Electron transport chains of lactic acid bacteria - walking on crutches is part of their lifestyle

Rob Brooijmans[1,2], Willem M de Vos[3]* and Jeroen Hugenholtz[2]

Address: [1]Top Institute (TI) Food and Nutrition, 6700 AN, Wageningen, The Netherlands; [2]Kluyver Centre for Genomics of Industrial Fermentation, 6710 BA, Ede, The Netherlands; [3]Laboratory of Microbiology, Wageningen University, Dreijenplein 10, 6703 HB, Wageningen, The Netherlands

* Corresponding author: Willem M de Vos (willem.devos@wur.nl)

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Abstract

A variety of lactic acid bacteria contain rudimentary electron transport chains that can be reconstituted by the addition of heme and menaquinone to the growth medium. These activated electron transport chains lead to higher biomass production and increased robustness, which is beneficial for industrial applications, but a major concern when dealing with pathogenic lactic acid bacteria.

Introduction and context

Many lactic acid bacteria (LAB) have been used for millennia for the production of a wide variety of fermented foods. Lactic acid fermentation increases the shelf-life of foods, while simultaneously making the foods easier to digest and improved in flavor [1,2]. Since they lack a heme biosynthesis pathway, this group of anaerobic Gram-positive bacteria cannot form endogenous electron transport chains (ETCs) and, thus, have long been considered as obligate fermentative bacteria.
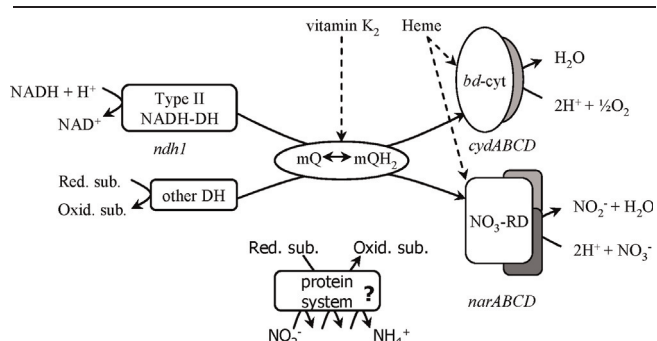
Major recent advances

For some LAB - that is, *Lactococcus lactis*, *Lactobacillus plantarum*, *Streptococcus agalactiae* and *Enterococcus faecalis* - it has been shown in some detail that the ETC activity can be reconstituted by addition of menaquinone and/or heme to the culture [3–9]. Below we describe this phenomenon in more detail.

Aerobic respiration in *L. lactis* and *S. agalactiae*

L. lactis and *S. agalactiae* show a doubling of biomass when cultivated in the presence of heme alone [4,7,9] or heme and menaquinone combined [6]. This can be explained in three ways: first, the oxidation of NADH by the ETC affects the redox-balance and, in *L. lactis*, results

in a shift from homo-lactic to a mixed-acid fermentation with acetate production, resulting in increased substrate-level ATP generation; second, a reduced acidification occurs when acetate and acetoin are produced instead of lactate, resulting in a more complete utilization of the available sugars; and third, extra energy is conserved by proton pumping, potentially conserving 2/3 (0.66) ATP molecules for each NADH molecule oxidized [7–9]. Together, these effects add up to give a dramatic improvement in growth [4,6,8,9].

Tolerance to different stresses is also much improved in the respiratory cell-state [10,11]. The reconstituted cytochrome *bd* shows active oxygen consumption, leading to low intracellular oxygen levels under aerated conditions and protection against oxidative stress [11]. This protection against oxidative stress during respiration was clearly shown in a microarray study that reported down-regulation of many stress-related genes [12]. Interestingly, a respiration-negative mutant (a *cydA* mutant) of *S. agalactiae* showed not only a lack of respiratory behaviour but also a decrease in virulence (as determined in a rat-model) and a decreased ability to grow in blood [6,13].

Figure 1. Model of the branched ETC of *Lb. plantarum* WCFS1

bd-cyt, *bd*-type cytochrome complex; DH, dehydrogenase; mQ/mQH$_2$, oxidized/reduced menaquinone; NO$_3$-RD, nitrate reductase; oxid., oxidised; red., reduced; sub., substrate.

Anaerobic respiration in E. faecalis *and* Lb. plantarum

As described above, several LAB show aerobic respiration via a rather simple and non-redundant ETC consisting of a NADH dehydrogenase, a menaquinol pool and a *bd*-type cytochrome [7,11,14] (Figure 1). *E. faecalis* and *Lb. plantarum* also contain components for anaerobic respiration. In *E. faecalis* the existence of a branched ETC that terminates not only in a cytochrome *bd*-type but also in a fumarate reductase has been suggested [15]. An association of the *E. faecalis frdA* gene with fumarate reduction was shown, although the complete *frdACBD* gene cluster, typical for fumarate reductase in *Escherichia coli* [16], was missing. Many LAB contain homologues of *frdA*, suggesting that this single gene may also be functional in other LAB [15].

Comparative genomics and genome-wide transcriptome analysis revealed that the *narGHJI* genes, encoding a nitrate-reductase A complex, are present and expressed in several lactobacilli; *Lb. plantarum* is able to reduce nitrate to nitrite only when both heme and menaquinone are supplied. Typically, reduction of nitrate by *Lb. plantarum* coincides with (transient) formation of nitrite and further reduction to ammonia, and a significant increase in biomass [17]. *Lb. plantarum* carrying a deleted *ndh1* gene failed to respire in the presence of heme and menaquinone under aerobic conditions [17]. However, this mutation did not abolish the nitrate reductase activity. This indicates that the ETC of *Lb. plantarum* is also branched at the level of electron donation (Figure 1).

Distribution of respiration in LAB

As described above, some LAB demonstrate respiratory behaviour when menaquinone and/or heme are added to the culture. In fact, many species of LAB have the *cydABCD* genes present on their genome that encode the *bd*-type aerobic cytochrome. Of the 45 completely

sequenced LAB genomes, these *cyd*-genes were found in about half the species [5]. In a recent screening activity (increased biomass formation during aerobic growth on media containing heme and menaquinone) it was shown that there are potential respirators among *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Carnobacterium*, *Enterococcus*, *Oenococcus* and *Leuconostoc* spp. [5]. However, very few LAB besides *Lb. Plantarum* – only *Lb. reuteri* and *Lb. fermentum* – contain the necessary *narGHJI* genes for anaerobic nitrate respiration [17].

Industrial and ecological relevance

Enhanced biomass production by adding heme-containing compounds to aerated cultures of LAB is of interest for industrial processes, especially with regard to starter culture production [10,18]. Anaerobic ETC activity, with nitrate or fumarate as terminal electron acceptors, could also be implemented in industrial fermentation. It can be argued that nitrate respiration may even be a normal activity for several strains of *Lb. plantarum*, *Lb. reuteri* and *Lb. fermentum* in the human gastrointestinal (GI) tract. Heme is present in all foods that are of eukaryal origin (heme can be found in all mitochondria) as well as in many bacteria but not LAB. Also, menaquinones are produced by many prokaryotes in the GI tract. In fact, most of the human nutritional vitamin K$_2$ requirement is covered by the gut microbiota [19,20]. In addition, nitrate and nitrite are part of the normal human diet [21–23] and derive from the consumption of green leafy vegetables or various meat products where these nitrogen compounds are used as preservatives [24].

Future directions

Many LAB have components of ETCs that enable them to use various intra- and extracellular electron donor and acceptor components for improved bioenergetics. The fact that these ETCs need the presence of exogenous cofactors such as heme and menaquinones to operate does not preclude that their activity is part of the normal lifestyle of LAB. Moreover, the presence of functional ETCs allows enhanced growth and functionality on a wider variety of substrates, which may provide new applications and even new health benefits by using LAB in the food, feed and pharmaceutical industries. Heme-sequestering by LAB should lead to a reduced risk of colonic cancer associated with eating red meat [22,25]. The reduction of nitrate via nitrite to ammonia in the GI tract by lactobacilli could lead to the transient production of nitric oxide. In fact, increased nitric oxide levels have been shown in the GI tract by combined dietary supplementation of nitrate and lactobacilli [26]. Nitric oxide production is known to counteract colonic inflammation [27] and has been implicated as a host defence mechanism against pathogens [28]. A final

functional and health benefit of this induced nitrate reduction would be to use this process for lowering or completely removing nitrate (and nitrite) in foods such as cheese, which contain unacceptable levels in some countries.

Abbreviations

ETC, electron transport chain; GI, gastrointestinal; LAB, lactic acid bacteria.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

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