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Review

Roles of flavoprotein oxidase and the exogenous heme- and quinone-dependent respiratory chain in lactic acid bacteria

Yuji YAMAMOTO[1]

[1]*Laboratory of Cellular Microbiology, School of Veterinary Medicine, Kitasato University, 23-35-1 Higashi, Towada, Aomori 034-8628, Japan*

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Lactic acid bacteria (LAB) are a type of bacteria that convert carbohydrates into lactate through fermentation metabolism. While LAB mainly acquire energy through this anaerobic process, they also have oxygen-consuming systems, one of which is flavoprotein oxidase and the other is exogenous heme- or heme- and quinone-dependent respiratory metabolism. Over the past two decades, research has contributed to the understanding of the roles of these oxidase machineries, confirming their suspected roles and uncovering novel functions. This review presents the roles of these oxidase machineries, which are anticipated to be critical for the future applications of LAB in industry and comprehending the virulence of pathogenic streptococci.

Key words: lactic acid bacteria, *Lactobacillus, Streptococcus,* oxygen, NADH oxidase, respiration, electron transport chain

INTRODUCTION

Lactic acid bacteria (LAB) are a diverse group of microorganisms characterized by their ability to produce lactate as the primary metabolic end-product through carbohydrate fermentation. These bacteria are commonly found in various natural environments, including plants, fermented food products, and the gastrointestinal tracts of humans and animals [1–3]. LAB play significant roles in various industrial processes, including food fermentation, probiotic production, and lactate and other chemical production. The most fundamental role of LAB in fermented foods is the conversion of sugars to lactate through fermentation. This process not only preserves food by creating an acidic environment that inhibits the growth of spoilage and pathogenic microorganisms but also imparts a tangy or sour taste to the final product [3, 4]. LAB are also often used as probiotics, which are "live microorganisms that, when administered in adequate amounts, confer a beneficial health effect on the host" [5, 6]. Probiotics can enhance gut health, promote immune system function, and relieve digestive disorders [6–8]. LAB strains, such as *Lactobacillus, Lacticaseibacillus*, and *Limosilactobacillus*, are commonly used in probiotic formulations in dairy products, dietary supplements, and functional foods. Pathogenic streptococci are also included in LAB [2, 9]. The streptococci in this group have the capability to cause various infections and diseases in humans and animals. These bacteria possess virulence factors that enable them to colonize host tissues, evade immune defenses, and cause various illnesses ranging from mild to severe [10]. Although food microbiologists frequently consider only benign LAB utilized in food production and probiotics as LAB, pathogenic streptococci belong to the criteria of LAB. Notably, both groups of bacteria have been found to be closely related based on phylogenetic studies [1, 11].

LAB are a class of bacteria that are gram-positive, non-spore-forming, and catalase-negative, and they exhibit low GC content [1–3]. As mentioned above, LAB produce lactate as their main metabolite. This indicates that LAB obtain energy by anaerobic fermentation. Members of this class of bacteria lack the ability to synthesize heme, a cofactor of cytochrome oxidases, which are the main components of the respiratory chain [2, 3]. Accordingly, energy generation by LAB depends on anaerobic lactic acid fermentation. Because of these characteristics, LAB are often considered to be anaerobic bacteria unrelated to oxygen. However, unlike other anaerobic bacteria, most LAB can also grow under aerobic conditions and consume oxygen via unique flavoprotein oxidases. Numerous studies on LAB have shown that flavoprotein oxidases can greatly improve the metabolic capabilities of this class of bacteria by allowing them to utilize oxygen as an electron acceptor [9, 12]. Furthermore, some LAB are known to perform respiratory metabolism when heme alone or heme and quinone are provided to their culture medium. It has become evident that a certain number of LAB perform respiratory metabolism, exceeding the initial estimates [12–14]. Greater understanding of the role of these flavoprotein oxidases and their

Corresponding author. Yuji Yamamoto (E-mail: yyamamot@vmas.kitasato-u.ac.jp)

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respiratory machinery is expected to enhance the effective use of LAB in the food industry by improving culture efficiencies and also lead to an understanding of the virulence of pathogenic streptococci. The next section of this review explains the roles of nicotinamide adenine dinucleotide (reduced) (NADH) oxidase and other flavoprotein oxidases in LAB. In a subsequent section, exogenous heme- or heme- and quinone-dependent respiratory metabolism is described. Although several reviews have already covered the respiratory metabolism of LAB [12–14], this review provides an overview of its historical context and recent findings, along with its relationship with other recently discovered electron transport systems.

FLAVOPROTEIN OXIDASES IN LAB

Flavoproteins are a diverse group of proteins that bind and utilize flavin cofactors such as flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These cofactors play essential roles in flavoprotein function by participating in various enzymatic reactions and electron transfer processes within cells [15]. Although some flavoproteins are components of the respiratory chain, most flavoprotein oxidases are non-respiratory oxidases that are widely distributed across different LAB. These include NADH oxidase, pyruvate oxidase, α-glycerophosphate oxidase, L-amino acid oxidase, and lactate oxidase [9]. The reactions catalyzed by these oxidases are listed in Table 1. α-Glycerophosphate oxidase, L-amino acid oxidase, and lactate oxidase are involved in the catabolism of their respective substrate. On the other hand, NADH oxidase and pyruvate oxidase have been proposed to have multiple functions, including regulation of the cellular nicotinamide adenine dinucleotide (NAD^+)/NADH ratio, ATP production, catabolism of sugar alcohols, oxidative stress resistance, the development of competence, reduction of the redox potential, and virulence. The functions of each flavoprotein oxidase in LAB are described below.

NADH OXIDASE

NADH oxidase is an enzyme that reduces oxygen using NADH. Two types of NADH oxidases have been reported in LAB: One catalyzes the two-electron reduction of oxygen to form H_2O_2 (H_2O_2-forming NADH oxidase), and the other catalyzes the four-electron reduction of oxygen to form H_2O (H_2O-forming NADH oxidases) [9, 16] (Table 1). The main function of NADH oxidase is to regulate the NAD^+/NADH ratio in vivo. LAB supply the NAD^+ needed for the smooth operation of glycolysis, usually through the conversion of pyruvate to lactate by lactate dehydrogenase (LDH), while NADH oxidase, using oxygen as an electron acceptor, can supply NAD^+ without producing organic acids such as lactate (Fig. 1). Owing to the function of NADH oxidase, the production of lactate decreases and the production of acetate, diacetyl, and acetoin increases when LAB are cultured under aerobic conditions compared with anaerobic conditions [9, 12]. This conversion of homolactic acid fermentation to mixed-acid fermentation by NADH oxidase is advantageous in terms of energy production. Acetic acid is synthesized via the catalytic action of several enzymes, including acetate kinase (Fig. 1), which produces ATP via substrate-level phosphorylation. This pathway is thought to contribute to the improved growth of many LAB under aerobic conditions [12, 17–22]. The use of oxygen as an electron acceptor by NADH oxidase allows the utilization of sugar alcohols, as these sugars contain more hydrogen atoms than glucose and other sugars. Research conducted on deficient strains of *Streptococcus mutans* indicated that the catabolism of sugar alcohols requires NADH oxidase [19].

NADH oxidase plays an important role in resistance to oxidative stress. Oxygen is a source of highly cytotoxic reactive oxygen species (ROS), including superoxide, hydrogen peroxide (H_2O_2), and hydroxyl radicals, which have deleterious effects on cellular components such as DNA, RNA, membranes, and proteins [2, 9]. Although less toxic than ROS, oxygen selectively inactivates certain cellular enzymes. For example, pyruvate formate-lyase, an important enzyme in the anaerobic synthesis of acetyl-CoA and formate in many bacteria, is highly sensitive to oxygen [23]. Additionally, pyruvate-ferredoxin oxidoreductase, which plays a crucial role in the energy metabolism of obligate anaerobes, is also easily inactivated by oxygen [24]. While NADH oxidase cannot directly scavenge ROS, it is thought to limit their harmful effects by removing oxygen from the immediate environment [2, 9]. Several H_2O-forming NADH oxidase-defective mutant strains are sensitive to oxygen, H_2O_2, and other ROS [17, 18, 20, 21, 25, 26]. Conversely, H_2O_2-forming NADH oxidase has been reported to be a source of H_2O_2 in LAB [27, 28]. However, the molecular mechanism underlying H_2O_2-forming NADH oxidase remains unclear. *S. mutans* Nox-1 is an H_2O_2-forming NADH oxidase for which the protein and gene were first identified in LAB; however, subsequent studies have shown that Nox-1 functions as an alkyl hydroperoxide reductase by acting with the AhpC subunit, which degrades H_2O_2 [29–31]. Nox-1 reportedly contributes to the degradation rather than the production of H_2O_2 in *S. mutans* [19]. On the other hand, the flavin reductase of *Lactobacillus johnsonii* has been identified as the enzyme that catalyzes NADH-dependent H_2O_2 production from oxygen in the presence of flavin. The construction of a strain deficient in flavin reductase revealed that this enzyme is the main source of H_2O_2 in the bacterium [32]. The flavin reductase gene appears to be

Table 1. Flavoprotein oxidases in lactic acid bacteria (LAB)

Flavoprotein oxidases	Reactions catalyzed
NADH oxidase	$NADH + H^+ + O_2 \rightarrow NAD^+ + H_2O_2$ (H_2O_2-forming oxidase) $2NADH + 2H^+ + O_2 \rightarrow 2NAD^+ + 2H_2O$ (H_2O -forming oxidase)
Pyruvate oxidase	pyruvate + O_2 + phosphate → acetylphosphate + CO_2 + H_2O_2
Lactate oxidase	lactate + O_2 → pyruvate + H_2O_2
α-Glycerophosphate oxidase	α−glycerophosphate + O_2 → dihydroxyacetone phosphate + H_2O_2
L-Amino acid oxidase	L-amino acid + H_2O + O_2 → 2-oxo acid + NH_3 + H_2O_2

NADH: nicotinamide adenine dinucleotide (reduced); NAD^+: nicotinamide adenine dinucleotide.

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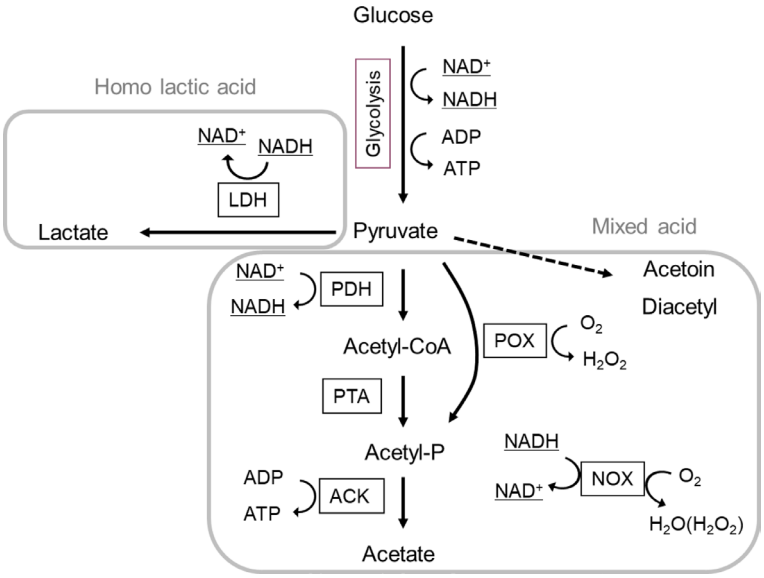

Fig. 1. Typical aerobic metabolic pathway for lactic acid bacteria (LAB) with homo-lactic acid fermentation.

Arrows indicate the flow of each metabolite. Enzymes involved in metabolic pathways: LDH: lactate dehydrogenase; PDH: pyruvate dehydrogenase; PTA: phosphotransacetylase; ACK: acetate kinase; POX: pyruvate oxidase; NOX: NADH oxidase; NADH: nicotinamide adenine dinucleotide (reduced); NAD^+: nicotinamide adenine dinucleotide.

broadly conserved within the acidophilus group of LAB, implying that it is the principal enzyme that exhibits H_2O_2-forming NADH oxidase activity and produces H_2O_2 in this group. Furthermore, enzymes that catalyze other reductive reactions have been reported to exhibit H_2O_2-forming NADH oxidase activity and serve as sources of H_2O_2 in other bacteria, such as bifidobacteria [33]. Therefore, other enzymes that catalyze reductive reactions in LAB may also exhibit H_2O_2-forming NADH oxidase activity and contribute to H_2O_2 production.

NADH oxidase affects fatty acid synthesis and membrane fatty acid composition. A strain of *Streptococcus agalactiae* lacking H_2O-forming NADH oxidase has been reported to have a lower fatty acid content and a lower ratio of unsaturated fatty acids to saturated fatty acids compared with the wild-type strain. The deficient strain requires the addition of unsaturated fatty acids or a source of unsaturated fatty acids (such as Tween 80) to the medium for aerobic growth [21]. Notable changes in the cell membrane unsaturated fatty acid content have been reported in *S. mutans* strains lacking H_2O-forming NADH oxidase [34]. Additionally, the cell membrane fatty acid composition in a *Streptococcus sanguinis* strain deficient in H_2O-forming NADH oxidase has been reported to exhibit a decrease in stearic acid and cis-vaccenic acid as well as an increase in palmitic acid compared with the wild type strain [35]. It is assumed that the loss of NADH oxidase in these streptococci affects the intracellular NAD^+/NADH ratio and alters fatty acid metabolism.

In *Streptococcus pneumoniae,* it has been reported that H_2O-forming NADH oxidase is necessary for competence development [36, 37]. The development of competence in *S. pneumoniae* is influenced by oxygen, and H_2O-forming NADH oxidase regulates transformability by activating ComE, which is a two-component regulator of competence development through post-translational modifications [38]. This suggests that the induction of competence using oxygen as a signaling molecule may be mediated by H_2O-forming NADH oxidase. In *S. pneumoniae*, H_2O-forming NADH oxidase has also been reported to localize to the cell surface and serve as an adhesion factor [39].

The NADH oxidases of *Lactococcus lactis* and *Streptococcus thermophilus* have been reported to be necessary for rapid fermentation of milk. Studies using H_2O-forming NADH oxidase-deficient strains in both bacteria have shown that this oxidase is the primary oxygen-consuming enzyme in those bacteria. A deficiency in this enzyme has been found to cause a delayed rate of dissolved oxygen consumption and fermentation of milk [40, 41]. Additionally, the H_2O-forming NADH oxidase of *S. thermophilus* is required to produce formate, an important nutrient for bacterial growth in milk. While formate has been known to be an important symbiotic factor provided by *S. thermophilus* to its partner, *Lactobacillus delbrueckii* subsp. *bulgaricus*, recent research has revealed that it also promotes the growth of *S. thermophilus* in milk [42]. It has been reported that the efficient production of formate by *S. thermophilus* relies on the presence of its NADH oxidase [41]. *S. thermophilus* pyruvate formate-lyase, the enzyme responsible for formate production, is highly sensitive to oxygen and is rapidly inactivated by it [23, 43]. H_2O-forming NADH oxidase is thought to protect pyruvate formate-lyase by removing oxygen from milk [41]. However, recent research has indicated that mere removal of oxygen is insufficient and that reduction of the redox potential is required to produce formate via pyruvate formate-lyase [42]. In *L. lactis,* though the relationship to formate production is not known, H_2O-forming NADH oxidase has also been reported to participate in the reduction of redox potential in milk. However, *L. lactis* H_2O-forming NADH oxidase primarily removes oxygen. Quinones and NADH dehydrogenase, which are components of the respiratory chain, reduce the redox potential [40]. The function of these enzymes as reductases in the absence of oxygen is of significant interest considering their role in anaerobic environments.

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NADH oxidases can also be used to control microbial metabolism. As mentioned above, NADH oxidase regulates the intracellular NAD^+/NADH ratio, and increased NADH oxidase activity reduces lactate levels and promotes the production of other metabolites [9, 12]. The accumulation of lactate and the resulting decrease in pH are recognized as the primary factors that impede the growth of LAB. To overcome this problem, NADH oxidase can be used to drive glycolysis without lactate production. This idea has previously been implemented in *L. lactis* by overexpressing H_2O-forming NADH oxidase via plasmids and, more recently, by conducting an aerobic fed-batch culture under glucose-limited conditions [44, 45]. In these studies, lactate accumulations were successfully reduced by 74–100% and 88%, respectively. These methods are expected to be used for high-density LAB culture in the future. On the other hand, it has been reported that the overexpression of H_2O-forming NADH oxidase enhances exopolysaccharide production by *Lacticaseibacillus casei* [46]. Additionally, there have been attempts to enhance the production of certain metabolites in *Saccharomyces cerevisiae* by overexpression of the H_2O-forming NADH oxidase of LAB. Although the effects vary depending on the strain and culture conditions of the *S. cerevisiae* used, studies have reported that overexpression of H_2O-forming NADH oxidase can increase ethanol production, stress tolerance capacity, acetoin production, and 2,3-butanediol production [47–49].

PYRUVATE OXIDASE

Pyruvate oxidase catalyzes the oxidative decarboxylation of pyruvate using phosphate and oxygen to yield acetyl phosphate, carbon dioxide, and H_2O_2 (Table 1). This enzyme plays a pivotal role in carbohydrate metabolism under aerobic conditions, especially in the production of the phosphoryl donor metabolite acetyl phosphate. Acetyl phosphate is a source of acetyl-CoA, which plays an integral role in multiple metabolic processes. Additionally, it generates ATP via its conversion to acetic acid via acetate kinase [2, 12, 50, 51]. Pyruvate oxidase is the primary enzyme that generates H_2O_2 in several LAB species, including *S. pneumoniae, S. sanguinis, Streptococcus gordonii, Streptococcus oligofermentans*, and *Levilactobacillus brevis* [51–54]. Interestingly, a recent study identified pyruvate oxidase from *L. bulgaricus* as a factor that inhibits the growth of *Porphyromonas gingivalis,* the bacterium responsible for periodontal disease. In this study, H_2O_2 produced by pyruvate oxidase inhibited the growth of *P. gingivalis* [55].

In addition to these roles, pyruvate oxidase of *S. pneumoniae* reportedly aids in the regulation of cell adhesion, capsular production, and pneumolysin release from bacterial cells [56–58]. The production of acetyl phosphate and H_2O_2 by pyruvate oxidase is thought to facilitate these functions. However, the details of this mechanism are currently unknown.

α-GLYCEROPHOSPHATE OXIDASE

α-Glycerophosphate oxidase plays a crucial role in glycerol metabolism. Glycerol is converted to α-glycerophosphate by phosphorylation and is then further oxidized to dihydroxyacetone phosphate. This oxidation is catalyzed by α-glycerophosphate oxidase and oxygen (Table 1). α-Glycerophosphate oxidase has been reported to be an enzyme required for aerobic glycerol metabolism in *Enterococcus faecalis*, *Enterococcus faecium*, and *Lacticaseibacillus rhamnosus* [59–61].

LACTATE OXIDASE

Lactate oxidase catalyzes the oxidation of lactate to pyruvate, producing H_2O_2 as a byproduct (Table 1). The enzyme has been reported to be involved in aerobic utilization of lactate in *S. pneumoniae* and *Streptococcus pyogenes* [51, 62]. In both bacteria, the lactate produced through lactic acid fermentation is oxidized to pyruvate by lactate oxidase. The pyruvate is then transformed into acetate, thus producing acetate and ATP. *Lactiplantibacillus plantarum* has been previously reported to possess lactate oxidase. However, lactate dehydrogenase facilitates the conversion of lactic acid to pyruvate in *L. plantarum* [63], rendering the function of lactate oxidase unclear. In *L. lactis*, lactate oxidase has been identified as an enzyme induced by high levels of copper (Cu), implying a potential role in Cu detoxification [64].

L-AMINO ACID OXIDASE

L-amino acid oxidases catalyze the oxidative deamination of amino acids, converting them into their corresponding α-keto acids, ammonia, and H_2O_2 (Table 1). These enzymes are involved in the catabolism of amino acids by breaking them down into their respective α-keto acids. In LAB, *S. oligofermentans* possesses a functional L-amino acid oxidase [65]. *S. oligofermentans* L-amino acid oxidases catalyze the oxidation of seven specific amino acids: L-aspartic acid, L-tryptophan, L-lysine, L-isoleucine, L-arginine, L-asparagine, and L-glutamine. This enzyme has also been reported to be the primary source of H_2O_2 in the bacterium in the presence of amino acids [65].

EXOGENOUS HEME-DEPENDENT RESPIRATION

In respiratory metabolism, electrons are transferred from "intracellular reducing substrates → membrane-bound dehydrogenases → quinones → cytochrome enzymes → oxygen molecules/other electron acceptors", resulting in a proton gradient and membrane potential that are used to synthesize ATP [66]. Most bacteria with respiratory metabolism, such as *Escherichia coli* and *Bacillus subtilis*, can synthesize all the necessary respiratory components. However, LAB cannot synthesize heme, which is an essential cofactor for cytochrome oxidase, and they can hence not carry out respiratory metabolism. Nevertheless, it has been known since the 1970s that respiratory metabolism can take place in *L. lactis* and *E. faecalis* when a heme source is added to their culture medium [67]. This observation suggests that these LAB possess all of the necessary respiratory chain components except for the heme synthesis capacity. Genetic studies of *L. lactis* and *E. faecalis* have revealed a simple respiratory chain with a minimal set of genes for respiratory metabolism, including type II NADH dehydrogenase, menaquinone as a quinone, and cytochrome *bd* oxidase as a cytochrome enzyme [68, 69] (Fig. 2). In comparison with *E. coli*, which has 11 dehydrogenases, three quinones, and 12 terminal oxidases as components of the aerobic/anaerobic respiratory chain [66], it becomes clear how simple the respiratory chain is in these LAB.

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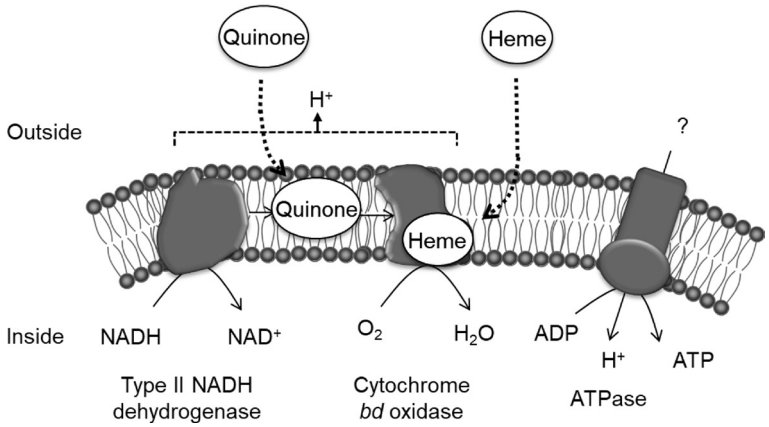

Fig. 2. Respiratory chain of lactic acid bacteria (LAB).

The LAB respiratory chain consists of a type II dehydrogenase, quinone, and cytochrome *bd* oxidase. LAB that are unable to synthesize heme rely on exogenous heme for respiration, whereas those unable to synthesize both heme and quinone use exogenous heme and quinone for respiration. In *L. lactis* MG1363, the genes encoding type II NADH dehydrogenases are *noxA* (llmg_1735) and *noxB* (llmg_1734), while the genes encoding cytochrome *bd* oxidase are *cydA* (llmg_1864) and *cydB* (llmg_1863) [13]. The quinone synthesis genes are encoded in the *menFDXBEC* operon (llmg_1828 to 1833) [13]. NADH: nicotinamide adenine dinucleotide (reduced); NAD+: nicotinamide adenine dinucleotide.

Heme-based respiration metabolism increases the growth yield of *L. lactis* compared with that in the case of fermentative metabolism [68]. Additionally, the shift of electron acceptors from organic acids to oxygen results in significant alterations in metabolites from homolactic acid to mixed acid fermentation [68], which is similar to the effect of NADH oxidase discussed earlier. In other bacteria with respiratory metabolism, increased growth is mainly attributed to increased ATP production via oxidative phosphorylation [66]. However, whether *L. lactis* generates ATP through oxidative phosphorylation during respiratory metabolism remains unclear [70]. Respiratory metabolism in *L. lactis* results in increased production of acetate by acetate kinase, which leads to additional ATP generation through substrate-level phosphorylation. Furthermore, the respiratory metabolism is expected to reduce ATP use, which is not directly linked to proliferation of LAB. Since ATP is used to expel protons accumulated during growth outside the cell via ATPase, the conversion of metabolites such as lactate to neutral substances such as acetoin and diacetyl through respiratory metabolism is thought to decrease the consumption of ATP. Additionally, the electron transfer system, which is responsible for respiratory metabolism, can expel protons without consuming ATP [70–72]. These respiratory metabolic effects have the potential to increase growth yield by increasing ATP production and/or decreasing consumption.

In addition to these metabolic alterations, the survival of cells undergoing respiration metabolism is dramatically improved. When *L. lactis* is stored at low temperatures, cells cultured under fermentative metabolic conditions do not survive for more than two weeks. Nevertheless, cells cultured under respiratory metabolic conditions exhibited a substantial increase in survival and maintained a high viable cell count even after two months [68]. This enhanced survival could be attributed to decreased lactate production and by avoiding an extreme decrease in pH of the medium as a result of respiratory metabolism [73]. In particular, activation of the pathway that produces acetoin, a neutral substance, has been found to contribute significantly to improved cell viability by avoiding acidification of the medium [72]. On the other hand, a reduction in oxygen stress has also been shown to be involved. The extent of protein oxidation and DNA damage, which are markers of oxidative stress, have been shown to be significantly reduced in cells cultured under respiratory metabolism. Additionally, the addition of antioxidant factors, catalase and iron chelators, also enhances the viability of cells in fermentative metabolism [73]. Because the respiratory chain is incapable of directly eliminating ROS, it is assumed to aid in mitigating oxygen stress by either limiting ROS production or by producing an anaerobic environment by excluding oxygen.

L. lactis is widely used as a cheese starter in food production. Although fermentative metabolism is commonly used to produce *L. lactis* starter cultures, respiratory metabolism is a highly advantageous alternative. In particular, respiratory metabolism increases the bacterial yield, which can significantly reduce culture costs. In addition, a significant increase in cell viability results in stable characteristics of the starter culture. This concept has been implemented in cooperation with a food ingredient company [14].

EXOGENOUS HEME- AND QUINONE-DEPENDENT RESPIRATION

The identification of respiratory chain components in *L. lactis* and *E. faecalis* allowed us to predict, based on genomic information, whether other LAB are potentially respiratory competent. Although respiratory metabolism had not previously been reported, investigation of the LAB genome revealed the existence of an alternative respiratory chain. *S. agalactiae* is a commensal bacterium that causes severe infections, including meningitis, septicemia, and pneumonia, particularly in neonates and immunocompromised individuals [74]. Although this bacterium has cytochrome *bd* oxidase activity, its respiratory metabolism has not been previously reported. A detailed analysis of the genome of *S. agalactiae* has revealed a defect not only in heme synthesis but also in quinone synthesis, thus suggesting

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that this bacterium requires exogenous heme as well as quinone for respiratory metabolism. Consistent with this hypothesis, respiratory metabolism has been observed when both heme and quinone sources were added to the culture medium [74] (Fig. 2).

Regarding exogenous quinone-dependent respiration, it has been hypothesized that quinones are acquired from other microbes or the host and employed in the respiratory chain. A respiration-deficient strain of *S. agalactiae* was reported to exhibit significantly decreased infectivity in a neonatal rat infection model, thus strongly suggesting that the bacterium obtains heme and quinone from the host and can perform respiration [74]. The quinone utilized to activate the respiration in the *in vitro* studies was menaquinone-4, which is also found in the animal body, albeit at varying concentrations in different tissues [75]. *S. agalactiae* can also utilize menaquinone-3 and demethylmenaquinone-3 produced by *L. lactis*. When *L. lactis*, which is capable of quinone synthesis, and *S. agalactiae* are co-cultured in the presence of heme and oxygen, respiration is observed in *S. agalactiae* [76]. In addition, *S. agalactiae* has been reported to be able to activate respiration by using 1,4-dihydroxy-2-naphthoic acid (DHNA), which is the precursor of demethylmenaquinone [77]. As DHNA is synthesized by intestinal bacteria, it may be acquired by *S. agalactiae* living in the gut [77]. Quinones or quinone precursors are molecules that other bacteria or hosts can supply and activate electron transfer systems such as respiration. This makes them of great interest in terms of host-bacteria or bacteria-bacteria interactions.

DISTRIBUTION OF RESPIRATORY METABOLISM IN LAB

The studies described above revealed that LAB have two types of respiratory metabolism: exogenous heme-dependent respiratory metabolism and heme- and quinone-dependent respiratory metabolism (Fig. 2). Interestingly, the genes involved in respiratory metabolism, especially cytochrome *bd* oxidase (*cydA* and *cydB*) and the transporters (*cydC* and *cydD*) involved in the maturation of cytochrome *bd* oxidase, are broadly present in LAB; approximately two-thirds of LAB have been reported to have these genes [14]. However, only a few LAB strains harboring these respiratory genes have been experimentally shown to exhibit respiratory metabolism. The following is a list of LAB for which respiratory metabolism or respiratory phenotypes have been reported.

In addition to *L. lactis*, *E. faecalis*, and *S. agalactiae*, *L. plantarum* and *Leuconostoc gasicomitatum* have been experimentally demonstrated to have respiratory metabolism through genetic studies (Table 2). *L. plantarum* has heme- and quinone-dependent respiratory metabolism, and the addition of both increases the rate of oxygen consumption and decreases lactic acid production, resulting in a mitigated pH drop in the culture medium and increased growth yields, whereas acetic acid production increased [78]. The stress tolerance capacity during respiratory metabolism is not clear, since acid stress tolerance decreases and oxygen stress tolerance increases; however, it is also strongly affected by heme catalase activation [79, 80]. *L. plantarum* has a nitrate reductase gene and is capable of anaerobic respiration using nitrates [78]. *L. gasicomitatum* is a heterofermentative LAB with heme-dependent respiration metabolism, and respiration metabolism has been shown to increase the growth rate and yield, as well as decrease ethanol production and increase acetoin and diacetyl production [81]. In addition to these two LAB species, Table 2 also lists LAB for which evidence has been reported for respiratory phenotypes in the presence of heme or heme and quinone. The respiratory phenotypes observed were as follows: 1) enhanced growth without a reduction in the pH of the medium, 2) elevated oxygen utilization, and/or 3) alterations in metabolites due to the addition of heme or heme and quinone [82–86]. Although the conditions and comparators varied among the experiments, they were regarded as representative of the respiratory phenotypes. Studies, primarily conducted by Zotta *et al.* [83, 85–87], have assessed the respiratory phenotypes of various LAB species and screened many strains from each single LAB species to assess differences between strains. Significant differences in respiratory metabolism phenotypes were found among the strains; in some species, the respiratory phenotype was found in only a small number of strains among those selected [83, 85–87]. Using synthetic media, studies have also reported that media components other than heme and quinone, such as Tween 80 as well as several amino acids, influenced the respiratory phenotypes [88, 89]. Since it cannot be ruled out that heme and quinone can affect LAB growth through metabolic pathways other than respiration, it is desirable to conduct additional genetic studies to establish proof of respiratory metabolism. Nonetheless, it is intriguing that some LAB strains have the potential to perform respiratory metabolism. Although the respiratory genes in LAB have been proposed to have been lost through regressive evolution and are considered

Table 2. List of lactic acid bacteria (LAB) with reported respiratory metabolism or respiratory phenotypes

Heam dependent respiration	Ref.	Heme and quinone dependent respiration	Ref.
*Lactococcus lactis***	[68]	*Streptococcus agalactiae***	[74]
*Enterococcus faecalis***	[69]	*Lactococcus garvieae**	[82]
*Streptococcus entericus**	[82]	*Lactiplantibacillus plantarum***	[78]
*Lactobacillus paralimentarius**	[82]	*Lacticaseibacillus casei**	[86]
*Leuconostoc gasicomitatum***	[81]	*Lacticaseibacillus rhamnosus**	[82]
*Leuconostoc mesenteroides**	[84]	*Lactobacillus johnsonii/gasseri**#	[83]
*Leuconostoc pseudomeseneteroides**	[87]	*Levilactobacillus brevis**	[82]
*Leuconostoc citreum**	[87]	*Levilactobacillus spicheri**	[85]
*Weissella minor**	[87]	*Limosilactobacillus reuteri**	[85]

**Species for which respiratory metabolism has been proven by genetic studies.
*Species for which respiratory phenotypes have been demonstrated experimentally.
#Experiments were conducted on strains identified as *L. johnsonii* or *L. gasseri* based on the analysis of their 16S rRNA gene sequences.

non-functional in many LAB [14], their active investigation suggests the potential for identifying additional LAB that have respiration metabolic capability.

EXTRACELLULAR ELECTRON TRANSFER AND ITS RELATIONSHIP WITH THE RESPIRATORY CHAIN

Recently, another metabolic system in LAB has been identified: extracellular electron transfer (EET), which is a biological process whereby bacteria exchange electrons with electron acceptors or donors outside their cell membranes [90]. EET in gram-positive bacteria consists of type II NADH dehydrogenase, enzymes required for menaquinone synthesis, small membrane proteins called EetA and EetB, and the cell wall-bound flavoprotein PplA [90]. They have been proposed to transport electrons from intracellular NADH to extracellular PplA via quinones located on the membrane. PplA subsequently reduces extracellular molecules such as iron [90]. The orthologs of these genes have been identified in LAB [90, 91], and their functions have been investigated. EET contributes to biofilm formation and reduction of ferric iron by *E. faecalis* [92–94]. In *L. plantarum*, the function of EET depends on exogenous quinone sources, as does respiratory metabolism. Tejedor-Sanz *et al*. reported that *L. plantarum* reduced ferric iron and carbon electrodes (anodes) in the presence of DHNA, a precursor of menaquinone [91]. They demonstrated that type II NADH dehydrogenase and *pplA* genes are required for ferric iron reduction, and only the type II NADH dehydrogenase gene contributes to anode reduction. According to the study, EET in *L. plantarum* promotes growth primarily by increasing ATP production at the substrate level [91]. They also analyzed the genome information of 1788 strains from 38 genera of LAB to determine the localization of EET-related genes and revealed a complete set of EET-related genes in 11 genera. Among LAB with EET-related genes, *Lactobacillus pentosus*, *L. casei*, *L. rhamnosus*, *E. faecium*, and *E. faecalis* have been found to exhibit ferric iron-reducing activity. In addition, *L. lactis*, which possesses all EET-related genes except *pplA*, did not exhibit ferric iron-reducing activity but demonstrated anode-reducing activity [91]. These findings suggest that several types of EETs play roles in a certain number of LAB.

EET and respiratory metabolism may appear similar because they share a number of quinones to deliver electrons across the membrane and they use extracellular electron acceptors. However, recent studies have suggested that these are, in fact, essentially independent systems that function separately within the cell [91, 93]. EET is another metabolic option for LAB, in addition to flavoprotein oxidases and respiratory metabolism, which warrants further investigation.

CONCLUDING REMARKS

This review describes the roles of flavoprotein oxidases and the respiratory chain in LAB. Although LAB primarily obtain energy through fermentative metabolism, these oxidase machineries enable metabolic flexibility by utilizing oxygen as an electron acceptor and by participating in various cellular processes. Additionally, they contribute to the development of anaerobic environments. Rapid fermentation of milk requires the elimination of oxygen and lowering of the potential by NADH oxidase and/or the respiratory chain. The removal of oxygen may also contribute to the robustness of cells cultured in respiratory metabolism. However, the full extent by which respiratory metabolism promotes LAB growth has yet to be fully explored. Additionally, while various physiological functions have been attributed to NADH oxidase and other flavoprotein oxidases, the details of their mechanisms of action remain obscure. Further analysis of flavoprotein oxidases and the respiratory chain, along with newly identified EETs, is required to better understand the behavior of LAB in both natural environments and industrial applications.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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