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Global transcriptional response of *Lactobacillus reuteri* to the sourdough environment

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Abstract

Lactobacillus reuteri is a lactic acid bacterium that is highly adapted to the sourdough environment. It is a dominant member of industrial type II sourdoughs, and is also able to colonize the intestinal tract of mammals, including humans, and birds. In this study, the transcriptional response of *L. reuteri* ATCC 55730 was investigated during sourdough fermentation by using whole-genome microarrays. Significant changes of mRNA levels were found for 101 genes involved in diverse cellular processes, such as carbohydrate and energy metabolism, cell envelope biosynthesis, exopolysaccharide production, stress responses, signal transduction and cobalamin biosynthesis. The results showed extensive changes of the organism's gene expression during growth in sourdough as compared with growth in chemically defined medium, and, thus, revealed pathways involved in the adaptation of *L. reuteri* to the ecological niche of sourdough. The utilization of starch and non-starch carbohydrates, the remodelling of the cell wall, characterized by reduced D-alanylation, and increased amounts of cell wall-associated polysaccharides, as well as the regulatory function of two component systems for cell wall biogenesis and metabolism were suggested by the gene expression data as being important for growth in sourdough. The impact of several *L. reuteri* genes for effective growth in sourdough was shown by implementation of mutant strains in sourdough fermentation. This study contributes to the understanding of the molecular fundamentals of *L. reuteri*'s ecological competitiveness, and provides a basis for further exploration of genetic traits involved in adaptation to the food environment.

Keywords: *Lactobacillus reuteri*; Sourdough fermentation; Gene expression; Whole genome microarray

Introduction

Sourdough results from the fermentation of cereals such as wheat or rye and has been used for centuries in the manufacture of numerous baked goods, particularly bread. It is responsible for the acidification of dough, development of aroma precursors and texture, and extension of product shelf-life [36]. The beneficial properties of sourdough are determined by the metabolic activity of the sourdough microbiota, mainly consisting of lactobacilli associated with yeasts [19,31,88]. Dependent on the type of fermentation, numerous *Lactobacillus* species have been shown to be

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highly competitive in sourdough [19]. The predominant species of type II sourdough fermentations, which are characterized by higher temperatures, longer fermentation times and higher water contents than type I doughs [89], are thermophilic and acid-tolerant lactobacilli such as *Lactobacillus pontis*, *Lactobacillus reuteri*, and *Lactobacillus amylovorus* [20,88]. In particular, strains of *L. reuteri* are highly competitive, persist in industrial fermentation processes over several years of continuous propagation, and are constituents of industrial sourdough starter cultures [26,55]. However, *L. reuteri* is not only adapted to the food fermentation environment, but is also a resident (autochthonous) member of the intestinal microbiota of animals and humans, capable of eliciting beneficial (i.e., probiotic) effects for the host organism [73,83].

A multitude of physiological features have been characterized as being responsible for the competitiveness of *L. reuteri* and other sourdough lactobacilli (reviewed in [28,33]). Most notably, the highly adapted carbohydrate and energy metabolism enables efficient exploitation of cereal carbohydrates, particularly maltose and sucrose, with a concomitant increase of energy yield through the use of external electron acceptors, such as fructose or oxygen [76,77]. Furthermore, the arginine deiminase (ADI) pathway permits protection against acidity by intracellular NH_3 production as well as extra ATP generation [18]. In addition, the secretion of antimicrobial substances such as organic acids and reutericyclin can provide a competitive advantage over the accompanying microbiota [27,56]. However, the genetic background responsible for the ecological performance of *L. reuteri* in sourdough fermentation is poorly understood. Recently, the draft genome sequence of *L. reuteri* ATCC 55730 [4], a strain isolated from human mother's milk, was determined, and it provided fundamental information on the genetic endowment of this species. On the basis of the genome information, 38 conditionally expressed genes of *L. reuteri* LTH5531 growing in a type II rye bran sourdough, mainly involved in cellular processes such as the stress response and metabolism of amino acids and nucleotides, were identified by applying in vivo expression technology (IVET) [15].

To gain further insight into the specific gene expression of *L. reuteri* and to complement the findings obtained with IVET, the transcriptomes of *L. reuteri* ATCC 55730 cells growing in sourdough and in chemically defined medium (CDML) were investigated in the present study by using whole-genome DNA microarrays. Strain *L. reuteri* ATCC 55730 was chosen as the object of study because of the availability of the genome sequence and its ability to dominate rye sourdough fermentation (E.H., personal observation), although it was not originally isolated from this environment. The results shed light on the cellular mechanisms beyond the well-investigated metabolic characteristics of lactobacilli and establish a basis for further research on the genetic fundamentals of the adaptation to the ecological niche of sourdough.

Materials and methods

Bacterial strains, media and culture conditions

Strains used in this study are listed in Table 1. *L. reuteri* strains were cultured microaerobically (2% O_2, 10% CO_2, 88% N_2) in MRS medium (Oxoid) at 37 °C or in the CDML at 40 °C. CDML was developed on the basis of a previously published synthetic medium for lactobacilli [24], but with the following modifications of the recipe ($g\,l^{-1}$): glucose 20, $FeSO_4 \cdot 7H_2O$ 0.01, $Co(SO_4)_2 \cdot 7H_2O$ 0.01, calcium citrate 0.05, folic acid 0.0005, *p*-aminobenzoate 0.0006, D-biotin 0.001, calcium panthothenate 0.003, riboflavin 0.003, nicotinic acid 0.003, thiamine 0.005, cyanocobalamin 0.005, myo-inositol 0.005, pyridoxine HCl 0.01, adenine 0.1, guanine 0.1, uracil 0.1, xanthine 0.1, and inosine 0.1. Calcium lactate and sodium acetate were omitted from the CDML recipe. To prepare CDML, salts were resuspended in distilled water and autoclaved. Amino acids, vitamins and glucose were each dissolved in distilled water with optional pH adjustment to facilitate complete solubility. The solutions were mixed, the pH was adjusted to 6.3 and the medium was then filter sterilized. *Escherichia coli* strains were cultivated aerobically at 37 °C in Luria–Bertani (LB) medium [70]. To determine the cell counts of the indigenous lactobacilli biota, MRS5 agar [55] containing $0.1\,g\,l^{-1}$ cycloheximide was used and the plates were incubated microaerobically at 30 as well as 37 °C for 48 h. For counting of yeasts, YGC agar [54,55] was used and the plates were incubated aerobically at 25 °C for 48 h. When required, erythromycin or chloramphenicol was added to the media at final concentrations of 10 or $5\,\mu g\,ml^{-1}$, respectively.

Sourdough fermentation

Type II sourdough fermentation was performed as described previously [55] but with modifications. Briefly, batches of dough were prepared from rye bran (8 g) and sterile tap water (21.36 ml) to provide a dough yield of 367 (mass of dough/mass of bran × 100). Erythromycin was added at a concentration of $10\,\mu g\,g^{-1}$ dough. Fermentation was started by the addition of 100 µl of inoculum (about 5×10^7 *L. reuteri* cells) followed by incubation in 50 ml plastic tubes at 40 °C in a water bath. After 24 h of incubation, the dough was propagated by back-slopping 1% of ripe dough followed by