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Daqu – a fermentation starter for Chinese liquor fermentation

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Introduction: Chinese liquor (Baijiu in Chinese) is distilled from alcohol produced from cooked sorghum by solid-state fermentation. For the alcoholic fermentation, a starter '*Daqu*' is used which serves as a source of microbial inoculum, microbial enzymes, and flavour components. *Daqu* exists in several categories, dedicated for specific flavours of liquor. In our joint research, we investigated the eco-physiology of the microbiota in one type of light-flavour *Daqu* named '*Fen – Daqu*'.

Methods: We used a combination of culture-dependent and culture-independent approaches and studied the metabolome by non-targeted ^{1}H-NMR (proton-Nuclear Magnetic Resonance) analysis.

Results: From a library of isolates among 109 bacteria *Brevibacterium* sp., *Bacillus licheniformis, Bacillus pumilus, Bacillus subtilis, Leuconostoc citreum, Pediococcus pentosaceus, Pseudomonas aeruginosa* and *Lactobacillus plantarum* predominated. Of 81 isolated fungi, the yeasts *Saccharomycopsis fibuligera, Pichia anomala, Issatchenkia orientalis,* and *Saccharomyces cerevesiae* and the filamentous genera *Absidia, Aspergillus, Mucor, Rhizopus, Rhizomucor* and *Penicillium* were encountered. AFLP analysis of the yeast isolates revealed a considerable diversity among the strains of *Pichia anomala* and *Issatchenkia orientalis.* Principal component analysis of ^{1}H-NMR metabolome data of stages representing progressing incubation of *Daqu* revealed clear separation of the samples obtained from different incubation stages. The major compounds that contributed to discrimination were acetate/alanine, arginine, ascorbate, betaine, choline, ethanol, fructose, galactose, glucose, glucitol, glycerate, lactate, maltose, mannitol, phenylalanine, proline, propylene glycol, threonine and tryptophan. These metabolites were regarded as the representative metabolites or biomarkers characteristic for each incubation stage and were related with microbiological changes of importance for quality control in *Fen-Daqu* production.

Conclusions:

1. Different types of *Daqu* could be distinguished by culture-independent DNA-PCR-DGGE (Denaturing Gradient Gel Electrophoresis) profiling, as well as by PCA (Principal Component Analysis) of ^{1}H-NMR data. This is of relevance for the authenticity (AOC: Appelation of Specified Origin) of specific *Daqu* types.
2. *Fen-Daqu* is obtained after a microbial succession in which bacteria, yeasts and filamentous fungi colonize the substrate. Simultaneously, the changes taking place in the metabolome are clearly distinguishable. This will enable further investigation of the impact of specific microbes on targeted metabolic markers which are specific for *Fen-Daqu*.