Aerobic Respiration Metabolism in Lactic Acid Bacteria and Uses in Biotechnology

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Abstract

The lactic acid bacteria (LAB) are essential for food fermentations and their impact on gut physiology and health is under active exploration. In addition to their well-studied fermentation metabolism, many species belonging to this heterogeneous group are genetically equipped for respiration metabolism. In LAB, respiration is activated by exogenous heme, and for some species, heme and menaquinone. Respiration metabolism increases growth yield and improves fitness. In this review, we aim to present the basics of respiration metabolism in LAB, its genetic requirements, and the dramatic physiological changes it engenders. We address the question of how LAB acquired the genetic equipment for respiration. We present at length how respiration can be used advantageously in an industrial setting, both in the context of food-related technologies and in novel potential applications.

INTRODUCTION

Lactic acid bacteria (LAB) are the workhorses of most food fermentation processes, and have been extensively studied for their metabolic and genetic properties. In the past two decades, they have been genetically manipulated, sequenced, and taxonomically distinguished, all of which has deepened our understanding of this diverse taxon. Great efforts have been applied to improve their functionality and stability, and to diversify their uses for probiotic exploration and medical biotechnology.

Several LAB species have a double metabolic life: They can switch from fermentation to aerobic respiration metabolism when provided with heme, and for some bacteria, heme and menaquinone (Duwat et al. 2001, Lechardeur et al. 2011, Yamamoto et al. 2005). An electron transport chain and evidence for cytochromes in heme-grown cultures were first reported in lactococci, leuconostocs, and some enterococci in the 1970s (Bryan-Jones & Whittenbury 1969, Sijpesteijn 1970, Whittenbury 1978), although the existence of cytochromes in lactococci was not consistently observed (Kaneko et al. 1990). In-depth studies on respiration metabolism and its physiological impact in various LAB came nearly thirty years later (Blank et al. 2001, Duwat et al. 2001, Duwat et al. 1998, Lechardeur et al. 2011, Yamamoto et al. 2005). A second class of LAB, requiring both heme and menaquinones, was then discovered (Brooijmans et al. 2009b, Yamamoto et al. 2005), leading to the realization that the majority of sequenced lactobacilli possess the genetic capacity for respiration. In *Lactococcus lactis*, where respiration metabolism is best documented, respiration impacts positively and significantly on bacterial biomass, resistance to oxygen, and long-term survival (Duwat et al. 2001, Gaudu et al. 2002). These findings opened new perspectives on LAB lifestyle and have yet to be understood and exploited to their full potential. Moreover, the ability to turn on or off LAB respiration metabolism simply by controlling heme and/or oxygen availability makes these bacteria valuable as conditional models for understanding the changes and benefits of respiration compared with analyses in more complex respiring bacteria like *Escherichia coli*.

Despite a complete documentation of the respiration capacity of several LAB and the genomic prediction of the generality of this finding, this subject has received little scientific attention. For those working on LAB, respiration constitutes a radical departure from conventional growth conditions; for specialists of the mechanistics of respiration metabolism, this class of bacteria, which relies on exogenous cofactors, has not yet emerged as a model.

Here, we discuss the genetics and physiology of respiration metabolism in LAB. As most studies to date were performed in *L. lactis*, this bacterium is highlighted. The relevance of respiration metabolism in LAB for biotechnological processes, either already realized or in ongoing or future applications, is discussed in depth. LAB are being developed as probiotics, i.e., for their functionalities in foods after they are ingested. Potential uses of respiration in the in vivo context are also considered.

For information, some recent reviews of LAB in biotechnology without respiration are informative for understanding the conventional uses of LAB (see Bron & Kleerebezem 2011, Douglas & Klaenhammer 2010, Pfeiler & Klaenhammer 2007, Teusink & Smid 2006). For comments and reviews on respiration, see Garrigues et al. 2006, Gaudu et al. 2002, Lechardeur et al. 2011, and Yamamoto et al. 2006. For industrial issues on implementation of lactococcal respiration starter cultures, see Høier et al. 2010 and Pedersen et al. 2005.

LACTIC ACID BACTERIA ARE DIFFERENT FROM OTHER RESPIRATION-PROFICIENT BACTERIA

Respiration metabolism is the predominant energy pathway in numerous bacteria, including the best known models, *E. coli* and *Bacillus subtilis*. Respiration in those bacteria is flexible, thanks to the

presence of multiple and redundant enzymes that assure respiration chain activity under different conditions (Richardson 2000).

Respiration in Lactic Acid Bacteria

The aerobic respiratory chain requires three main membrane components, as described in the model bacteria. (*a*) An NADH dehydrogenase acts as an electron donor. (*b*) A quinone then delivers electrons from the dehydrogenase to a final acceptor enzyme complex. In gram-positive bacteria, this function is mediated by menaquinones, which are synthesized by a series of enzymes encoded by the *men* and *isp* genes. (*c*) The acceptor protein complex is a cytochrome oxidase whose activity is heme dependent. In addition to these essential features, the protons extruded by the respiratory chain can be utilized by the F_0F_1 ATP synthase to generate ATP (Gennis & Stewart 1996).

The stripped-down respiratory chain of respiration-competent LAB puts them in a class apart from the better-studied aerobic bacteria (Richardson 2000) (**Figure 1**). First, LAB require exogenous heme, as they lack enzymes for heme biosynthesis. Some LAB also lack *menFDXBEC*, the menaquinone biosynthesis genes (Rezaiki et al. 2008), which means that two exogenous components are needed to activate respiration (Lechardeur et al. 2011, Yamamoto et al. 2005). The absence of these required cofactors means that respiration metabolism is strongly dependent on the environment. Second, respiring LAB all encode a single type of cytochrome oxidase, the quinol oxidase CydAB. This enzyme complex is known to work at low oxygen concentrations and may contribute to oxygen elimination from the bacterial environment (Rezaiki et al. 2004). Third, the role of the F_0F_1 ATPase to recover extruded protons for ATP production is not yet proven, although recent data suggest that this may occur (Koebmann et al. 2008). Finally, unlike *E. coli* and *B. subtilis*, which use the Krebs cycle to produce NADH, LAB require a sugar carbon source and glycolytic activity to generate NADH. Thus, in some way, fermentation metabolism is likely needed prior to, or during, LAB respiration growth.

Given these restrictions, why would LAB maintain such a conditional respiration system? The answer may lie in the significant energy and survival gains observed when respiration is active (see below). Interestingly, as studied in *L. lactis* and *Streptococcus agalactiae*, respiration gene expression appears to be constitutive, with a slight increase late in growth (Duwat et al. 2001, Pedersen et al. 2008, Yamamoto et al. 2005). This means that respiration metabolism can be rapidly turned on when heme, or heme and menaquinones, becomes available in the local environment.

Major Impacts of Respiration on Lactic Acid Bacteria Behavior

Many LAB are genetically equipped for respiration growth (**Table 1**). As mainly studied in *L. lactis*, and confirmed in other LAB, the most striking effects of respiration growth are the increased bacterial cell count yield and long-term survival (**Figure 2**).

The basis for greater cell growth and survival are explained by the following physiological changes.

Respiration chain NADH oxidase. The respiration chain generates an NADH oxidase activity, which recycles NAD^+. The NADH:NAD^+ ratio has profound effects on activities of key metabolic enzymes that use NADH or NAD^+ as cofactors. In particular, lactate dehydrogenase, the key enzyme involved in lactic acid production, requires NADH as a cofactor. As such, it is in direct competition with the respiration chain NADH oxidase (**Figure 1**).

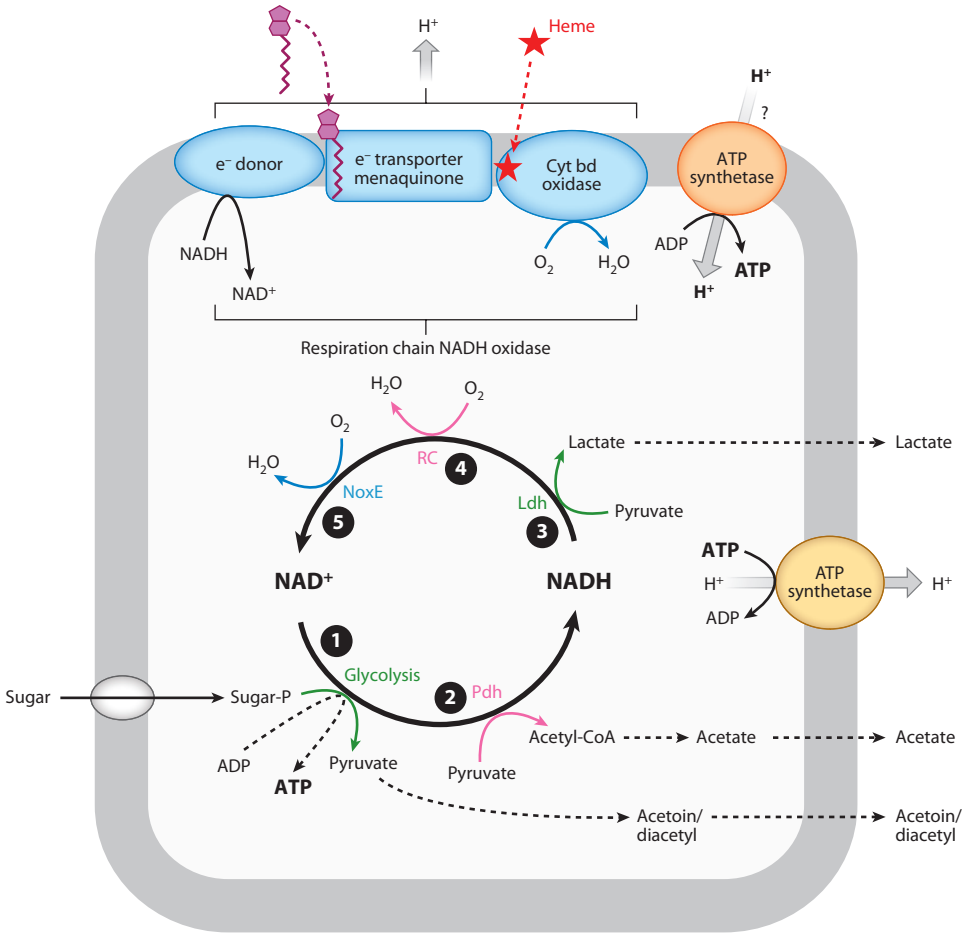

Figure 1

Central carbon metabolism and respiration in lactic acid bacteria (LAB). The membrane respiration chain (RC) comprises an electron donor [putatively encoded by *noxAnoxB* (Brooijmans et al. 2009a, Gaudu et al. 2002)], menaquinones (MKs) [encoded by *men* operon genes or provided exogenously (Yamamoto et al. 2005)], and a terminal electron acceptor [the cytochrome oxidase encoded by *cydAcydB* (Duwat et al. 2001, Lechardeur et al. 2011)]. Heme (*red star*) must be added exogenously (*red dashed arrow*) to activate cytochrome oxidase. In some LAB, MKs (*schematic blue molecule*) must also be added to activate respiration (*blue solid arrow*) (Yamamoto et al. 2005). RC activity results in H^+ expulsion. The ATPase might import H^+, which generates ATP, in addition to its known role during fermentation of expulsing H^+ (Blank et al. 2001, Koebmann et al. 2008). LAB lack a complete Krebs cycle. Thus, NADH, which is needed for the respiratory chain, is produced by carbon catabolism. Once phosphorylated, ① sugar is catabolized to pyruvate via glycolysis with production of ATP and NADH. ② Pyruvate dehydrogenase (Pdh) provides extra NADH from pyruvate when oxygen is present. Acetyl-CoA is converted further to acetate. ③ Lactate dehydrogenase (Ldh) oxidizes NADH into NAD^+ by conversion of pyruvate into lactate, thus maintaining glycolytic activity during fermentation. When oxygen is present, NADH is also oxidized by additional enzymes: ④ membrane NADH oxidase of the respiration chain or ⑤ by the cytoplasmic H_2O-forming NADH oxidase (NoxE), both of which out-compete Ldh activity for NADH. Pyruvate buildup leads to synthesis of acetate or the neutral acetoin and diacetyl.

When respiration is activated by heme addition, lactic acid production is diminished with a higher final pH. Instead, the yields of neutral metabolic byproducts acetoin and diacetyl are massively increased, e.g., as reported for *L. lactis* (Duwat et al. 2001, Kaneko et al. 1990). Production of these compounds might serve as a metabolic safety valve to redirect pyruvate. Interestingly, pyruvate conversion to acetoin accounts for about 20% of the glucose carbon used during respiration growth (Duwat et al. 2001).

Oxygen elimination. Respiration leads to the conversion of atmospheric oxygen, plus H^+, to water; in *E. coli*, for example, 70% of intracellular water is generated from respiration activity (Kreuzer-Martin et al. 2005); less O_2 in the cell, as determined in *L. lactis*, reduces the risk of oxidative damage (Rezaiki et al. 2004).

Table 1 Lactic acid bacteria classification of predicted or demonstrated respiration proficiency[a]

	Streptococcaceae	Lactobacillaceae
Respire with exogenous heme	*Enterococcus casseliflavus* ***Enterococcus faecalis*** *Enterococcus gallinarum* *Enterococcus italicus* *Eremococcus coleocola* ***Lactococcus lactis*** *Lactococcus garviae* *Leuconostoc argentinum* *Leuconostoc citreum* *Leuconostoc fallax* *Leuconostoc gasicomitatum* *Leuconostoc kimchii* ***Leuconostoc mesenteroides*** *Weissella cibaria* *Weissella paramesenteroides*	
Respire with exogenous heme + menaquinone	*Oenococcus oenii* ***Streptococcus agalactiae*** *Streptococcus dysgalactiae* *Streptococcus parauberis* *Streptococcus pseudoporcinus* *Streptococcus uberis*	*Lactobacillus antri* *Lactobacillus brevis* *Lactobacillus buchneri* *Lactobacillus casei* *Lactobacillus coryniformis* *Lactobacillus crispatus* *Lactobacillus fermentum* *Lactobacillus gasseri* *Lactobacillus hilgardii* *Lactobacillus johnsonnii* *Lactobacillus oris* *Lactobacillus paracasei* ***Lactobacillus plantarum*** *Lactobacillus reuteri* *Lactobacillus rhamnosus* *Lactobacillus salivarius* *Lactobacillus ultunensis* *Lactobacillus vaginalis*

(*Continued*)

Table 1 (*Continued*)

	Streptococcaceae	Lactobacillaceae
Never respire[b]	*Enterococcus faecium* *Streptococcus equi* *Streptococcus gallolyticus* *Streptococcus gordonii* *Streptococcus infantis* *Streptococcus mitis* *Streptococcus mutans* *Streptococcus parasanguinis* *Streptococcus pneumoniae* *Streptococcus pyogenes* *Streptococcus sanguinis* *Streptococcus suis* *Streptococcus thermophilus*	*Lactobacillus acidophilus* *Lactobacillus delbrueckii* *Lactobacillus iners* *Lactobacillus sakei*

[a]Lactic acid bacteria (LAB) are classified for respiration proficiency using as criteria presence or absence of *cydABCD* genes. No LAB as known to date are genetically equipped for heme synthesis, although some encode an incomplete heme biosynthesis pathway. Only some LAB synthesize menaquinones. Here LAB are classified according to their capacity to respire aerobically in the presence of exogenous heme, or heme and menaquinone. [b]Nonrespiring LAB lack *cyd* genes. Closely related species are not necessarily in the same category with respect to respiration potential or required cofactors. Species in bold were tested experimentally, whereas the others carry the *cyd* genes. Species in each category are used in food fermentations.

Menaquinone redox activity. In the absence of heme, electrons released by menaquinones facilitate reduction of Cu^{2+} to Cu^{1+}, which can enter the cell and lead to reactive oxygen species (ROS) formation, and to O_2 reduction to O_2^- (superoxide, a ROS) (Rezaiki et al. 2008). By using the electrons released by menaquinones for respiration metabolism, the risk of radical formation is decreased.

Energy gain. Respiration may be an efficient means of generating, or at least saving, energy by avoiding the need to extrude protons via the F_0F_1 ATPase, which is energetically costly (Blank et al. 2001). Respiration chain activity extrudes protons without additional energy cost.

ESSENTIAL AND ACCESSORY GENETIC REQUIREMENTS FOR LACTIC ACID BACTERIA RESPIRATION

Determining the genetic basis for LAB respiration is facilitated by studies in other bacteria. However, accessory genes seem to be less conserved and are being identified one by one. Those characterized to date are involved in heme transport or metabolism.

Essential Respiration Components

Data thus far indicate that *L. lactis* encodes a nonflexible set of respiration chain functions. The membrane NADH dehydrogenase(s) likely encoded by *noxB* and *noxA* is the electron donor (Brooijmans et al. 2009a, Gaudu et al. 2002), the menaquinones synthesized by the *men* genes promote electron transfer (Rezaiki et al. 2008, Tachon et al. 2009), and the cytochrome bd oxidase, encoded by *cydA* and *cydB*, is the final acceptor, which in turn donates the electron for oxygen reduction (**Figure 1**). To our knowledge, cytochrome bd oxidase is the sole terminal oxidase used for LAB aerobic respiration. *Lactobacillus plantarum* is also equipped for anaerobic respiration,

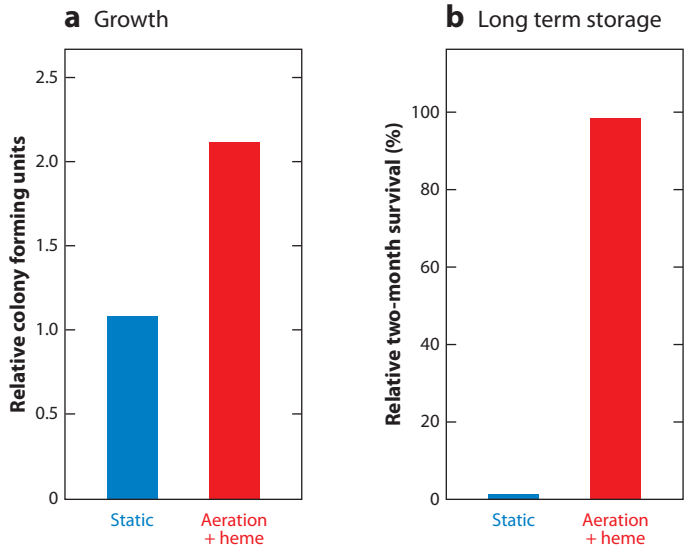

Figure 2

Lactococcus lactis is more robust when grown aerobically with heme. Wild-type lactococci are grown to saturation in rich medium in conventional (static; *blue bar*) or aeration plus heme (aeration + heme; *red bar*) conditions in a rich medium (M17 plus 1% glucose, or industrial medium containing lactose). (*a*) Colony forming units (CFUs) under the respiration conditions are essentially double that under conventional static growth. (*b*) If cultures are stored at 4°C over a two-month period, survival is at or near 100%. In contrast, survival of conventionally grown cells typically drops by several logs (often $> 10^5$-fold). Such results are typical of dozens of experiments (B. Cesselin, P. Gaudu, A. Gruss, unpublished data; Duwat et al. 2001). Respiration metabolism and, where tested, similar effects on growth and survival were obtained with other LAB, including *Enterococcus faecalis*, *Streptococcus agalactiae*, *Lactobacillus plantarum*, and *Leuconostoc mesenteroides* (Brooijmans et al. 2009b; Huycke et al. 2001; Yamamoto et al. 2005; C. Foucaud & A. Gruss, unpublished results).

which also requires heme, using nitrate reductase (Nrd) as the terminal enzyme to deliver the electron to nitrate rather than to O_2 (Brooijmans et al. 2009b). Similarly, *Enterococcus faecalis* can use fumarate as an electron acceptor for an anaerobic respiration (Huycke et al. 2001).

L. lactis, *E. faecalis*, and *Leuconostoc mesenteroides* all carry the complete set of menaquinone biosynthesis genes and have all been confirmed to undergo respiration in the presence of heme (Duwat et al. 2001, Huycke et al. 2001, Winstedt et al. 2000; C. Foucaud & A. Gruss, unpublished data). This condition is not enough to activate respiration in certain LAB, which additionally require menaquinones. Being lipophilic, exogenous menaquinones are readily assimilated into membranes and thus can be provided by the environment. The formal demonstration that respiration can be activated by adding both heme and menaquinones was first made in *S. agalactiae*, and predicted to be applicable to numerous lactobacilli, as later confirmed in *L. plantarum* (Brooijmans et al. 2009b, Yamamoto et al. 2005). Thus, more generally, based on current data, LAB can be classified into three categories: (*a*) respiration activated by heme addition, (*b*) respiration activated by heme and exogenous menaquinones, and (*c*) nonproficient for respiration (**Table 1**).

Accessory Respiration Components

As a bioactive molecule, heme may be involved in jobs other than respiration. For example, certain LAB, including those lacking respiration capacity, encode a heme-dependent catalase,

thus justifying a heme requirement (e.g., respiring bacteria, such as *E. faecalis*, *Lactobacillus brevis* and *L. plantarum*, and nonrespiring bacteria, such as *Lactobacillus. sakei*) (Frankenberg et al. 2002, Wolf et al. 1991). *L. sakei* may encode a cytochrome P450 homolog, possibly involved in lipid oxidation and turnover (Chaillou et al. 2005). However, in *L. lactis*, the only known use for heme is in respiration, and heme-binding proteins identified to date seem needed solely to coordinate this basic function (although future studies may disprove this observation). The different functions involved in heme metabolism are summarized below.

Heme uptake. Respiration in LAB would presumably comprise machinery to deal with heme trafficking and insertion into the cytochrome bd oxidase. Given that no LAB synthesize heme, a heme uptake system is required. In *L. lactis*, this may involve the *fhuDBAR* operon (Gaudu et al. 2003; P. Gaudu, E. Van West, A. Gruss, unpublished data). However, despite the existence of *fhu* homologs in different bacteria, it appears that heme uptake mechanisms are not sufficiently conserved to make functional predictions.

Heme-binding proteins. Insertion of heme into membrane cytochrome oxidases may involve chaperone proteins. The identities of these functions are mainly unknown in LAB. One candidate heme chaperone is AhpC, the peroxiredoxin reductase needed for oxygen stress response, which was proposed to be a heme chaperone that protects intracellular heme from degradation (Lechardeur et al. 2010). Another is the CydCD complex, which is implicated in cytochrome oxidase (CydAB) assembly. CydCD is thought to contribute to a reducing environment by transporting cysteine and glutathione, which might facilitate CydAB-heme interactions (Cruz-Ramos et al. 2004, Pittman et al. 2002, Pittman et al. 2005).

Management of heme pools. Although heme is essential for oxygen sensing and numerous enzymatic reactions (see above), it is a source of intracellular toxicity in its free form (Kumar & Bandyopadhyay 2005). Thus, efficient systems for heme homeostasis are expectedly required to avoid the toxic effects of heme.

Heme efflux. *L. lactis* seems to lack the enzymatic equipment to degrade heme for iron recovery (but see below). However, it encodes a dedicated heme efflux system (YgfCBA, now called HrtRBA) (Pedersen et al. 2008; D. Lechardeur, B. Cesselin, U. Liebl, M.H. Vos, A. Fernandez, C. Brun, A.Gruss, P. Gaudu, submitted) that effluxes excess heme to avoid its accumulation. Another system, discovered and named Pef in *S. agalactiae* and comprising two putative efflux pumps, also effluxes heme, but is less stringent than HrtRBA, as it also effluxes protoporphyrin IX, the iron-free porphyrin (Fernandez et al. 2010). It is notable that among the numerous LAB encoding HrtRBA and/or Pef homologs, some do not have respiration potential, raising the question of whether LAB assimilate and use heme for purposes other than respiration.

Heme degradation. Another means of controlling intracellular heme is by degrading it. In addition to diminishing heme pools, degradation is considered as a means of iron capture, as shown for bacterial pathogens (Hammer & Skaar 2011, Wandersman & Delepelaire 2004). Heme oxygenases, which degrade heme for iron capture, are present in opportunist pathogens but not in LAB. Interestingly, a heme-degradation deferrochelatase, which is involved in removing iron from the protoporphyrin shell, but leaves it intact, was recently discovered in *E. coli* as encoded by two genes, *efeB* and *yfeX* (Letoffe et al. 2009). Homologs of YfeX, which is cytoplasmic, are absent in *L. lactis*, but present in leuconostoc and in several lactobacilli. We speculate that heme-utilizing bacteria carrying *yfeX* may additionally make use of the liberated iron. Interestingly, there is no strict correlation between the presence (or absence) of *yfeX* and respiration capacity. Finally, heme

degradation may occur when cells are in the presence of reducing agents and oxygen. For example, glutathione or hydrogen peroxide might catalyze hydrolysis of the protoporphyrin ring to liberate iron (Atamna & Ginsburg 1995, Lechardeur et al. 2010).

Other genes involved in LAB respiration metabolism have thus far escaped identification. Surprisingly, random mutagenesis screening for respiration-defective bacteria did not turn up functions other than those directly involved in biosynthesis of the respiration chain components (L. Rezaiki, P. Gaudu, A. Gruss, unpublished results; Brooijmans et al. 2009a). Possibly, biochemical approaches, which do not rely on bacterial growth, may be better adapted for identifying novel functions.

Respiration may be considered as a highly regulated process, as it occurs only when heme is added to cells. Regulation is exerted at the level of heme homeostasis, i.e., levels must be strictly controlled in order to avoid toxicity, but to permit sufficient amounts of heme to mediate respiration growth. Inactivation of CcpA, regulator of carbon catabolite repression, had a slight positive effect on expression of *cydABCD* and a negative effect on predicted NADH dehydrogenase (*noxA*) (Zomer et al. 2007). However, *ccpA* deletion should also disturb metabolic pools and thus impact on the respiration capacity as a growth defect in the mutant was observed under this condition (Gaudu et al. 2003).

METABOLIC CHANGES DURING LACTIC ACID BACTERIA RESPIRATION

Respiration and Carbon Metabolism

Proteome and trancriptome analyses led to a remarkable observation: Despite the spectacular effects of *L. lactis* respiration on cell density and long-term survival (Duwat et al. 2001, Pedersen et al. 2008, Rezaiki et al. 2004), gene expression changes attributable to respiration are rather limited (Pedersen et al. 2008, Vido et al. 2004). By deduction, the major changes occurring via respiration are attributed to a shift in metabolite pools engendered by electron transport chain activity (**Figure 1**). Specifically, pools of NADH, a common electron donor in several enzymatic reactions, are depleted by respiration activity (Blank et al. 2001, Duwat et al. 2001, Pedersen et al. 2008). As a consequence, the NADH:NAD^+ ratio, a driving force in determining the dominant metabolic pathways, is decreased. For example, less NADH correlates with decreased lactate production under respiration conditions compared with fermentation (Duwat et al. 2001). Concomitant with lower lactate dehydrogenase activity (the enzyme requires NADH), pyruvate is rerouted to acetoin and acetate (Duwat et al. 2001, Pedersen et al. 2008). In both a *L. lactis* subsp. *cremoris* model strain MG1363 and a subspecies *lactis* industrial strain, CHCC2862, acetoin and acetate accumulation late in respiration growth also coincided with slightly increased NAD-dependent pyruvate dehydrogenase (Pdh complex) and acetolactate synthase (Als) activities (Vido et al. 2004; B. Cesselin, M.B. Pedersen, C. Garrigues, P. Gaudu, unpublished results). The knowledge that cofactors are responsible for the numerous metabolic changes in the cell has led researchers to consider the importance of metabolites, which cannot be detected by transcriptome and proteome analyses, in reprogramming cell activity.

Studies in *E. faecalis* and *L. plantarum* indicated that in addition to using O_2 (via a cytochrome bd oxidase), these bacteria may also use fumarate and nitrate, respectively, as electron acceptors. Such anaerobic respiration still requires heme as a cofactor (Brooijmans et al. 2009b, Huycke et al. 2001). In *L. plantarum*, NADH dehydrogenase inactivation abolished aerobic respiration but had no effect on anaerobic nitrate respiration, indicating that other electron donors are present in anaerobiosis. Thus, although the set of respiration enzymes in LAB seem limited, some LAB may

use respiration metabolism in different conditions to optimize growth according to environmental availability of substrates. As such, respiration may prevail when heme, and in some bacteria, heme and menaquinones, is available together with an electron acceptor (oxygen, nitrate, or fumarate).

Respiration and Nitrogen Metabolism

L. lactis is endowed with an extracellular protease, PrtP, and several cytoplasmic peptidases to degrade proteins and peptides in response to growth in poor medium (den Hengst et al. 2005, Guedon et al. 2005). In contrast, peptidases are much less produced in medium rich in amino acids, as assessed (via branched chain amino acids) by the regulator CodY (den Hengst et al. 2005, Guedon et al. 2005). Recent studies identified other regulators of nitrogen homeostasis, such as CcpA, the carbon catabolite control regulator of carbon metabolism (Zomer et al. 2007), and GlnR, which controls its own expression, ammonium/ammoniac uptake (via amtB-glnK), glutamate synthase (via glnA), glutamine transport (via glnP), and other functions (Larsen et al. 2006). Studies in respiration conditions suggest that regulation of nitrogen homeostasis in *L. lactis* is more complex than presently considered: (*a*) In the case of CodY, data suggest the existence of another regulator in respiration conditions. Indeed, the CodY-regulated pepO1 gene was upregulated under respiration conditions even in a CodY deletion mutant (Vido et al. 2004). (*b*) Concerning GlnR, low glutamine levels in spent respiration culture medium (Vido et al. 2004) might explain observed amtB-glnK induction via the GlnR pathway; however, expression of other genes of the GlnR regulon was not affected (Pedersen et al. 2008). Studies under respiration growth conditions may uncover additional mechanisms of nitrogen pool management in lactococci.

An intriguing observation is the high amount of proline detected in the medium during respiration growth (Vido et al. 2004). This might be connected to PepO1 expression, as this peptidase cleaves proline-rich peptides. Control of proline homeostasis might explain the expulsion; however, cells usually tolerate high proline concentrations in the cytosol (Takagi 2008), leaving in question the reason for its expulsion. Intracellularly, proline can stabilize proteins and prevent the loss of viability during stress conditions. An interesting consequence of proline extrusion may be its beneficial effects on proline-auxotroph bacteria in coculture (Deutch 2011, Li et al. 2010). Indeed, respiration alleviates some fermentation stress of respiration-negative bacteria by decreasing the acidity of the medium and scavenging oxygen (**Figure 3**) (Rezaiki et al. 2004). Proline extrusion during respiration growth may contribute to this effect and reveal a new positive use as a helper for bacterial survival.

Respiration and Menaquinones

Menaquinones are redox membrane-associated compounds with a naphthoic ring linked covalently to a hydrophopic isoprenoic chain that corresponds to a repeated isoprenoid unit. As a central respiratory chain component, menaquinones drive electrons from reducers (such as dehydrogenases) to terminal reductases (such as CydAB). Inactivation of naphthoic ring synthesis abolishes *L. lactis* respiration growth (unless exogenous menaquinone is supplied), thus confirming its nonredundant role (Brooijmans et al. 2009b, Huycke et al. 2001, Rezaiki et al. 2008). Curiously, *L. lactis* produces menaquinones with different size chain lengths: MK-3 and MK-9 are the most represented species (MK-3 designates three isoprenyl residues in the side chain, etc.) in contrast to *E. coli* (MK-8) or *B. subtilis* (MK-7), which reportedly produce a single major menaquinone species (Brooijmans et al. 2009b, Rezaiki et al. 2008). One can speculate that each menaquinone species might participate in specific functions, as their production was maintained even in the absence of oxygen. Besides CydAB, these molecules shuttle electrons to protein complexes like nitrate reductase and fumarate reductase for anaerobic respiration in *L. plantarum* and

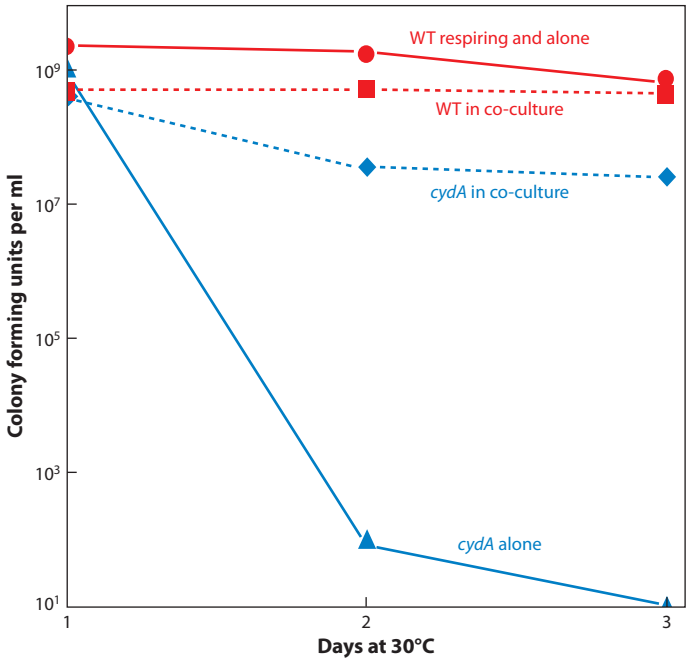

Figure 3

Respiring lactic acid bacteria (LAB) can improve the environment for nonrespiring LAB in coculture. In experimental conditions, differentially marked wild-type (WT) and *cydA* (nonrespiring mutant) *Lactococcus lactis* strains were grown separately or together in co-culture. The nonrespiring *cydA* strain grew less well, and survived poorly when maintained in aerobic medium with heme over a three-day period. In contrast, the respiring WT strain thrived. Remarkably, the *cydA* strain fared much better when grown in coculture with the WT strain, as determined by plating experiments. Adapted with permission from *Molecular Microbiology* (Rezaiki et al. 2004).

E. faecalis, respectively (Brooijmans et al. 2009b, Huycke et al. 2001) (note that *L. plantarum* uses exogenous menaquinones to perform this function). Moreover, they reduce extracellular compounds: oxygen, copper, iron, and redox-sensitive dyes (Rezaiki et al. 2008, Tachon et al. 2009). Interestingly, menaquinone reduction of copper was far more efficient than that of oxygen or iron in *L. lactis*. In addition, copper reportedly stimulated aerobic growth, as did heme (Kaneko et al. 1990, Kaneko et al. 1991); although pH was not increased as in respiration, it is tempting to describe this phenomenon as a respiration on copper. Moreover, menaquinone (particularly short chain moieties) produced by *L. lactis* can cross-feed other bacteria, such as *S. agalactiae*, to support its respiration (Rezaiki et al. 2008). Their remarkable reducing capacity and potential for diffusion into the environment may also be of interest in food production where natural vitamins have added value. As illustrated above, menaquinones can impact on the redox state and further on the development of secondary flora and sensorial quality in products like cheese (Tachon et al. 2009), and may also confer human/animal health benefits (see below).

In *L. lactis* MG1363, the total menaquinone pool was nearly constant and independent of the energetic growth mode (fermentation or respiration) when examined in M17 medium. However, the menaquinone chain length did vary according to the growth conditions: Short-chain menaquinones accumulated late in growth, and amounts may increase under low oxygen tension (Brooijmans et al. 2009a, Rezaiki et al. 2008). This phenomenon may be correlated with its various demonstrated reducing capacities, which may be implicated in bacterial fitness.

These observations underline the importance of menaquinones in cellular processes and suggest their applications in dairy technologies.

TAXONOMIC RELATIONSHIP BETWEEN RESPIRATION-COMPETENT LACTIC ACID BACTERIA: GAIN-LOSS OF GENES

Compared with bacterial species fully equipped for respiration metabolism, some LAB species maintain the capacity to respire at minimum genetic cost, i.e., without carrying the genetic and enzymatic baggage required for synthesizing all the respiration chain components. Heme, and in some cases menaquinone, must be obtained from the environment. This observation leads to the question of whether respiration metabolism in this rudimentary form was acquired by horizontal transfer or whether this capacity was lost in the rest of the LAB, converting them into strictly fermenting bacterial species.

A bioinformatics survey of the presence/absence of genes dealing with respiration metabolism in LAB was performed (**Figure 4**). Lactobacillale species are placed within a phylogenetic tree based on 16S sequences, using *B. subtilis* as an outgroup (Wu et al. 2009). At the root of Lactobacillales, all other Bacilli (e.g., *Listeria*, *Staphylococcus*, *B. subtilis*, and *Bacillus cereus*) have respiratory metabolism, which leads to the simple suggestion that respiration was progressively lost in both families of LAB. On these trees, genes were grouped into three functional categories: C for the *cydAB*-type cytochrome bd oxidase, the unique terminal acceptor used in LAB for aerobic respiration; M for the menaquinone biosynthetic pathway (menaquinones are the unique quinones produced by low GC% gram-positive bacteria); and H for the heme biosynthetic pathway, needed as cytochrome bd oxidase cofactor.

The simplest hypothesis leading to the present-day LAB respiration situation appears to be evolution via successive steps of functional losses (**Figure 4**): The most ancient is the loss of H genes, followed by sporadic loss of M genes in some branches, with only *E. faecalis*, *Leuconostoc*, and *L. lactis* of this study having conserved the entire pathway of M genes. Among species having lost H and M, there was then a sporadic loss of the C genes, leading to only two Streptococcaceae among six analyzed with a respiration potential (i.e., *S. agalactiae* and *L. lactis*) (Duwat et al. 2001, Yamamoto et al. 2005). Remarkably, a majority (11 out of 15) of lactobacilli maintained the C genes and, therefore, a respiration potential. In one exception, *S. agalactiae* seems to have reacquired the *cydAB* operon. An acquisition (i.e., horizontal transfer; **Figure 4**) would fit the phylogenetic relationships in these trees. This scenario has to be taken with caution, in view of difficulties encountered for building solid phylogenies of Lactobacillales (Makarova et al. 2006, Zhang et al. 2011). Nevertheless, a similar scenario built on the above-mentioned published trees would lead to the same overall conclusion of successive loss of functions, starting with H, then M, then C groups of genes. Large-scale comparative genomics of Lactobacillales has already led to the conclusion that this clade suffered massive gene losses and that its common ancestor was probably microaerophilic and living in rich environments, rendering certain metabolic pathways dispensable (Makarova et al. 2006). We propose that respiration potential is conserved in numerous LAB that inhabit heme-containing animal and plant environments. In those contexts, respiration is the preferred lifestyle of these LAB and might confer a strong fitness advantage.

If the *cydABCD* genes in the LAB species were progressively lost rather than acquired, phylogenetic trees built on the corresponding open reading frames would expectedly be superimposable with those built on housekeeping proteins, which is usually the case. A caveat concerns the acquisition of *cydAB* genes by *S. agalactiae*. Because they behaved as ancestral genes rather than horizontally acquired genes from a distant donor, their origins on the *S. agalactiae* genome remain to be investigated.

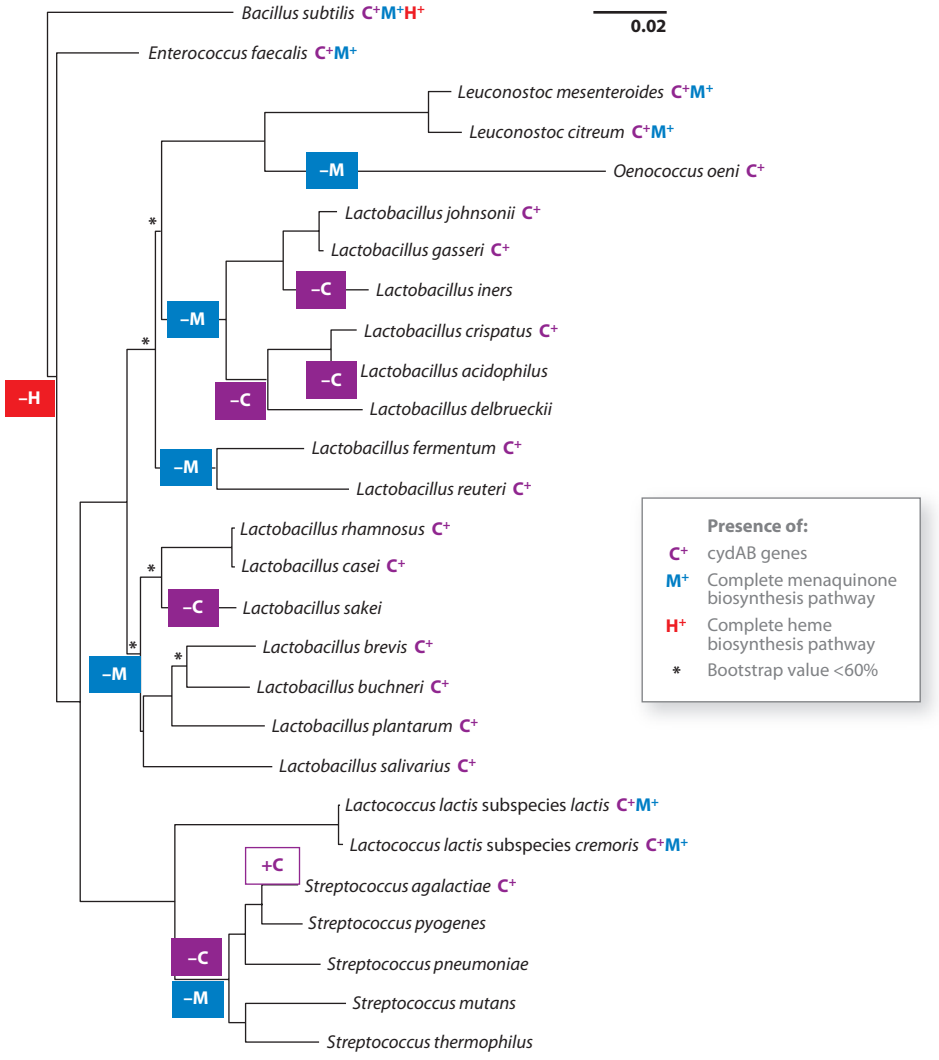

Figure 4

Gene gain-loss among lactic acid bacteria respiration genes. Phylogenetic trees built on 16S sequences were constructed directly on the Ribosomal Database interface (Cole et al. 2009). The KEGG compilation was used to determine these characteristics in each species. Eleven ancestral species (see Wu et al. 2009) belonging to the Bacilli class were used to ascertain the M+C+H+ character at the root of this clade: all were C+ and H+, and all but *Bacillus clausi* and *Bacillus halodurans* were M+. A scenario for the successive loss of H, M, and C functions is shown on the branches of both trees (*colored squares*), the putative addition of *cyd* genes in *Streptococcus agalactiae* is indicated by a white +C square. Scale bar represents the number of substitutions per nucleotide.

To test further the progressive loss scenario, a comparison of gene synteny, i.e., conservation of gene organization, can be made. Among the most closely related Lactobacillaceae, *Lactobacillus johnsonii* and *Lactobacillus gasseri*, the genes encoding cytochrome bd oxidase are in a chromosomal region exhibiting good synteny. Both *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* have lost the entire operon, together with 7 and 23 additional genes, respectively, relative to *L. johnsonii*.

In both species, one endpoint of the deletion is the same, arguing for an ancestral loss event. Interestingly, in both cases, the deletion is accompanied by an insertion of several other genes, in place of the deleted portion (3 and 19 genes, respectively, none of which are related to mobile genetic elements). Among the Streptococcaceae, a simple insertion or deletion involving the *cyd* operon and three additional genes is visible when comparing *S. agalactiae* with its closest neighbor, *S. pyogenes*. However, with more distant relatives, synteny of the region is lost, making the directionality of the event, i.e., deletion versus insertion, impossible to predict. These analyses of the LAB respiration components are consistent with the more general suggestion that LAB have undergone major losses, and possibly, minor gains via horizontal gene transfer during their evolution (Makarova et al. 2006).

The phylogenetic scenario is consistent with experimental observations showing that *cyd*+ bacteria manage to respire by acquiring the missing components heme and in some LAB, menaquinones, which is consistent with the hypothesis that the *cyd* genes are lost last. This could make sense if (*a*) menaquinones are needed uniquely for energy consumption or if (*b*) their presence is disadvantageous when bacteria do not respire. In favor of the second possibility, results showed that in nonrespiring conditions, menaquinones mediate reduction of copper, and to some extent oxygen and iron, such that wild-type *L. lactis* is more copper-sensitive than a *menB* mutant (Rezaiki et al. 2008). In addition, a search in bacterial databanks indicated that a complete menaquinone biosynthesis pathway is present only in bacteria that are respiration proficient. This suggests that the primitive respiration capacity of LAB is somehow a stable state (i.e., the *cyd* genes are maintained longer than the other genes of the respiration chain). The altered metabolism of respiration-proficient LAB may allow them to evolve differently in environments containing heme, or heme and menaquinone.

APPLICATIONS OF RESPIRATION IN FOOD TECHNOLOGY AND IN VIVO APPLICATIONS

Practical and commercial applications of LAB respiration growth are still being developed and are likely to grow with better understanding of its potential. Increasing demands for probiotics and other health benefits open new opportunities for applying respiration growth to technology and health issues. These prospects are discussed below and summarized in **Table 2**. This section deals with real R&D issues encountered during the different stages of development of a new technology in the food industry.

Table 2 Potential and real applications for lactic acid bacteria (LAB) respiration

Field of application	Benefits/impacts of respiration[a]
Starter culture	Higher cell count
	Prolonged survival
Food products	Greater acetoin/diacetyl production for aroma
Biotechnology	Less stressful conditions for protein production
	Heme-inducible reporter and expression system
Health, diet	Vitamin K_2 (menaquinone) production by some LAB
	Lower heme toxicity in gut due to heme utilization
	More robust and long-lasting probiotic cultures
Plant biology	Acetoin is a plant-signaling molecule that stimulates growth

[a]Most of the indicated applications are speculations of the authors. See text for discussion of each suggested benefit of LAB respiration.

Table 3 Uncertainty when commercializing inventions: focus on respiration

Process Step	Uncertainty
Technical	■ Long way from proof of principle in lab scale (low cell density) to industrial scale (high cell density). Many parameters/variables differ, e.g., media and oxygen transfer.
	■ Physiological state of respiration-grown cells is dramatically different than cells from conventionally grown cultures. Possible changes in performance or product stability, including the ratio between different strains in mixed cultures.
	■ Validation of the new culture product must be performed in large-scale dairy fermentations, e.g., including maturation of cheeses made.
Market	■ Need to assure customer acceptance of new products, which should have the same properties.
	■ New raw materials may be required. Must comply with legislation and religious constraints, especially when producing cultures for global distribution.
Resources	■ Building new factories or retrofitting existing ones to allow sparging with oxygen is costly and may cause delays.
	■ Total investment required, i.e. both personnel and equipment/factories, to change to a radically new production method.
Organization	■ Resistance to radically new technologies, which break with the dogma of lactic acid bacteria being produced under anaerobic conditions.
	■ Clarification of the responsibility of the project, both for the initial proof of principle experiments, pilot plant validation, and industrial implementation.
	■ Legal issues, such as setting up agreements and patent filing.

Industrialization of Respiration Technology to Lactic Acid Bacteria Starter Cultures

Major scientific inventions are made in universities, research institutions, and companies around the world. Although there is great interest in commercializing these inventions, success is the exception rather than the rule. Success means innovation, i.e., an invention plus commercialization, such as in the case of implementation of the respiration technology for *L. lactis*.

When commercializing inventions, the bottlenecks can generally be split into four groups, i.e., technical, market, resources, and organization (Leifer et al. 2000) and are summarized in **Table 3**. Selected points are discussed in more detail in the sections that follow.

In all disciplines, the way from invention to actual commercialization can be painfully long. Considering the numerous obstacles and costs of development, radical inventions must have a high potential to actually succeed. In the case of respiration technology, the benefits were potentially great and justified the time (several years) and investment costs before the technology could be successfully implemented. As with most radically new technologies, the first implementation is often imperfect. The respiration technology was further improved by combining in-depth characterization of LAB physiology in respiration with 'omic technologies.

Understanding the physiology of respiration. The essential aspects of the respiration technology were investigated in the late 1990s and implemented in the early 2000s at Chr. Hansen A/S

(Pedersen et al. 2005). This was done through several projects and required numerous man-years of work. The primary focus was on *L. lactis*, which was the species used for the initial proof of principle (Duwat et al. 2001, Duwat et al. 1998). Knowing that respiration technology would dramatically change bacterial physiology compared with conventional anaerobic fermentations, as discussed above, numerous investigative experiments were carried out to ensure that respiration starter cultures performed as expected when inoculated in milk or other foods for fermentation (Pedersen et al. 2005). Just after the respiration findings, one LAB genome sequence became publicly available: *L. lactis* subsp. *lactis* IL1403 (Bolotin et al. 2001). The transcriptome platform became the first whole genome platform used to investigate LAB respiration versus anaerobic growth (Pedersen et al. 2008, Pedersen et al. 2005).

From an industrial point of view, bacterial yield improvement for starter cultures is an important focus. The initial invention of respiration growth for starter culture preparation led to approximately a doubling in the yield on the industrial scale (Kringelum et al. 2005). The yield increase was pushed further by combining physiological studies and transcriptome results.

Further improvements of a novel technology. The initial microarray analysis on respiration was done comparing one condition with another, e.g., respiration against anaerobic fermentation, as above. For optimization of fermentations, it is, however, common to carry out time-course microarray analysis. For such analysis, multiple microarrays are produced at selected time-points during fermentation, with a common sample as reference.

With a time-course microarray series, the complexity of the analysis increases dramatically, as you have to consider observed changes in gene expression to essentially all other time-points. In this case, the analysis furthermore had to be put in the context of the novel respiration technology.

Initial time-course analyses were made with relatively few arrays and because of the choice of sample points, did not reveal any clear way of increasing biomass yield. In a later experimental setup, we analyzed a complete fermentation, with ten samples points, from early exponential growth at around OD_{600} 2 to stationary phase at around OD 47 (**Figure 5**).

The full dataset from the respiration time course gives a complex picture. A means of extracting useful information is by looking at individual genes and pathways, or optionally by using various bioinformatics clustering tools. Many genes remained largely unregulated throughout respiration growth, e.g., glycolytic genes converting lactose into lactic acid, or genes involved in nucleotide interconversion (genes coding for enzymes interconverting nucleotide bases, nucleosides, and nucleotides). One result stood out: Genes involved in purine de novo synthesis, with IMP as the product, were induced up to 100-fold at OD 10–15, whereas no signal was detected before this point (see **Figure 5**). This led us to hypothesize that cells were starved for purines early on in growth (Kringelum et al. 2005).

Inosine and IMP are purine sources available at relatively low cost. When we ran new respiration cultures with these compounds added, we surprisingly found that the already increased yield of the respiratory culture was almost doubled from OD 45 to 76 and actually giving exactly double the amount of product per fermentor volume (Kringelum et al. 2005).

To validate the basis for these observations, we investigated respiration cultures to which purine sources were added. Indeed, in these new conditions, purine de novo synthesis genes were no longer induced, showing that the cells were no longer starved for purines (M.B. Pedersen, unpublished data).

Remarkably, purine sources had no positive effects when added to an anaerobic fermentation *L. lactis* culture, nor when added to the respiration-negative *Streptococcus thermophilus* (Kringelum et al. 2005). The yield increase is thus uniquely related to growth under respiration. This effect remains to be characterized.

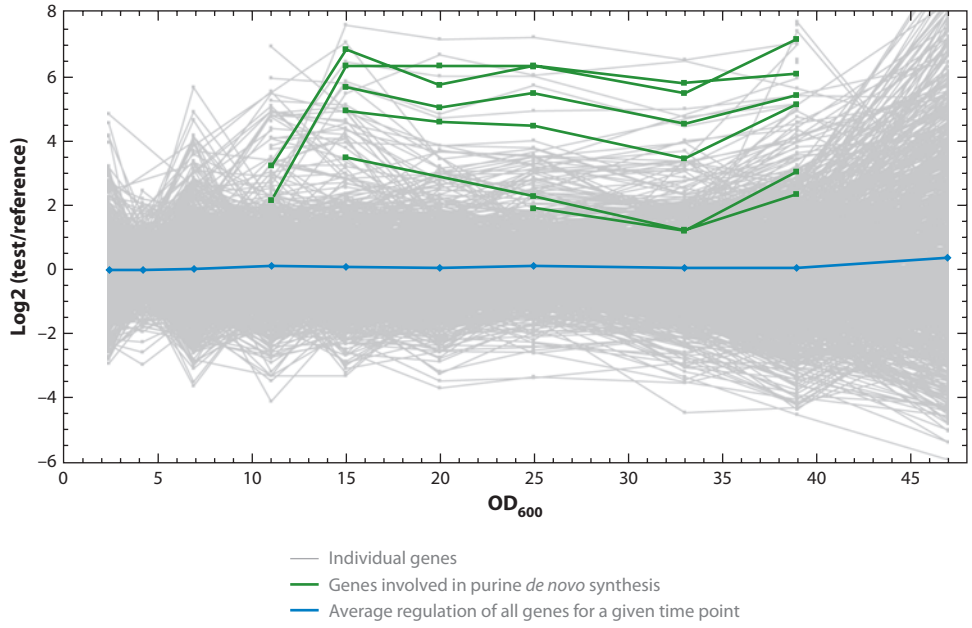

Figure 5

Microarray time-course analysis of respiration fermentation. The strain *Lactococcus lactis* subsp. *lactis* CHCC2862 was grown in industrial production medium under respiratory conditions. Ten microarrays were produced from OD_{600} from 2 to 47 as indicated on the x-axis. $OD_{600} = 4$ was chosen as the reference for each sample point. The y-axis indicates the $\log_2$ regulatory value of the "Test" (Cy5 label) divided by "Reference" (Cy3 label), both minus the background. The y-axis scale is from −6 (64-fold downregulation) to +8 (256-fold upregulation). The arrays were produced and analyzed as described (Pedersen et al. 2008), except that each dataset was Lowess-normalized (i.e., standard parameters). The value of the blue line is essentially 0 because of normalization.

Another promising means to further improve the yield of respiration starters was through using a more slowly catabolized sugar source in the fermentation (Koebmann et al. 2008). When changing from lactose, a sugar that is catabolized relatively fast by industrial strains, to maltose, which is more slowly catabolized, there was indeed a positive effect on yield in industrial scale fermentations. However, the resulting acidification activity of the starter cultures subsequently inoculated in milk was highly reduced, rendering the concept not industrially applicable for starter production (H. Møllgaard & M.B. Pedersen, unpublished results). Microarray analysis revealed that the gene expression pattern of maltose grown cells resembled that of cells in the early stationary phase. Given that cells in stationary phase are less metabolically active than growing cells, one hypothesis is that this is the cause of the lower activity (M.B. Pedersen, unpublished results).

The development of respiration technology stimulated new applications and ideas, some of which relate to more general process optimization. A direct application is the expansion of respiration starter cultures to other LAB (**Table 1**). Respiration activity reduces both oxidative stress and acid stress, two important conditions to be alleviated in industrial processes (Duwat et al. 2001, Rezaiki et al. 2004). Respiration growth was found to improve survival of nonrespiring cocultured strains (Rezaiki et al. 2004). An exciting prospect is to prepare strains together to confer benefits to the dependent species (**Figure 3**). Indeed, studies showed that survival of a *cydA* lactococcal mutant is much improved by coculture with a respiring wild-type strain in the presence of heme

and oxygen. This in *trans* advantage of respiration on nonrespiring bacteria may be of interest in starter culture and food applications for improving robustness of an otherwise fragile strain. Finally, investigations on process optimization have resulted in developments regarding improved cryoprotectants and increased acidification activity of products, which improved performance of respiration cultures (Kringelum et al. 2004). The research fusion between upstream questions and downstream development has proven fruitful in discovering and developing novel applications through to production.

Is implementation of respiration technology worth the investment? The switch of starter culture technology from conventional fermentation methods to respiration is a high-risk investment. The respiration technology became lucrative only when there were further improvements, when further research assured the generality of success, and when the technology could be extended to most *L. lactis* cultures. At that point, benefits began to outweigh development and capital costs and the greater complexity of the respiration technology. To accommodate the increased sales of cultures, a 40 million Euro factory was built by Chr. Hansen A/S in 2008 to handle respiratory fermentations most optimally, overall showing the expected longevity of the respiration technology.

Other Spin-Offs from Respiration

Food products. To date, respiration technology has not been applied to foods, although this is a tempting prospect. Diacetyl/acetoin are much-used flavoring compounds in foods, which are added to increase the aroma, e.g., of butter, cookies, and popcorn. Aerobic growth in heme was previously suggested as a means of obtaining good yields of these volatile compounds (Kaneko et al. 1990, Kaneko et al. 1991). An alternative strategy could be to prepare fermented foods in respiration conditions to obtain naturally aromatic food products. Such developments would have to be monitored to control for bacterial balance and for outgrowth of nonstarter LAB that may persist in pasteurized milk.

Biotechnology. Bacteria used for protein production are selected in part for their growth in relatively cheap and simple media and for the simplicity of protein purification. The choice of LAB for protein production has been limited by relatively low bacterial and protein yields in part because of acid accumulation in the medium. Despite this, LAB remain an attractive choice, as they are harmless and secrete relatively few proteins into the medium. To date, reported attempts of protein production in respiration conditions have not improved yields relative to conventional fermentation (Berlec et al. 2008, Mifune et al. 2009). Further development may be required to fine-tune conditions of expression and to develop the appropriate genetic tools.

An interesting spin-off of respiration studies was the identification of a tightly regulated and highly induced gene, *ygfC*. It was recently developed as an easily manageable heme-inducible system that could be used for regulated protein expression. This inducible system has potential applicability in bacteria that do not synthesize their own heme (Garrigues et al. 2011; Pedersen et al. 2008; D. Lechardeur, P. Gaudu, M.B. Pedersen, A. Gruss, in preparation).

Health. Validation of health claims for probiotics is currently a popular area of study with high commercial stakes. The proposed uses of LAB respiration metabolism presented below are suggestions of the authors and remain to be validated.

Foods rich in natural vitamins may give added health value to fermented products. Menaquinone-producing LAB, such as *L. lactis*, may constitute a source of vitamin K_2 in host

(Conly et al. 1994). The best known beneficial effect of vitamin K_2 is on bone health, but vitamin K_2 may also, unlike vitamin K_1, decrease the risk of cardiovascular disease (Geleijnse et al. 2004, Vermeer et al. 2004). Some work has been done to further improve the amount of vitamin K_2 produced by *L. lactis* (Garault et al. 2008, Pedersen 2009).

Heme, which is commonly present in the diet, may be a source of toxicity to the host, and may explain the increased risk of cancer associated with diets rich in red meat (Corpet 2011). Heme uptake and utilization by LAB might help reduce free heme in the gut.

Numerous LAB being currently developed as probiotics, particularly among the lactobacilli, are genetically equipped for respiration metabolism. Improved growth and stability upon respiration growth make this mode of preparation a clearly useful option for the preparation of probiotic cultures.

Plant health. In plants, volatile substances like acetoin are reportedly supplied by bacteria and act as plant growth promoters (Ryu et al. 2003). Remarkably, nodules, which are abundant in the roots of leguminous plants, produce high amounts of the heme-rich leghemoglobin (Appleby 1984), which is an excellent activator of *L. lactis* respiration (A. Gruss, unpublished), and as discussed, respiration metabolism reprograms LAB to produce acetoin and diacetyl. One can speculate that in a leguminous plant environment, lactococcal respiration is activated when bacteria are in contact with root nodules. Production of acetoin during lactococcal respiration would act as part of the signaling pathway, leading to faster plant growth. It is tempting to consider novel uses of lactococci as plant probiotics, which when sprayed on plants, would stimulate their growth. Although such positive effects remain to be tested, this proposal raises the broad potential of using findings from one field for totally novel applications.

CONCLUSION

Our aim in this chapter is to reveal how LAB, used for centuries in dairy, meat, and vegetable fermentations, live in the real world and profit from the natural resources to optimize their lifestyle. The cost of respiration is low because the LAB simply use environmental ready-to-wear rather than do-it-yourself products (i.e., heme and menaquinones) to equip themselves for more robust growth. The respiration capacity of numerous LAB opens the door for more efficient production of these industrial bacteria and for the development of novel uses in situations where good growth, prolonged survival, less oxygen, and a more neutral pH could constitute a benefit.

DISCLOSURE STATEMENT

M.B.P. is affiliated with the Chr. Hansen A/S company, which has been involved in respiration research since 2000 and has provided financial support for research in the INRA laboratory. We have taken care to report objective findings as issued from the laboratories of the authors and from the literature.

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