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Current Opinion in Systems Biology

Systems biology of lactic acid bacteria: For food and thought

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Abstract

Lactic acid bacteria (LAB) ferment plants, fish, meats and milk and turn them into tasty food products with increased shelf life; other LAB help digesting food and create a healthy environment in the intestine. The economic and societal importance of these relatively simple and small bacteria is immense. In this review we hope to show that their adaptations to nutrient-rich environments provides fascinating and often puzzling behaviours that give rise to many fundamental evolutionary biological questions in need of a systems biology approach. We will provide examples of such questions, compare the (metabolic) behaviour of LAB to that of other model organisms, and provide the latest insights, if available.

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Current Opinion in Systems Biology 2017, **6**:7–13

This review comes from a themed issue on **Systems biology of model organisms (2017)**

Edited by **Jens Nielsen** and **Kiran Raosaheb Patil**

For a complete overview see the Issue and the Editorial

Available online 18 July 2017

http://dx.doi.org/10.1016/j.coisb.2017.07.005

Keywords

Lactic acid bacteria, Lactococcus, Lactobacillus, Food fermentation, Systems biology, Biotechnology.

Introduction

With over 100 billion Euros annually [1], the economic value of foods fermented by such small bacteria – LAB have genome sizes of only 2–3 Mbp-is impressive. Yogurt, cheese and sauerkraut, but also breads, hams, and olives, or pickles, soy- and fish sauce require the metabolic activities of LAB. Many of these foods have a long history, and the associated industry is rather traditional and wary of revolutionary changes in production processes, let alone genetic modifications to improve traits. Rather, the industry takes advantage of the enormous diversity in species and strains with different functionalities, such as flavour production profiles or texturing properties. Furthermore, one may change pH and temperature a bit, or change some ingredients perhaps.

It is also not so easy to decide what to change, or how to change it: foods are chemically complex and undefined, and often the fermentation is not carried out by a single strain, but a complex mixture of LABs. Finally, analyses in sticky, solid and inhomogeneous food matrices can be tedious, and they often are. Therefore, in contrast to the industrial biotechnology field that produces biobased chemicals, largely on the basis of monocultures in relatively well-defined growth media, research in LAB has much less adopted engineering and systems approaches – although metabolic engineering activities in LAB are ongoing [2–4].

We believe this is a pity, from both sides: Systems biology has a lot to offer to the LAB field, and the LABs have a lot to offer to the systems biology field. LABs provide questions, challenges, and biological examples of adaptations to environments rich in nutrients and full of stress [5]. They can offer interesting and relevant cases to test the generality of findings in other, better studied, model organisms, *Escherichia coli* in particular. Systems biology on the other hand, can provide structure to, and understanding of, the complex systems comprising LAB. In this review, we will describe some features of LAB physiology that we believe are interesting from a systems biology perspective, and we will describe our current understanding and open questions. We hope this will attract more systems biologists to these fascinating microorganisms.

Metabolic adaptation to rich nutrient environments: auxotrophies and division of labour

Environments in which LAB thrive are rich in sugars and protein; fats, vitamins and nucleotides are also often available. Consequently, most LAB are auxotrophic for a large number of amino acids and vitamins. The loss of function in the presence of some specific nutrients may be caused by genetic drift or provides a selective advantage. Recent works make strong cases for the latter. It was shown experimentally in *E. coli* that introduction of auxotrophies and subsequent exchange of amino acids provided the auxotrophic mutant an increased growth rate over the wildtype [6]. These traits

are evolvable in laboratory evolution experiments and make the mutants depend on cross-feeding [7].

The differences between LAB species and even strains from the same species, however, is large and although it was explained in general by niche adaptation for *Lactococcus lactis* strains [8], the diversity in amino acid metabolism and corresponding auxotrophies remains puzzling as the environments in which LAB are isolated often appear equally rich in amino acids, peptides and/or proteins. This matters for LAB applications as their catabolic products are flavour compounds [9], or bitter or health-promoting [10] peptides. The physiological role of such catabolism is not always clear: its wide spread occurrence may be a consequence of our selection for flavour production, but it may also contribute to stress and energy metabolism [11], chemical warfare, host–microbe interactions [12] or possibly geographic spreading through attracted insects.

In Table 1 the experimentally-determined auxotrophies for amino acids are shown for a number of representatives of LAB species. However, defining such auxotrophies is nontrivial by dependencies on the presence of other nutrients: also in humans the dichotomy between essential and non-essential amino acids was questioned and condition-dependent essential amino acids introduced [13]. For example, if glutamine is present, glutamate is not required, but almost all LAB require either glutamine or glutamate, because they cannot synthesize its precursor α-ketoglutarate *de novo*, by lack of a complete TCA cycle [11]. Alternatively, the presence of some amino acids can provide feedback inhibition on the synthesis of another amino acid. This happens e.g. for aromatic and branched chain amino acids [14]. Therefore, auxotrophies predicted from genome-scale metabolic models require careful experimental validation, as recently done extensively for *Enterococcus faecalis* [15] and *Streptococcus pyogenes* [16].

The similarities between auxotrophies of LAB species and humans perhaps suggest an underlying cause or constraint. Methionine or cysteine are always required, as is histidine (except for *Lactobacillus plantarum*); Aromatic and branched chain amino acids, especially valine, are often needed for growth, or at least for fast growth [14]. Why these amino acids? Are these the most expensive to make, or require complex co-factors or vitamins, and are the genes therefore most easily lost? A comparative study in lactobacilli showed a great diversity for different amino acids in true loss of biosynthetic genes and loss of activity by mutations [17]. However, the growth of all tested lactobacilli could not be restored without glutamate, even after mutagenesis and selective plating. The collective loss by LAB of the ability to synthesize such a key amino acid as glutamate is mysterious.

Table 1

Amino acid requirement for selected LAB and man (in black box). Green indicates that growth is possible (but often at a lower rate) in the absence of the amino acid, red means no or extremely poor growth is observed. Based on studies of specific strains of S.t. [18], L. l. [19], L.p. [14], S.p. [16], E.f. [16], H.s. [13] and L.a. [17]. Note that auxotrophies between different strains of the same species can differ.