful cocoa bean fermentation processes. Simultaneously, complex enzymatic reactions are initiated in the cocoa beans that are responsible for the formation of the necessary colour and flavour precursors of well-fermented dry cocoa beans.

Although current knowledge concerning the microbial species diversity and activities present in spontaneous cocoa bean fermentation processes worldwide is available, the development and implementation of appropriate starter cultures for controlled cocoa bean fermentation processes has not been started yet by the cocoa industry. The present study focused on the functional roles and development of starter culture strains of LAB and AAB for a controlled cocoa bean fermentation process to evaluate their impact on the quality of fermented dry cocoa beans and chocolates produced thereof. Therefore, a multiphasic approach was performed, based on microbiological analyses, both culture-dependently and culture-independently, in combination with metabolite target analysis (various chromatography techniques) of samples taken during the whole cocoa bean fermentation and/or drying processes, followed by chocolate making from the respective fermented dry cocoa beans and sensory evaluation of the respective chocolates.

Applying this research approach, the research group IMDO developed and implemented on-farm controlled cocoa bean fermentation processes initiated with a yeast/LAB/AAB starter culture to produce high-quality fermented dry cocoa beans for the manufacture of standard bulk chocolates. A controllable cocoa bean fermentation process encompasses not only good agricultural and fermentation practices but also the addition of an appropriate starter culture. The starter culture strains used were selected based on their functional roles necessary for and their prevalence during successful cocoa bean fermentation. Therefore, a small-scale cocoa bean vessel fermentation method was developed to speed up the research on the usefulness and selection of starter cultures for cocoa bean fermentation. The selected non-citric acid-converting *Saccharomyces cerevisiae* strain fermented the cocoa pulp carbohydrates (glucose) into ethanol in a fast and consistent way and produced the necessary flavours for standard bulk chocolates. Simultaneously, the selected *Lactobacillus fermentum* strain fermented the residual carbohydrates (glucose and fructose) into lactic acid, acetic acid, and mannitol, and converted citric acid present in the cocoa pulp during the initial stages of the controlled cocoa bean fermentations into lactic acid and acetic acid as well as flavour compounds in a fast way. The ethanol and lactic acid thus produced were rapidly oxidised into acetic acid by the selected *Acetobacter pasteurianus* strain. The addition of a (yeast)/LAB/AAB starter culture led to a reduction in fermentation time from six to four days of fermentation and made chocolate production with standard flavour profiles possible. A starter culture preparation composed of strains of *S. cerevisiae*, *L. fermentum*, and *A. pasteurianus* is ready for commercial exploitation now.

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