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Microbial Ecology of Cereal Fermentations

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Summary

Fermented cereal preparations are used to initiate and perform the essential acidification of rye flour containing doughs and also have a tradition in wheat doughs and various cereal batters. Starters containing living bacteria and also dried preparations are produced non-aseptically; their hygienical safety and high quality must be ensured by employing well defined, traditional process conditions which control the microbial ecology.

The main flora of a traditional sourdough preparation consists of strains of ***Lactobacillus sanfrancisco,*** *and* ***L. pontis****. Whereas these organisms exhibit a specialized and effective maltose utilization by using maltose phosphorylase, typical sourdough yeasts as* ***Candida milleri*** *are maltose negative. The sourdough lactobacilli furthermore can excrete glucose and use electron acceptors as the fructose present in the flour to gain additional energy. These special metabolic features and internal parameters as the redox potential affect the composition of the metabolites formed and thus the composition of the microflora. Other effects on the microbial ecology result from external parameters. The increased temperature often used with cereal batters results in changes in the microflora. Such batters are used for the production of sour mashes for beer brewing or dried sourdough preparations. Additional lactobacilli are found which may even dominate including* ***L. amylovorus, L. reuteri, L. johnsonii*** *and* ***L. acidophilus****. These strains are furthermore adapted to the high acid content of such habitats. The knowledge of the intrinsic microbial capabilities helps set up formula and technology in cereal fermentations and control microbial ecology, the metabolites and thus the quality of the product.*

Keywords: sourdough, lactobacilli, yeasts, ecology, metabolism

Introduction

Sourdough has a special status among all fermented products of the food industry in that it is, by definition, not consumed directly but produced as an intermediate product used for bread manufacture *(1)*. It is produced in traditional processes which vary with the inheritent habits in a country or region and thus, a variety of baked goods can be produced from them. In contrast to the time consuming traditional methods which maintain the specific flora, sourdough can also be produced in spotaneous, one-step and continuous fermentation systems to provide sourdough ready for demand. Modern concepts of sourdough production furthermore involve chemical acidification or application of starters containing organisms which do not belong to the competitive flora in traditionally treated doughs. Many species have been isolated from spontaneous sourdough fermentations which are fast acidifyig agents but may rather be mere contaminants from the environment as they are lost upon prolonged propagation of a sourdough. The application of such organisms including e.g. *L. plantarum* and *Saccharomyces cerevisiae* is tempting from a time-saving economic aspect, but it can lead to significant loss in the variery of regional specialties. Knowledge, exploitation and improvement of the key features of the typical sourdough microbes present in traditional preparations is required to attend to the consumers' and industries' demands.

Microflora of Traditional Rye and Wheat Sourdoughs

The sensorial properties of traditionally prepared sourdough bread are highly appreciated. In the respective fermentations a distinct combination of lactobacilli at cell numbers of 10^8–10^9 cfu/g and yeasts of approxi-

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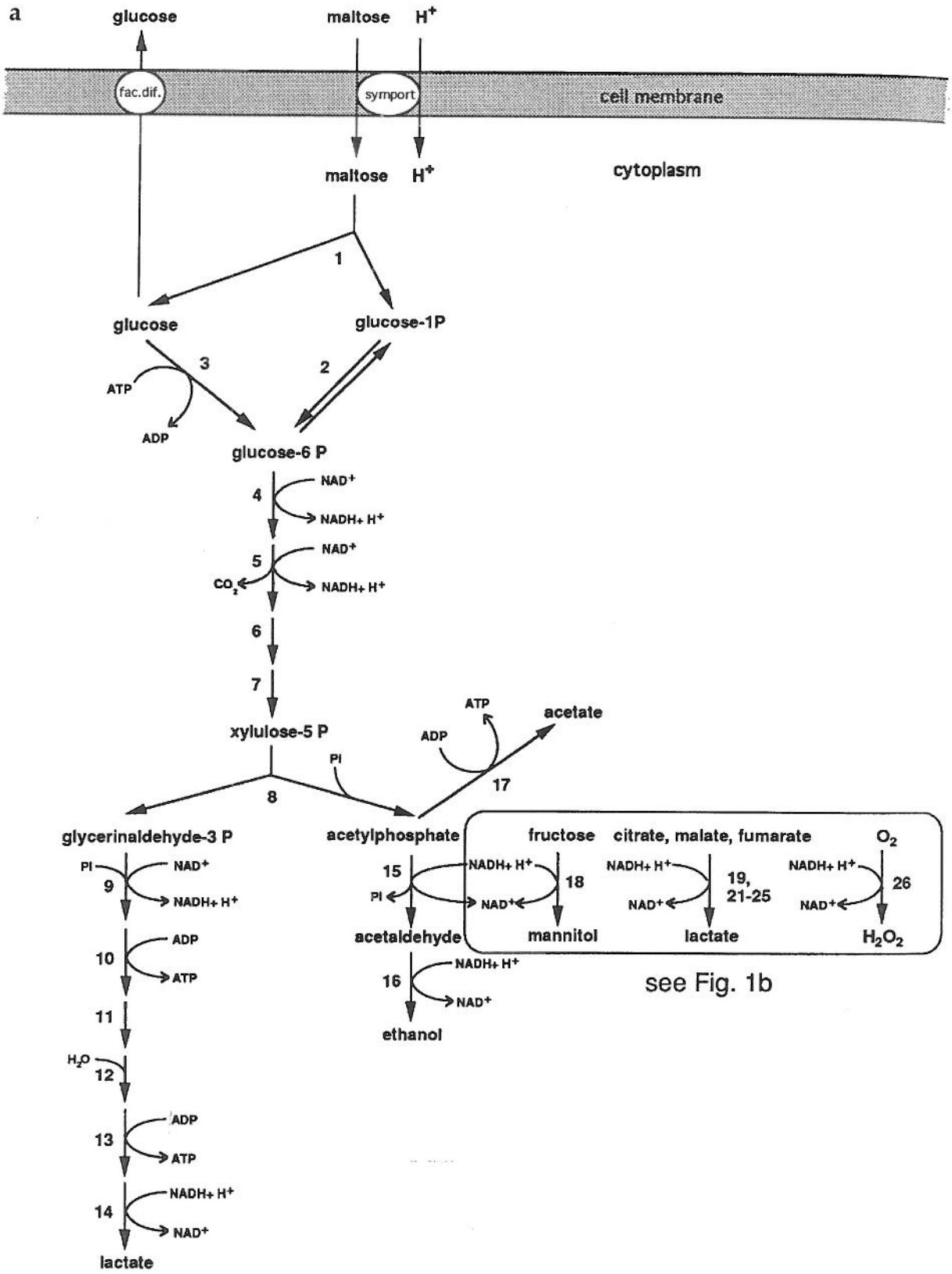

Fig. 1a. Metabolic key reactions of sourdough lactobacilli. Metabolism of maltose.
The organisms and enzymes involved are given in the following list.
1 maltosephosphorylase: *L. sanfrancisco, L. pontis, L. reuteri, L. fermentum*; 2 phosphoglucomutase: *L. sanfrancisco, L. pontis, L. reuteri, L. fermentum*; 3 hexokinase: all lactic acid bacteria; 4–8 enzymes of the phosphogluconate pathway: all heterofermentative lactic acid bacteria; 8 phosphoketolase: all heterofermentative lactic acid bacteria; 9–14 enzymes of the Embden-Meyerhof glycolytic pathway: all lactic acid bacteria; 15 phosphotransacetylase: all heterofermentative lactic acid bacteria; 16 alcohol dehydrogenase: all heterofermentative lactic acid bacteria; 17 acetate kinase: all heterofermentative lactic acid bacteria.

mately 10^6 cfu/g is found, comprising *Lactobacillus sanfrancisco, L. brevis, L. fermentum, L. fructivorans* and the yeasts *Candida milleri* and *C. krusei (2,3)*. Some of the strains intially classified as *L. brevis* recently were allotted to a new species designated as *Lactobacillus pontis (4)*.

Metabolism of Sourdough Lactobacilli

Despite many efforts in the investigation of technical aspects of sourdough production the factors decisive for the competitiveness of the dominant strains are still poorly understood. For control of the process, however, this knowledge is of paramount importance as sourdough fermentations proceed under non-aseptic conditions and over long periods. Recent work focussing on the metabolism of maltose and the utilization of electron acceptors as oxygen or fructose by sourdough lactobacilli has provided first insight into the action of ecological parameters on the development of the sourdough microflora. The major pathways of this metabolism are depicted in Fig. 1.

Sourdough is rich in starch and polyfructosanes which are enzymatically degraded to the fermentable carbohydrates maltose, fructose and little glucose. The sourdough lactobacilli effectively metabolize maltose by the use of maltose phosphorylase which produces glucose-1-phosphate without the expenditure of ATP *(5)*. The resulting glucose is excreted into the medium where it can induce glucose repression in competitors. This reaction may hinder their use of other carbohydrates in the dough e.g. maltose, without affecting maltose metabolism in *L. sanfrancisco* or *L. pontis*. Furthermore, it was demonstrated that strains of *L. sanfrancisco* readily consumed fructose by its preferential use as an electron acceptor when maltose was available in excess *(6)*. Under the latter conditions the cells do not form ethanol, instead they gain an additional ATP by formation of acetate from acetyl-phosphate. This reaction becomes possible as NAD is regenerated by reducing fructose to mannitol *(7)*.

Therefore, the sensorial properties of sourdough bread can be affected by an increase of acetate formation by addition of fructose to the flour.

Yeasts in Sourdough Fermentations

The selection of specific yeasts, namely *C. milleri* and *C. krusei (8)*, and their role in sourdough fermentation still remains to be elucidated. In addition to these yeasts

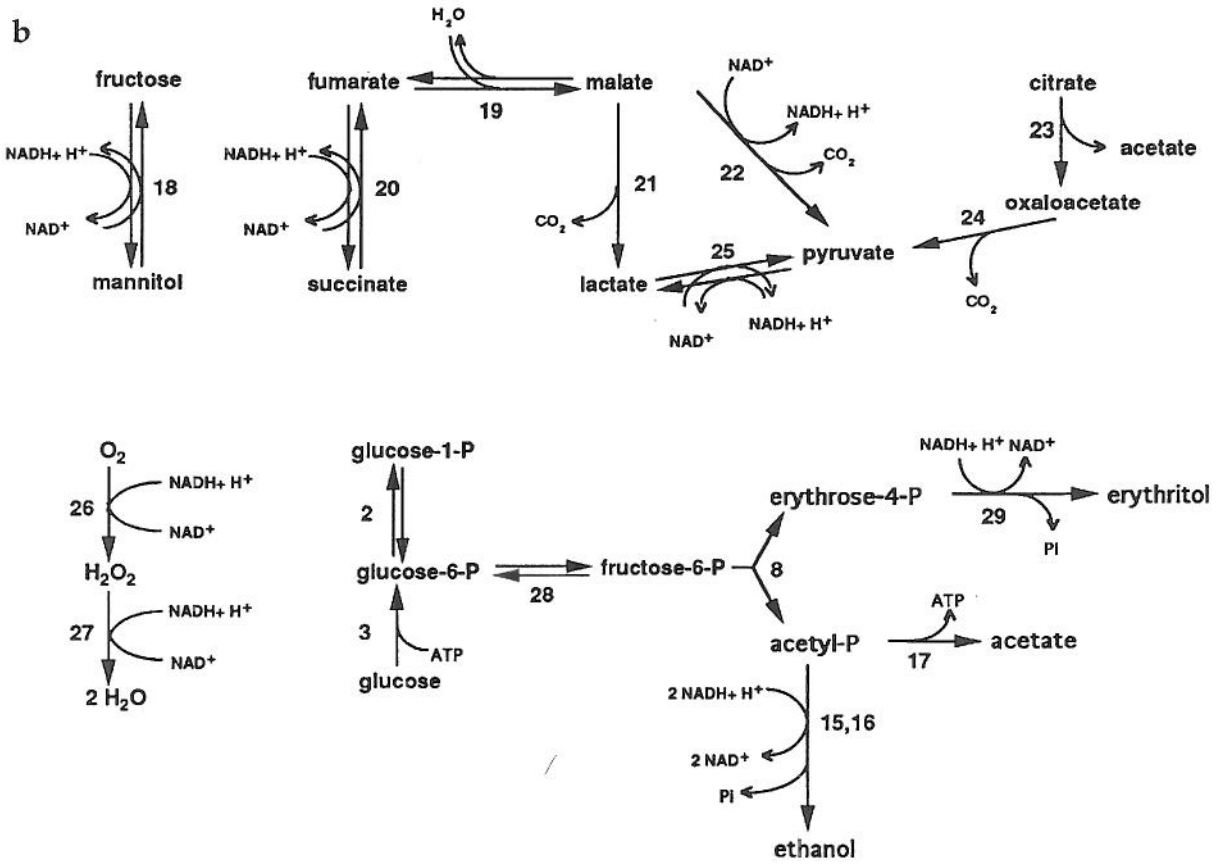

Fig. 1b. Metabolic key reactions of sourdough lactobacilli. Fate of potential electron acceptors upon maltose fermentation. The organisms and enzymes involved are given in the following list.
18 mannitol dehydrogenase: *L. sanfrancisco, L. pontis, L. fermentum*; 19 fumarase: *L. sanfrancisco, L. pontis, L. reuteri, L. amylovorus, L. fermentum*; 20 succinate-dehydrogenase: *L. pontis, L. reuteri, L. amylovorus, L. fermentum*; 21 malolactic enzyme: *L. sanfrancisco*; 22 malate enzyme: not found; 23 citrate-lyase: *L. sanfrancisco, L. amylovorus, L. fermentum*; 24 oxaloacetate-decarboxylase: *L. sanfrancisco, L. amylovorus, L. fermentum*; 25 lactate-dehydrogenase: all lactic acid bacteria; 26 NADH-H_2O_2-oxidase: *L. sanfrancisco*; 27 NADH-peroxidase: *L. sanfrancisco*; 28 glucosephosphate-isomerase: all lactic acid bacteria; 29 erythritol-dehydrogenase and erythrose-4-P-phosphotransferase: *L. sanfrancisco*.

Saccharomyces cerevisiae has been found in sourdough preparations. However, this yeast is lost upon continuous propagation of sourdough in a traditional way even if it is inoculated at high levels. The selection of *C. milleri* and *C. krusei* might be due to their high resistance to undissociated acetic acid present in sourdough at low pH. Furthermore, they do not compete for maltose as they cannot ferment it. They are poor fermenters and only ferment glucose which is taken up by a high affinity transport system. The finding of *S. cerevisiae* in some sourdoughs may also be due to misidentification as there is no reliable system for the identification and classification of yeasts from this habitat.

Despite their low numbers as compared to lactobacilli they can contribute significantly to the leavening of the dough by production of carbon dioxide. On the other hand, it was shown that sufficient leavening is achieved by the application of single strains of *L. sanfrancisco.*

Microbial Ecology in Alternative Processes

Although the conditions in other cereal fermentations may be somewhat different, a combination of maltose-fermenting lactobacilli and maltose-negative yeasts may be found. This was demonstrated for sorghum batters used for the production of Sudanese Kisra *(9).* Whenever other genera were found, including *L. amylovorus* and *L. reuteri*, the principled ecological situation is similar. In modern technologies used for the production of dried sourdough preparations the fermentation conditions may also be different resulting in a change in the ecological situation. Sometimes temperatures of up to 40 °C and relatively high water content resemble an environment as found in the production of sour mashes used for the production of (wheat) beer. As a consequence the spectrum of organism involved in these fermentations includes *L. pontis, L. reuteri* but also *L. amylovorus* and *Weissella confusa* in addition to not yet identified species. Taxonomic investigations include M13 typing and sequencing of 16S rRNA as classic methods give misleading results.

Conclusion

It has become obvious that the ecology of cereal fermentations still harbours some surprises and new genera to be discovered. Their metabolic features exhibited *in situ* determine the habitats' parameters and therefore the development of the emerging microflora. Future work will therefore include molecular monitoring systems for the estimation of flora development as well as metabolic studies on the discovered organisms.

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Mikrobna ekologija fermentiranih žit

Povzetek

Fermentirani kisli nastavki se uporabljajo za zakisovanje rženih test, pa tudi v pšeničnih in drugih zamesih. Sveže ali suhe bakterijske starter kulture se proizvajajo neaseptično, vendar njihovo higiensko neoporečnost in visoko kvaliteto zagotavljajo točno definirani pogoji proizvodnje. Glavna mikroorganizma klasičnih kislih test sta ***Lactobacillus sanfrancisco*** *in* ***L. pontis.*** *S pomočjo maltoza fosforilaze izkoriščata maltozo kot enega osnovnih virov ogljika v testu, medtem ko značilna kvasovka kislih test,* ***Candida milleri*** *te sposobnosti nima. Laktobacili kislih test lahko izkoriščajo tudi fruktozo kot dodatni vir energije. Te metabolične posebnosti, kakor tudi značilni notranji fizikalno-kemijski parametri (npr. redoks potencial) in zunanji dejavniki (npr. temperatura) vplivajo na sestavo in metabolizem mikrobne asociacije kislih test. Povišana temperatura se uporablja pri pripravi suhih kislih nastavkov in kisle sladice v pivovarstvu. Tu lahko prevladujejo drugi sevi, npr.* ***L. amylovorus, L. reuteri, L. johnsonii*** *in* ***L. acidophilus.*** *Prilagojeni so na nizke pH vrednosti okolja. Razumevanje mikrobne ekologije omogoča optimizacijo tehnologije fermentacije žit, sintezo željenih metabolitov in kakovost končnih izdelkov.*