Review

CellPress

Microbial lactate utilization: enzymes, pathogenesis, and regulation

Tianyi Jiang[1,2,3], Chao Gao[2], Cuiqing Ma[2], and Ping Xu[1]

[1] State Key Laboratory of Microbial Metabolism and School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China
[2] State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, People's Republic of China
[3] School of Municipal and Environmental Engineering, Shandong Jianzhu University, Jinan 250101, People's Republic of China

Lactate utilization endows microbes with the ability to use lactate as a carbon source. Lactate oxidizing enzymes play key roles in the lactate utilization pathway. Various types of these enzymes have been characterized, but novel ones remain to be identified. Lactate determination techniques and biocatalysts have been developed based on these enzymes. Lactate utilization has also been found to induce pathogenicity of several microbes, and the mechanisms have been investigated. More recently, studies on the structure and organization of operons of lactate utilization have been carried out. This review focuses on the recent progress and future perspectives in understanding microbial lactate utilization.

Lactate utilization in microbes

Lactic acid is one of the most important α-hydroxy acids in nature. As the end product of anaerobic glycolysis, it plays important roles in many biological processes in microbes. Lactate is produced from carbohydrates with the regeneration of NAD^+ in many microorganisms. The process can be important for survival, especially for the lactic acid bacteria, which use carbohydrates for growth and energy [1]. Lactate utilization is the pathway for microorganisms to oxidize lactate, and this pathway is present in both prokaryotic and eukaryotic microbes. Lactate can provide energy for growth by being a substrate for electron transport when it is oxidized to pyruvate [1]. In habitats that are rich in lactate, this process can allow the survival of some microbes through the use of lactate as a carbon source. In lactic acid bacteria, lactate produced during fermentation is also utilized to produce energy for cells when glucose becomes a limiting factor for growth [2,3].

There are several proteins involved in microbial lactate utilization, with the key step being the oxidization of lactate by lactate dehydrogenase. There are two types of lactate dehydrogenases in microbes, NAD-dependent lactate dehydrogenases (nLDHs) and NAD-independent lactate dehydrogenases (iLDHs). nLDHs use NADH as cofactor, whereas iLDHs utilize flavins. Although nLDHs can also catalyze the oxidization of lactate *in vivo*, *in vitro* experiments may require high pH and a high substrate concentration [3]. The iLDHs, also called respiratory lactate dehydrogenases, are generally considered to be the enzymes responsible for lactate oxidization in microbes [1,4]. Their encoding genes often appear close to other genes involved in lactate utilization, including lactate permease, and regulatory protein encoding genes [5].

New developments continue to be made in the study of microbial lactate utilization. The key enzymes of this process are used in biocatalysis and lactate determination techniques. Lactate utilization has also been found to be involved in the pathogenesis of some microbes, and the lactate utilization operons from several species have been carefully studied. Therefore, research into lactate utilization not only provides insights into the diversity of microbial lactate utilization mechanisms, but also assists in the production of valuable chemicals and in understanding the microbial infections. Here we comprehensively review and summarize recent work on lactate utilization and specifically focus on the characterization and applications of lactate oxidizing enzymes, the mechanisms of lactate utilization-induced microbial pathogenicity, and the regulatory mechanisms of microbial lactate utilization operons.

Microbial NAD-independent lactate dehydrogenases

Categorization of iLDHs

As the enzymes that are mainly responsible for lactate oxidization *in vivo*, iLDHs can be divided into L-iLDHs and D-iLDHs, according to their chiral specificity [1]. Despite their apparent functional similarities, the two groups can greatly differ in terms of enzyme properties or protein structures. The iLDHs in bacteria have been categorized by Garvie [1], and there have been many further investigations into them ever since. There are also various novel enzymes that further increase the number of iLDH types. Here we categorize L-iLDHs and D-iLDHs according to their natural electron acceptors. This classification is based on the proposal that L-iLDHs or D-iLDHs that use the same natural electron acceptor are homologous and have similar sequence features (Figure 1A). However, this proposal lacks decisive evidence and needs further confirmation.

Most of the L-iLDHs belong to the family of α-hydroxy acid-oxidizing flavoproteins. They use flavin mononucleotide (FMN) as a cofactor and can be classified into three groups on the basis of the types of electron acceptors that

Corresponding authors: Xu, P. (pingxu@sjtu.edu.cn); Ma, C. (macq@sdu.edu.cn).
Keywords: lactate utilization; NAD-independent lactate dehydrogenase; biosensor; biocatalysis; pathogenesis; regulation.

0966-842X/
 http://dx.doi.org/10.1016/j.tim.2014.05.008

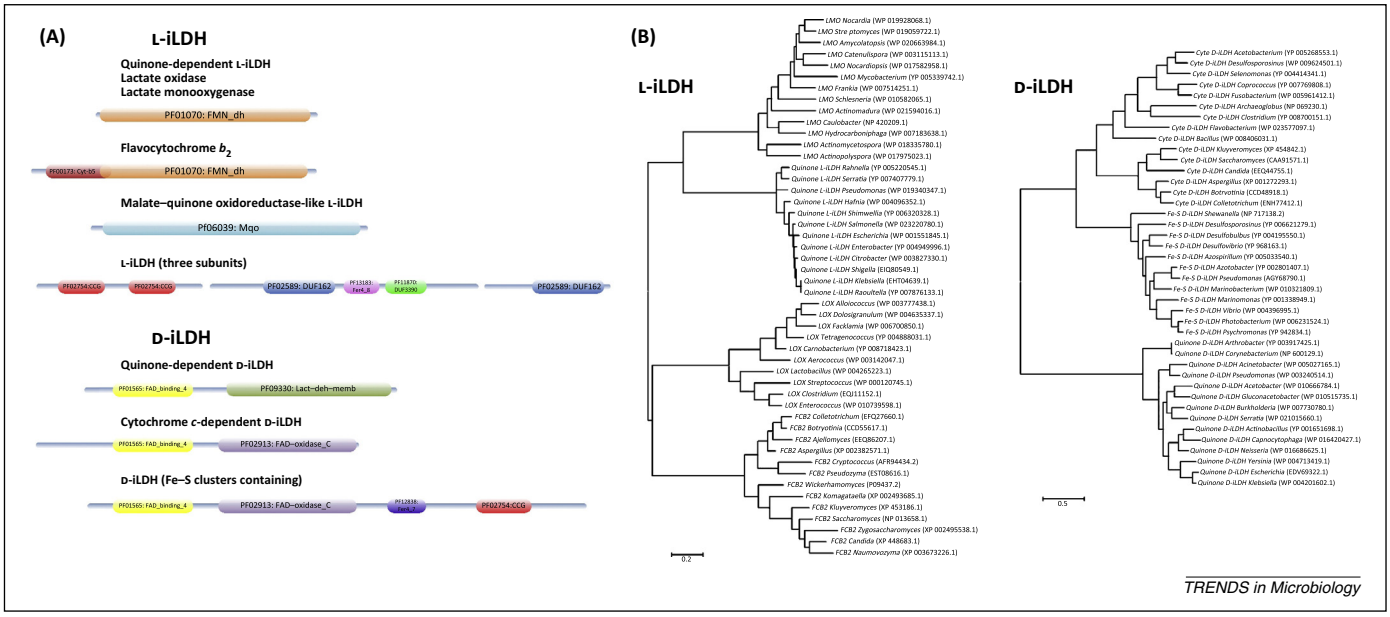

Figure 1. Categories of NAD-independent lactate dehydrogenases (iLDHs). **(A)** Sequence features of identified iLDHs. The analysis is based on the Pfam (protein family) database. The accession numbers and names of domains in the Pfam database are given. Detailed descriptions of the domains can be found in the Pfam database (http://pfam.sanger.ac.uk/). Generally, iLDHs with the same type of electron acceptor have a similar domain organization, so it is possible to speculate the type of an iLDH by its domain organization. However, detailed experimental demonstration is needed for final confirmation. **(B)** Phylogenetic trees of iLDHs. As for L-iLDHs, lactate oxidase (LOX), lactate monooxygenase (LMO), flavocytochrome b_2 (FCB2), and quinone-dependent L-iLDH (quinone L-iLDH) from various genera were analyzed. The malate–quinone oxidoreductase-like L-iLDH and multi-subunits L-iLDH were not included because the former was only a sporadic case and the latter does not show any similarity with any other characterized L-iLDHs. As for D-iLDHs, the quinone-dependent D-iLDH (quinone D-iLDH), cytochrome *c*-dependent D-iLDH (Cytc D-iLDH), and Fe–S clusters containing D-iLDH (Fe-S D-iLDH) from various genera were analyzed. The phylogenetic tree was constructed using Mega 5 software (using the neighbor-joining method). The scale at the bottom indicates sequence divergence. The accession numbers (from the National Center for Biotechnology Information, NCBI) are given in parentheses.

they utilize. The first group contains the enzymes that are membrane-associated proteins and use membrane quinones as external electron acceptors [3,6]. The second group directly uses O_2 as an electron acceptor, and can be further subdivided into lactate oxidase (LOX) and lactate monooxygenase (LMO). They differ in their products of lactate oxidization (the former produces pyruvate and H_2O_2, whereas the latter produces acetate, CO_2, and H_2O), and this is caused by the different stabilities of their intermediates, reduced flavin enzyme and pyruvate [7,8]. However, LMO has a closer phylogenetic relationship to quinone-dependent L-iLDHs than LOX (Figure 1B). The third group is found in fungi and is called flavocytochrome b_2 (FCB2). Its natural electron acceptor is cytochrome *c*. The most distinctive feature of this enzyme is that it has an additional cytochrome domain that contains a protoheme IX prosthetic group [9] (Figure 1A). The above classification cannot account for all L-iLDHs. First, some L-iLDHs in lactic acid bacteria have relative high sequence identity with the LOX, but their natural electron acceptor has not been identified. It was reported that these enzymes may not be responsible for lactate oxidizing *in vivo* [3,10]. Second, it was found that an enzyme originally annotated as a malate–quinone oxidoreductase in *Staphylococcus aureus* actually oxidizes L-lactate, but not malate [11] (Figure 1A). Although it seems to be an isolated case, this is an example where an enzyme with a different annotated function may be a lactate oxidizing enzyme. Third, a recently discovered enzyme complex with three subunits was found to function as an L-iLDH in *Shewanella oneidensis*. None of the three subunits has similarity with other characterized L-iLDHs. They contain several iron–sulfur cluster-binding motifs, but no flavin-binding motif in the protein sequence [5,12,13] (Figure 1A). The indispensable role of each subunit in L-lactate oxidization has been demonstrated by mutagenesis [5]. Although this novel L-iLDH has not been further characterized, the large difference between it and typical L-iLDHs suggests that it has a different catalytic mechanism.

In comparison to L-iLDHs, all characterized D-iLDHs use flavin adenine dinucleotide (FAD) instead of FMN as a cofactor for electron transfer, and they have sequence features of the FAD-binding oxidoreductase/transferase type 4 family [14,15] (Figure 1A). The characterized D-iLDHs may use quinones or cytochrome *c* as an electron acceptor, but to date there are no reports of a D-iLDH that uses O_2 directly. The D-iLDHs from *Escherichia coli* and *Corynebacterium glutamicum* are quinone dependent [16,17]. The D-iLDHs of this type appear in bacteria (Figure 1B), and they have a special membrane-binding domain at the C terminus (Figure 1A). The cytochrome *c*-dependent D-iLDHs are found in fungi, bacteria, and archaea [18,19]. The bacterial and archaea enzymes have a close phylogenetic relationship, whereas the eukaryotic ones are found in a separate branch of the phylogenetic tree (Figure 1B). There is also a specific C-terminal domain in cytochrome *c*-dependent D-iLDHs (Figure 1A). Additionally, a novel type of D-iLDH from *S. oneidensis*, with an unknown natural electron acceptor, and with an additional C-terminal multi-domain, has also been recently identified. The C-terminal multi-domain contains several iron–sulfur cluster-binding motifs, whereas its N-terminal multi-domain exhibits similarity with cytochrome *c*-dependent D-iLDHs [5] (Figure 1A), indicating that these two types of D-iLDHs have a close phylogenetic relationship (Figure 1B). This arrangement makes the enzyme seems to

be a fusion protein. Unexpectedly, its homolog from *Campylobacter jejuni* was found to utilize L-lactate *in vivo*, although the purified enzyme exhibited higher activity towards D-lactate than L-lactate [13]. In addition, this enzyme also reduces pyruvate to D-lactate in *S. oneidensis* [20]. Therefore, the physiologic function of this enzyme is still obscure.

Structures and catalytic mechanisms of iLDHs

Among the iLDHs, LOX from *Aerococcus viridans*, FCB2 from *Saccharomyces cerevisiae*, and D-iLDH from *E. coli* have had their structures solved. The structures of the two L-iLDHs are homologous whereas the D-iLDH structure does not show much similarity to them.

FCB2 has been extensively studied for many years [9], and is the first of these enzymes to have its structure determined. Variant enzymes or enzymes in complex with different ligands have also been crystallized and analyzed [21–25]. Based on the abundant structural information, its catalytic mechanism has been thoroughly investigated. As a typical flavin-dependent α-hydroxy acid dehydrogenase, the mechanism of its reductive half-reaction has long been debated by biochemists as to whether it involves the formation of a carbanion intermediate, or whether it occurs by direct hydride transfer [26]. Variants of FCB2 with mutated active site residues were constructed and analyzed to identify their roles in the catalytic reaction [21–23]. The key residues affecting the substrate specificity of FCB2 were also identified. Specific mutations were identified that made FCB2 a more efficient catalyzer of mandelate than of lactate [27,28]. Crystallographic studies of the mutant enzyme in complex with the mandelate oxidation product supports the hydride transfer mechanism [21]. Nevertheless, this topic remains debatable as the results obtained from other research, such as the elimination reactions of halogenated substrates catalyzed by FCB2, suggest that there is a carbanion intermediate during the reaction [26]. The electron transfer in FCB2 has also been investigated, and in spite of a covalent linkage between the domains, it is similar to a transient electron transfer complexes. Work in this area has been recently reviewed [9]. It has been proposed that cytochrome *c* binds to the enzyme following domain movements that expose the edge of the heme moiety. Thus, the mobility of the heme domain is considered to be essential for the enzyme's function [9,29].

The structure of *A. viridans* LOX shows a great deal of similarity to other flavin-dependent α-hydroxy acid dehydrogenases, although there are some minor differences [30,31]. From analyzing the conformation of a manually docked L-lactate, two novel residues (Arg181 and Tyr215) have been proposed to interact with the substrate. As the latter residue is not conserved in this protein family, it was suggested to confer some unique properties to LOX [30]. LOX crystallized in complex with ligands or under different conditions have also been used to investigate the catalytic mechanism. The involvement of these two novel residues in substrate binding was confirmed by determining the structure of the enzyme–pyruvate complex [32]. In work that used acidic condition for LOX crystallization, the structure obtained was believed to mimic an intermediate that occurs after the protonation of His265 (the critical active site base). It was proposed that a flip action of His265 at this stage would trigger a large structural rearrangement, facilitating O_2 in electron transfer. In support of this, the bound molecular O_2 was discovered in the complex [7].

The structure of the *E. coli* D-iLDH is the only reported D-iLDH structure to date. It belongs to the FAD-binding-4 structural family, which all have similar FAD-binding domain folds but little sequence conservation along the entire sequence alignment [15,16,33]. The structure of the *E. coli* D-iLDH was divided into a FAD-binding domain, a cap domain, and a membrane-binding domain. However, 50 residues were missing from the membrane-binding domain in the structure because they exhibited discontinuous and untraceable electron density. Some positive residues in this region, along with several others observed in this domain, were proposed to form an electropositive surface. This surface could interact with the phospholipid head groups of the cell membrane through electrostatic forces, and contribute to association with the membrane [16]. However, no further investigations of ligand binding in this enzyme have been carried out, and its catalytic mechanism has not been studied as thoroughly as that of L-iLDHs.

Applications of iLDHs in lactate determination

Lactate determination is required in medical diagnoses and in various industrial processes, such as food and beverage production, and quality control [34]. To develop lactate biosensors, both nLDHs and iLDHs have been utilized. However, nLDHs need the addition of NAD^+ as cofactor, which requires either an additional immobilization step or addition of the cofactor to the solution. Additionally, biosensors based on nLDHs have been found to be less sensitive in the determination of lactate concentration. This is because the oxidization of lactate by NAD^+ reduction is an unfavorable reaction, owing to the low oxidizing power of NAD^+ [34,35]. Therefore, an essential advantage of iLDHs is that they have internal flavin cofactors [34,36,37]. Among the iLDHs, LOX, FCB2, and cytochrome *c*-dependent D-iLDHs have been used to sense lactate. As for the quinone-dependent iLDHs, most of them are membrane-associated, and the need for using detergents during their purification would be unfavorable for enzyme immobilization.

L-Lactate-specific LOX is the enzyme most widely used in biosensor applications for L-lactate determination. Its unique property in using O_2 directly as electron acceptor can further facilitate lactate determination. Generally O_2 reduction or H_2O_2 oxidization is detected using an electrode [34,38]. In addition, O_2 can be substituted by artificial electron acceptors in anaerobic operations [39]. Compared to biosensors using L-nLDHs, the LOX-based biosensors have wider linear ranges and lower detection limits [36]. The *Pediococcus* sp. LOX is the most widely used enzyme, and the LOX from *A. viridans* that has a known structure, *Streptococcus* sp., and *Enterococcus* sp. have also been used, although sporadically [34]. Unfortunately, similar procedures cannot be used for D-lactate determination owing to the lack of a corresponding

D-iLDH. However, there are also potential disadvantages of LOX-based biosensors. First, some easily oxidized substances will interfere with the detection of H_2O_2 because it needs a high working potential [34,35,37]. Second, the long-term operational and storage stability of LOX is limited [36,38]. These problems can be partly overcome by improving the working conditions of the enzyme, for example, by adding stabilizers, changing the immobilized materials, etc. [40–42]. In addition, it is possible to change the inherent properties of enzymes using protein structural information and protein engineering [30].

FCB2 is a good alternative enzyme for LOX in L-lactate determination. The FCB2 from *S. cerevisiae*, *Hansenula anomala*, and *Hansenula polymorpha* have all been used to date [34,35,43]. Artificial electron acceptors with low redox potentials are commonly used as mediators, and thus the biosensor can be operated at a low potential [35]. It has also been reported that both the storage and operational stability of FCB2 is better than that of LOX, although this advantage is not widely reported [35,36]. Nevertheless, there are also disadvantages of this enzyme. It has a low saturation limit of L-lactate, and is prone to inhibition by D-lactate and pyruvate. Furthermore, FCB2 was reported to be highly sensitive to pH, especially acidic media [34].

As for D-lactate determination, most of the biosensors designed so far are based on D-nLDHs [34], and only recently has a study investigated the use of a cytochrome *c*-dependent D-iLDH [44]. The use of *S. cerevisiae* D-iLDH has been investigated with the cheap artificial electron acceptor phenazine methosulfate as a mediator. Because several properties of this enzyme, including the stability, chiral specificity, and the working potential, were as good as or better than those of D-nLDHs, it has the potential to be an alternative enzyme in D-lactate determination [44].

Applications of iLDHs in biocatalysis

The other important applications of iLDHs are in the syntheses of 2-oxoacids or chirally pure 2-hydroxyacids through 2-hydroxyacids oxidization. Whole cells or crude cell extracts containing iLDH activities are mostly used as the biocatalysts and cheap racemic 2-hydroxyacid mixtures are used as the starting materials.

Biocatalysts with both L- and D-iLDH activities can be used for the production of 2-oxoacids. Pyruvate production by this route is the most widely investigated. Although biotechnological production of pyruvate can also be achieved by fermentation or lactate oxidization through nLDHs, the former has the disadvantages of low yield and difficulties in product recovery, whereas the latter requires the addition or regeneration of the NAD^+ cofactor [45,46]. The use of LOX in this process is also limited because H_2O_2 will further metabolize pyruvate to acetate, CO_2, and water [46–50], and the elimination of H_2O_2 is relatively complicated [51]. Biocatalysts using quinone-dependent iLDHs are most promising (Figure 2, route a). Electron acceptors of these enzymes can be regenerated artificially (purified enzyme), or by use of the electron transport chain (whole cells or crude cell extracts) [47,52]. Except for pyruvate, a similar route of 2-oxobutyrate production through 2-hydroxybutyrate oxidization by iLDHs has also been established (Figure 2, route b) [53]. This is because the reported quinone-dependent iLDHs also have a relative high activity towards 2-hydroxybutyrate [48,54,55].

Biocatalysts with iLDH activity towards one isomer can be used for the chiral separation of racemic 2-hydroxyacid mixtures because most iLDHs, especially the L-iLDHs, have strict chiral specificity [54,55]. Biocatalysts can be prepared by selectively inactivating the iLDH activity towards one isomer. This route has been applied in the preparation of D-lactate and D-2-hydroxybutyrate using a *Pseudomonas stutzeri* strain [56,57] (Figure 2, routes c and d). The D-iLDH and L-iLDH in this strain have different temperature sensitivities, and thus the D-iLDH activity can be completely inactivated by heat treatment, without affecting L-iLDH activity. Alternatively, strains with heterogeneous expression of iLDHs can be utilized. For example, an engineered *P. stutzeri* L-iLDH with enhanced L-mandelate-oxidizing activity was expressed in *E. coli* and the strain was used for the preparation of D-mandelate (Figure 2, route e) [58]. In these routes, 2-oxoacids are also produced, and they can be separated with the chirally pure 2-hydroxyacids by simple ion exchange procedures [56,57].

Although these biocatalysts are efficient and easy to operate, there are also some underlying problems. First, 2-oxoacids could be further metabolized in microbes. This problem can be resolved by adding EDTA to complex the metallic ions of the 2-oxoacids degradation enzymes, thereby blocking their activities [49,56,58]. Another problem is the high cost in preparing the cells containing iLDH activities, because the activity needs to be induced by lactate in some strains [45,59]. To develop more economic applications with these strains, mutants with constitutive iLDH activities could be utilized so that the biocatalysts can be prepared using cheaper carbon sources [45]. As for the chiral separation routes, so far they are limited to the preparation of D-isomers using L-iLDHs, whereas the utilization of D-iLDH activities for L-isomers preparation, although feasible, has not been investigated. A reason could be that some of the above mentioned routes are based on heat inactivation of D-iLDH, and only L-iLDH activity can be isolated in this way [56,57]. For similar purposes, mutating the L- or D-iLDH encoding gene would be a promising solution to develop biocatalysts that are much easier to prepare.

Microbial pathogenicity induced by lactate utilization

As a ubiquitous carbon source in nature, lactate can be utilized by many pathogenic microbes. It has been found that lactate utilization contributes to the pathogenicity and infection processes of several pathogenic microbes. Although some may utilize similar mechanisms, disparate mechanisms have also been identified.

Lactate utilization-induced pathogenesis of Neisseria *spp.*

Lactate utilization-induced pathogenesis has been most clearly elucidated in *Neisseria gonorrhoeae* and *Neisseria meningitidis*, the pathogenic strains that cause gonorrhea and meningitis, respectively. Lactate is abundant in human urogenital tract and nasopharynx that *N. gonorrhoeae* and *N. meningitidis* inhabit, respectively [60,61], and there

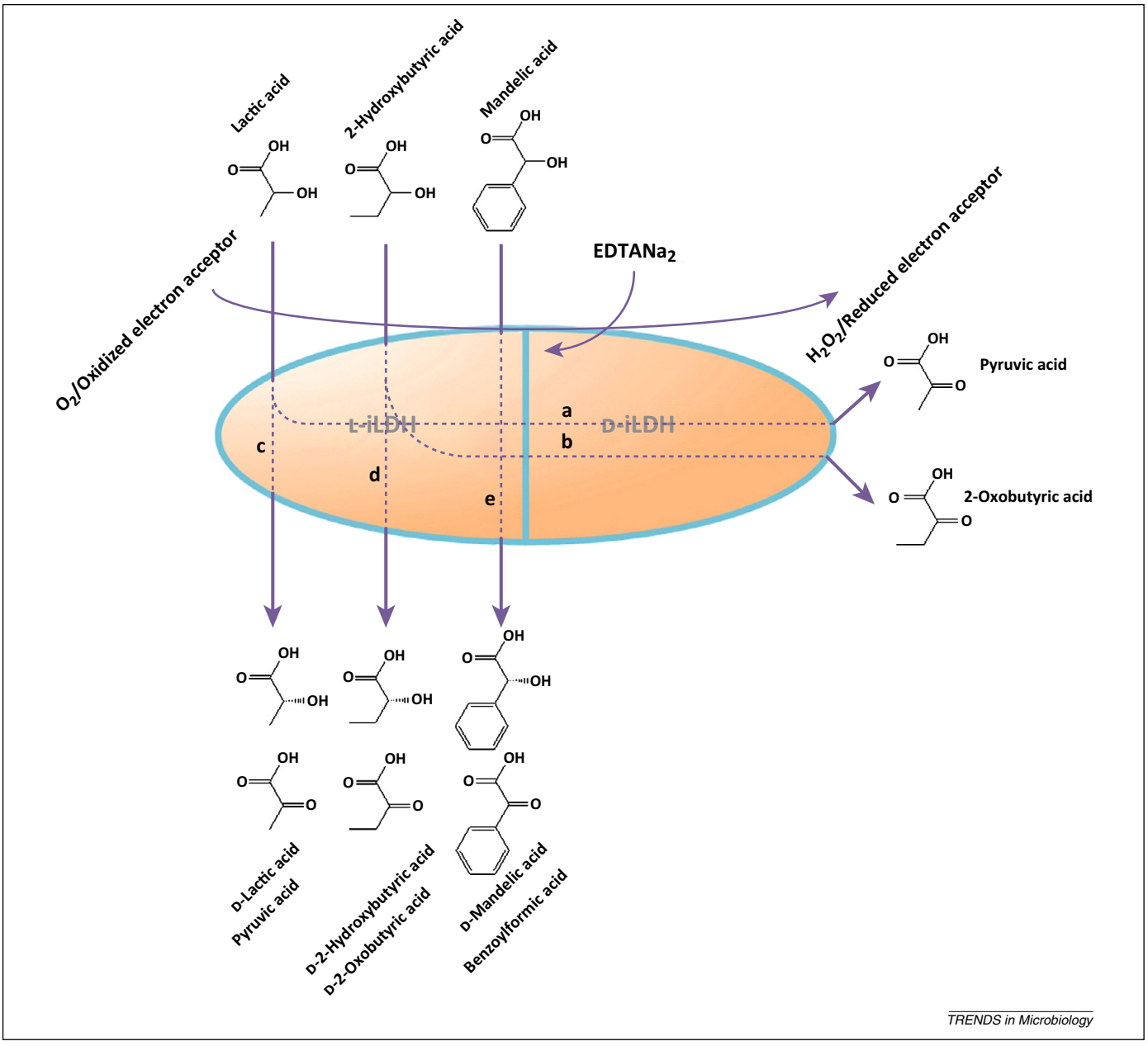

Figure 2. The applications of NAD-independent lactate dehydrogenases (iLDHs) in biocatalysis for 2-oxoacid and chiral 2-hydroxyacid production. Routes a and b indicate the production of 2-oxoacids from 2-hydroxyacids using both L- and D-iLDH activities. Routes c, d, and e indicate the chiral separation of racemic 2-hydroxyacid mixtures for the production of D-isomer (top) and 2-oxoacids (bottom) using L-iLDH activity. Different electron acceptors are utilized depending on the type of enzyme. EDTA is added to stabilize the 2-oxoacids.

are several lactate utilization-induced pathogenic mechanisms that are utilized by them. First, in *N. gonorrhoeae*, lactate utilization was found to enhance resistance to O_2-dependent bactericidal mechanisms by competing for O_2 [62,63] (Figure 3, mechanism A). Second, lactate utilization leads to a general stimulatory effect on metabolism, which consequently enhances the growth rate of these strains (Figure 3, mechanism C). Third, lactate utilization can also enhance the synthesis of specific pathogenic determinants (Figure 3, mechanism D) [60,61]. There have been reviews of these mechanisms [60,61], and more detailed descriptions of these mechanisms are given in Box 1. These proposed mechanisms were investigated by using mutant strains that are unable to utilize lactate. It was from *N. meningitidis* that a lactate permease-deficient mutant strain was first obtained. As expected, the mutant showed a greater susceptibility to complement-mediated lysis and impaired nasopharyngeal colonization [64,65]. Similar work has been carried out with *N. gonorrhoeae*. In this case, the mutant was significantly attenuated in its ability to colonize and survive in the genital tract, whereas the complemented mutant exhibited no defect for colonization [66]. These results confirmed the indispensable role of lactate utilization in their pathogenicity.

Lactate utilization-induced pathogenesis of other species

Other identified microbes that have lactate utilization-induced pathogenesis include *Haemophilus influenzae* and *S. aureus*. It has long been recognized that serum or

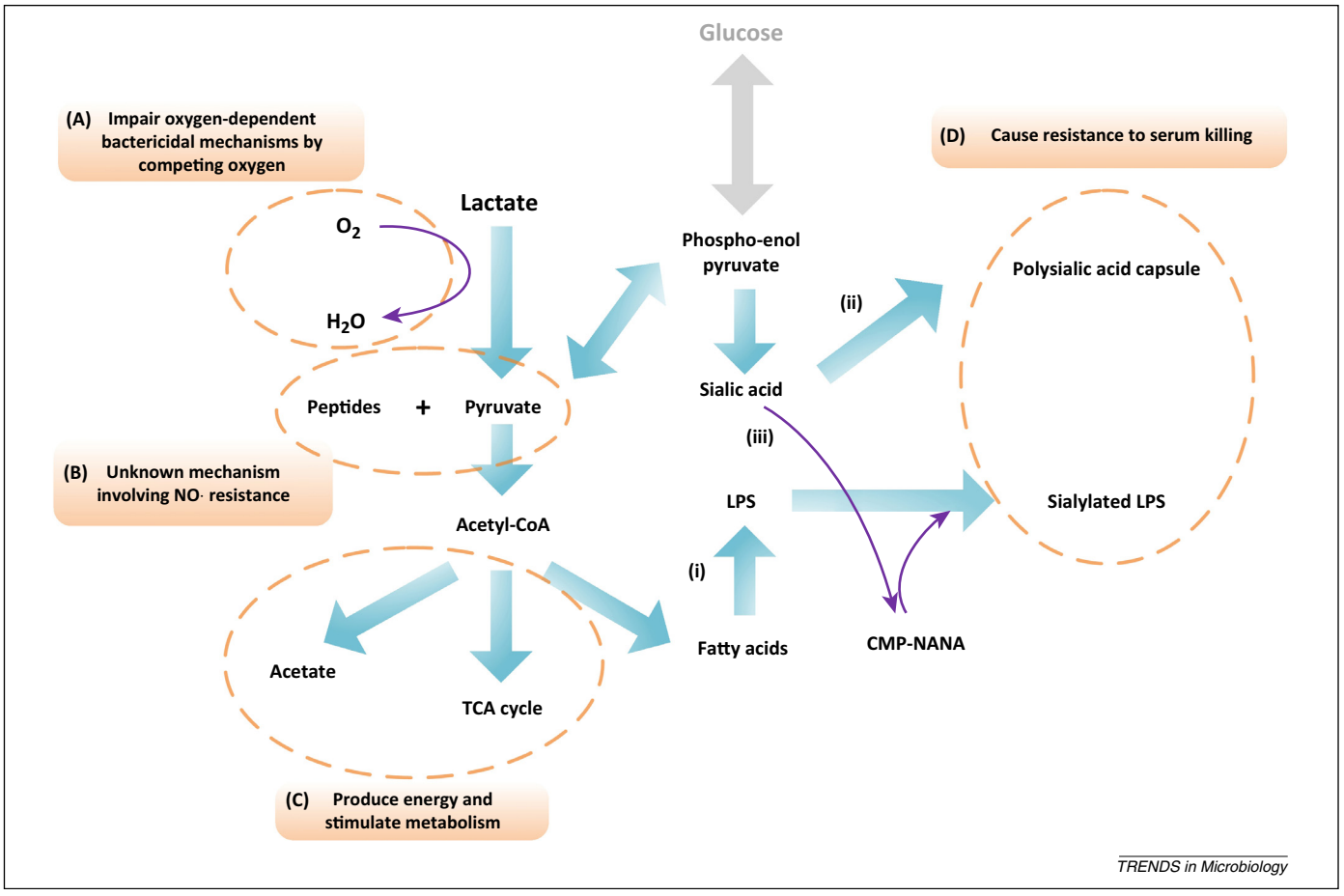

Figure 3. Lactate utilization induced microbial pathogenic mechanisms. Mechanism **(A)** (identified in *Neisseria gonorrhoeae*) indicates that lactate utilization competes for O_2, which is needed for O_2-dependent bactericidal mechanisms. Mechanism **(B)** (discovered in *Staphylococcus aureus*) indicates that the pyruvate produced from lactate oxidization enhances the resistance of *S. aureus* to NO• stress in combination with peptides. However, the details of this mechanism have not been determined. Mechanism **(C)** (identified in *N. gonorrhoeae* and *Neisseria meningitides*) indicates that lactate utilization provides a more direct and faster pathway for energy production than glucose utilization (which is indicated by a gray arrow, whereas the detailed reaction steps are omitted). It therefore leads to a general stimulatory effect on metabolism. Mechanism **(D)** (identified in *N. gonorrhoeae* and *N. meningitides*) indicates that lactate utilization also provides a more direct and faster pathway for the synthesis of specific pathogenic determinants (polysialic acid capsule and sialylated LPS). Pathway (i) is present in both species whereas pathways (ii) and (iii) [the synthesis of polysialic acid capsule and its own cytidine 5′-monophosphate-*N*-acetyl neuraminic acid (CMP-NANA)] are present only in *N. meningitides*.

nasopharyngeal secretions can increase the resistance of *H. influenzae* to bactericidals and antibodies. Two mechanisms that may account for this resistance were proposed following examination of various *H. influenzae* strains, and both mechanisms involve lactate utilization [61]. In one of the mechanisms, both glucose and lactate are required; in this case, lactate utilization is likely to function in a manner similar to that of *N. gonorrhoeae*. The other mechanism involves an increase in capsular polysaccharide, and it may be similar to that in *N. meningitides* [61]. Mutation of lactate permease has also been obtained while screening for potentially essential genes in the pathogenicity of *H. influenzae* [67]. Although further investigations have not been reported, this mutant provides candidate material for further research.

As for *S. aureus*, an important human pathogen that is able to infect nearly every host tissue, a correlation between its virulence and lactate utilization has also been recently proposed. The pathogenic mechanism involves its uncommon resistance to radical nitric oxide (NO•), a key broad-spectrum antimicrobial host effecter that is essential in the destruction of pathogenic microorganisms [11,68]. Initially, researchers found that *S. aureus* produces L-lactate exclusively after exposure to NO•, and exhibits markedly elevated nLDH activity. This phenomenon prompted investigations into identifying a unique L-nLDH that is induced by NO• stress. Coincidentally, this enzyme does not exist in some other staphylococcal species that are sensitive to NO• stress. Therefore, it was proposed that when *S. aureus* is exposed to NO• stress, it ceases to respire and shifts metabolism to the fermentation of L-lactate using the induced L-nLDH, and that this alteration is important in maintaining redox homeostasis by regenerating NAD^+. This is believed to contribute to the resistance to NO• stress, and to the pathogenicity of the strain [68]. However, after prolonged NO• exposure, *S. aureus* specifically utilizes L-lactate [11]. Although the presence of L-iLDH activity in *S. aureus* had been known for some time, the enzyme had not been identified until a recent research found a quinone-dependent L-iLDH, which was originally annotated as a malate–quinone oxidoreductase. An L-iLDH-deficient mutant was found to be defective for growth in murine cardiac tissue under NO• stress. To understand the mechanism, *in vitro* experiments were carried out using a defined medium. The results showed that glycolytic carbon sources can support *S. aureus*

Box 1. Mechanisms of lactate utilization-stimulated pathogenesis of *Neisseria* spp.

Early research demonstrated that when interacting with human neutrophils, *N. gonorrhoeae* was able to utilize host-derived lactate to enhance their O_2 metabolism, which decreases neutrophil formation of reactive O_2 intermediates, by competing for molecular O_2. This action will therefore enhance the resistance of strains to O_2-dependent bactericidal mechanisms [62]. This mechanism was further demonstrated, because the increased L-iLDH activity of the strain was observed when it was subjected to an *in vitro* oxidative stress condition, and the stressed strain was shown to use L-lactate more readily than other carbon sources [63].

Later research focused on another lactate-stimulated resistance mechanism involving lipopolysaccharide (LPS) sialylation. Lactate in blood cells could enhance the ability of host-derived cytidine 5′-monophosphate-*N*-acetyl neuraminic acid (CMP-NANA) to sialylate *N. gonorrhoeae* LPS. The sialylated LPS is a determinant that causes resistance to complement mediated killing (Figure 3, mechanism D). This enhanced resistance may be caused by a variety of potential mechanisms [60,61].

Further research has indicated that lactate utilization has a general stimulatory effect on *N. gonorrhoeae* metabolism. This stimulation consequently enhances the growth rate of strains *in vivo* (Figure 3, mechanism C). In fact, the resistance induced by increased LPS sialylation was also speculated to be the result of increased availability of LPS and sialyltransferase. This effect only appears when lactate and glucose are both present, whereas lactate alone causes slow growth despite the rapid utilization of lactate [61]. Furthermore, the lactate carbon was predominantly incorporated into fatty acids, the precursors of LPS [Figure 3, mechanism D, pathway (i)]. Based on these facts, mechanisms of lactate utilization-induced enhancement of pathogenicity were proposed. When lactate is oxidized to pyruvate, it enters two pathways: gluconeogenesis and the formation of acetyl-coenzyme A. When glucose is present, gluconeogenesis from lactate does not occur and only lactate is used to produce acetyl-coenzyme A. The additional acetyl-coenzyme A then produces more energy and LPS. Compared to the metabolism of glucose, lactate utilization produces acetyl-coenzyme A and LPS more directly and faster, and hence stimulates the metabolism and pathogenicity of the strain [60,61].

As for *N. meningitidis*, the stimulation of metabolism and LPS sialylation were also identified and unique mechanisms were proposed. This strain can synthesize its own CMP-NANA [Figure 3, mechanism D, pathway (iii)] for LPS sialylation. Furthermore, polysialic acid capsules can be synthesized [Figure 3, mechanism D, pathway (ii)], and it also mediates resistance to complement-mediated killing. Lactate utilization also provides a more direct pathway for their synthesis [60,65].

$NO^{\bullet}$ resistance while gluconeogenic sources cannot. Interestingly, there was an exception in that the combination of L-lactate or pyruvate with peptides could also support *S. aureus* growth under $NO^{\bullet}$ stress. The authors proposed that this lactate utilization manner provides an additional insurance of the $NO^{\bullet}$ resistance, and that it is necessary for the full virulence of *S. aureus* [11] (Figure 3, mechanism B). Although the underlying mechanism has not been elucidated, the common presence of these two required components (lactate and peptides) in host tissues suggests that this is a viable explanation. Later research by *in vivo* NMR studies confirmed efficient lactate utilization when glucose becomes limiting in *S. aureus*. It was proposed that this may endow *S. aureus* with a metabolic advantage in its ecological niche [4].

Regulation of microbial lactate utilization

While investigating lactate oxidizing enzymes and lactate utilization induced pathogenesis in different microbes, it was noticed that the regulatory mechanisms of lactate utilization are distinct, despite the high degree of conservation of the enzymes [69]. For example, in *E. coli* and *C. glutamicum*, D-iLDH activities are constitutively expressed, whereas L-iLDH activities must be induced by L-lactate [1]. In *N. meningitidis* and *N. gonorrhoeae*, both L- and D-iLDH activities are constitutively expressed [70]. By contrast, the L- and D-iLDH activities in various *Pseudomonas* species are induced by either isomer of lactate [48,59,71]. To clarify these differences, the corresponding lactate utilization operons were identified and characterized.

The structures of lactate utilization operons have been described in *E. coli*, *C. glutamicum*, and *Pseudomonas aeruginosa* [71–73]. As shown in Figure 4A, all of these operons contain the *lldR* gene that encodes the regulatory protein LldR, the regulatory sequence, and the structural genes. Lactate is the factor that induces the transcription of the operons. By comparing the expression of certain activities with the structure of corresponding operon, it is obvious that the variations in iLDH expression patterns are due to the differences in structural genes. Corresponding to the previous observations, only the L-iLDH encoding gene *lldD* is located in this operon in *E. coli* and *C. glutamicum* [72,73]. In comparison, the L- and D-iLDH encoding genes (*lldD* and *lldE*) of *P. aeruginosa* are all in this operon [71] (Figure 4A). Based on the known gene sequences, comparative genome analysis was performed to find the lactate utilization operons in further species [5,71,74]. A notable finding was that the lactate permease encoding gene *lldP* is the most conserved component of lactate utilization operons. By virtue of the current abundant genomic information available, *lldP* can act as a marker to identify the lactate utilization operon in other microbes. Novel enzymes involved in lactate utilization could also be identified by this approach [5,13].

To further clarify the regulatory mechanisms of lactate utilization, the regulatory proteins from *E. coli*, *C. glutamicum*, and *P. aeruginosa* were characterized. They exhibit high sequence similarity and all belong to the FadR subfamily of the GntR transcription factor family [71–73,75]. Homologs of them exist in many distantly related species [71,73]. There are also transcriptional regulators that belong to other families involved in lactate utilization that have not been characterized in detail, such as LysR-like regulators [5,69]. Further investigations showed that although the FadR-like regulators have conserved domain features (Figure 4B), their regulatory mechanisms are not identical. It was supposed that the presence or absence of L-lactate in *E. coli* would control the role of LldR, either as a repressor or an activator, in lactate utilization operon transcription. When L-lactate is not present, the binding of two LldR molecules on two regulatory sequences leads to DNA looping and the repression of transcription. When L-lactate is present, it binds to the LldR, promoting a conformational change that destabilizes the DNA loop, enabling transcription. In addition, LldR may also interact

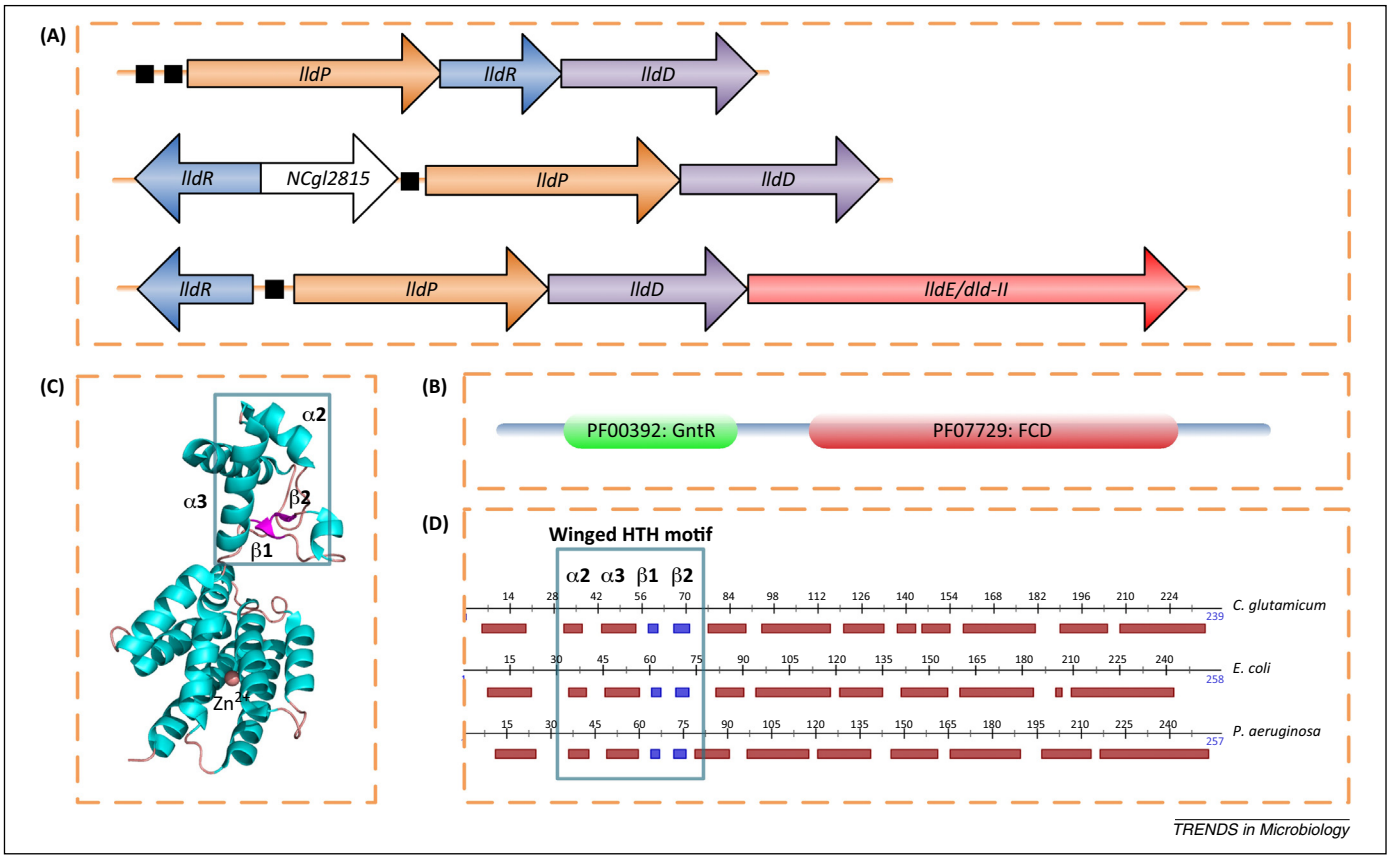

Figure 4. Comparison of identified lactate utilization operons and their regulatory proteins. **(A)** The structures of lactate utilization operons from *Escherichia coli* (top), *Corynebacterium glutamicum* (middle), and *Pseudomonas aeruginosa* (bottom). *NCgl2815* encodes a putative protein with unknown function. The black squares indicate the binding sites (predicted or experimentally demonstrated) of regulatory proteins. **(B)** Sequence analysis of LldR proteins based on the Pfam (protein family) database. The characterized LldR proteins have similar sequence features. Detailed descriptions of the domains can be found in the Pfam database (http://pfam.sanger.ac.uk/). **(C)** Protein structure of *C. glutamicum* LldR. The panel indicates the helix-turn-helix (HTH) structure including two α-helices and two β-sheets. Zn^{2+} in the C-domain is displayed as a ball. **(D)** Comparison of the secondary structures of LldR proteins. The secondary structures were predicted using the PredictProtein server (https://www.predictprotein.org/). These proteins also have similar secondary structures, with a winged HTH motif (indicated by *C. glutamicum* LldR).

with RNA polymerase or other transcriptional regulators to activate transcription at this level [72]. In *C. glutamicum*, the binding site of LldR was experimentally demonstrated. Although only one site was found, two LldR–DNA complexes were observed. The binding site overlaps the transcriptional start site of the operon, which leads to a sole repression mechanism involving interference of the RNA polymerase–promoter interaction. As with *E. coli* LldR, only L-lactate can bind *C. glutamicum* LldR [73]. However, when L-lactate was present, no additional activation was observed [72]. In comparison, both L-lactate and D-lactate can bind to the LldR from *P. aeruginosa* and induce the transcription of the operon [71]. Considering that the D-iLDH-encoding gene is also located in this operon in *P. aeruginosa*, it can be assumed that *P. aeruginosa* LldR has evolved to adapt its own genome arrangement.

The only LldR structure resolved to date is that of *C. glutamicum* [76]. The protein consists of two monomers. The typical structure of a FadR family member with N- and C-domains was observed in the monomer. The N-domain contains a motif formed by two helices, a turn that connects them, and a winged anti-parallel β-sheet (Figure 4C,D). The structure resembles the typical prokaryotic DNA-binding motif with a helix-turn-helix (HTH) structure [76,77]. The C-domain is a helical bundle that consists of seven helices (Figure 4D), and it is likely to be responsible for dimerization and ligand binding. The residues involved in DNA binding and dimer formation in LldR are highly conserved among various LldR homologues. The presence of Zn^{2+} in the *C. glutamicum* LldR is a novel feature of GntR family proteins (Figure 4C), and the Zn^{2+}-binding residues are also highly conserved. This structure further elucidates the regulatory mechanism for lactate utilization. However, the structure of LldR in complex with lactate has not been obtained; this structure will provide further insight into the mechanism of protein conformation change during the regulatory process [76].

LldR proteins can also be used to develop lactate detection techniques. Recently, a genetically-encoded Förster resonance energy transfer (FRET)-based lactate sensor based on LldR was designed. FRET sensors are fusion proteins composed of a ligand-binding moiety, a recognition element, and a fluorescent pair with overlapping emission and excitation spectra. Binding of the test molecule causes a conformational change in the recognition element that affects the relative positions of the fluorescent proteins, causing a change in fluorescence intensity. LldR was used as the recognition element in this lactate sensor. The sensor was expressed in eukaryotic cells to

dynamically detect lactate levels. Although this procedure seems complex, it provides a solution to the problem of detecting intracellular lactate noninvasively, in real time, and with single cell resolution. This lactate sensor has the potential to be used in medical diagnosis [78].

Concluding remarks and future perspectives

Studies of microbial lactate utilization, including its enzymes, induced pathogenesis, and regulatory mechanisms, have provided not only insights into this important microbial catabolism process, but can also aid in improving people's health and quality of life. Although some of the reviewed subjects have rather long research histories, new findings are constantly arriving, and further studies still need to be carried out (Box 2).

As for the lactate oxidizing enzymes, further characterization will be helpful in the development of more efficient and easily operable lactate determination techniques and biocatalysts. Screening for novel enzymes with improved practical properties, such as high temperature stability and catalytic efficiency, is an effective approach. Another promising approach is the use of rationally engineered enzymes. This approach has been extensively applied to various enzymes in modern biotechnology. Therefore, more structural information is required, and this should be the focus of future studies. However, some lactate oxidizing enzymes are peripheral membrane proteins, making them relatively difficult to study. In view of work carried out on mandelate dehydrogenase, which also belongs to the α-hydroxy acid-oxidizing flavoproteins family, it is possible to reconstruct them as soluble proteins for study [79]. As an alternative, advanced operating conditions and engineering processes will also facilitate these applications.

The reviewed studies highlight the importance of microbial lactate utilization on pathogenicity. Considering the abundance of lactate in humans, it can be predicted that there are many other pathogenic microbes that require lactate utilization for invasion and replication. Studies similar to those described above could identify more correlations of microbial lactate utilization with human disease. Furthermore, it may be possible to treat these diseases by using drugs that specifically block lactate utilization in these pathogenic microbes. As the structures of several proteins involved in microbial lactate utilization have been resolved, rational drug design on these targets is feasible.

Box 2. Outstanding questions

- What are the structures and the catalytic mechanisms of quinone-dependent L-iLDHs and cytochrome *c*-dependent D-iLDHs?
- How to engineer iLDHs to develop biocatalysts and biological recognition elements for biosensors with better practical properties?
- Does lactate utilization also play important roles in the colonization processes of pathogenic microorganisms other than the reviewed ones? Are their mechanisms similar?
- Are there drugs that could specifically block the lactate utilization pathway to cure or prevent diseases caused by those pathogenic microorganisms?
- How does the regulation of lactate utilization interact with the entire regulatory network?

The identification of microbial lactate utilization operons provides a new approach for studying the above-mentioned subjects. For example, in designing drugs to block lactate utilization of pathogenic microbes, LldR is a promising target. Similar studies have been carried out on FadR [80], which belongs to the same family as LldR, and thus these sorts of studies are likely to be worthwhile. Another example is in obtaining biocatalysts with constitutively expressed iLDH activities or sole iLDH activity towards one lactate isomer; it may be possible to construct pertinent mutants based on the structure of lactate utilization operons. However, the regulatory mechanism of the operons themselves needs to be further characterized, because present studies lead to different conclusions and hypotheses. The structures of lactate utilization operons vary in different species, and these should be characterized further [5]. Further experimental data regarding the LldR DNA binding sites will also be helpful in clarifying differences in regulatory mechanisms. Furthermore, more structural information about LldR proteins is needed to elucidate the differences in their functions and properties. For example, the structure of LldR in complex with lactate will explain why different inducers are utilized by these proteins. More broadly, the involvement of other regulatory proteins in this operon, or LldR in other operons, needs to be further investigated because both of these situations have been observed [72,76]. It is also noteworthy that D-lactate can be an inducer of the *P. aeruginosa* lactate utilization operon that contains the D-iLDH-encoding gene, whereas the *E. coli* and *C. glutamicum* operons that only include L-iLDH-encoding genes cannot be induced by D-lactate. Therefore, as a broad question, it is still unclear as to why different expression patterns of lactate oxidizing activities are utilized by different microbes.

In summary, although lactate utilization in microbes is a simple pathway with only a few steps, it is rather important to microbial physiological acclimation mechanisms, and the detailed mechanisms involved are diversified. To better understand these mechanisms and to better utilize relevant research findings in medicine and pharmacy, further studies should be carried out.

Acknowledgments

We gratefully acknowledge the support for our previous research from the National Basic Research Program of China (grant number 2011CBA00800), the National Natural Science Foundation of China (grant numbers 31270856, 31270090, and 31170052), and the Chinese National Programs for High Technology Research and Development (grant number 2011AA02A202).

References

1 Garvie, E.I. (1980) Bacterial lactate dehydrogenases. *Microbiol. Rev.* 44, 106–139
2 Goffin, P. *et al.* (2005) Lactate racemization as a rescue pathway for supplying D-lactate to the cell wall biosynthesis machinery in *Lactobacillus plantarum*. *J. Bacteriol.* 187, 6750–6761
3 Goffin, P. *et al.* (2004) Major role of NAD-dependent lactate dehydrogenases in aerobic lactate utilization in *Lactobacillus plantarum* during early stationary phase. *J. Bacteriol.* 186, 6661–6666
4 Ferreira, M.T. *et al.* (2013) Effect of oxygen on glucose metabolism: utilization of lactate in *Staphylococcus aureus* as revealed by *in vivo* NMR studies. *PLoS ONE* 8, e58277
5 Pinchuk, G.E. *et al.* (2009) Genomic reconstruction of *Shewanella oneidensis* MR-1 metabolism reveals a previously uncharacterized

machinery for lactate utilization. *Proc. Natl. Acad. Sci. U.S.A.* 106, 2874–2879
6 Stansen, C. *et al.* (2005) Characterization of a *Corynebacterium glutamicum* lactate utilization operon induced during temperature-triggered glutamate production. *Appl. Environ. Microbiol.* 71, 5920–5928
7 Furuichi, M. *et al.* (2008) X-ray structures of *Aerococcus viridans* lactate oxidase and its complex with D-lactate at pH 4.5 show an alpha-hydroxyacid oxidation mechanism. *J. Mol. Biol.* 378, 436–446
8 Maeda-Yorita, K. *et al.* (1995) L-Lactate oxidase and L-lactate monooxygenase: mechanistic variations on a common structural theme. *Biochimie* 77, 631–642
9 Lederer, F. (2011) Another look at the interaction between mitochondrial cytochrome c and flavocytochrome b_2. *Eur. Biophys. J.* 40, 1283–1299
10 Zhao, R. *et al.* (2013) NAD-dependent lactate dehydrogenase catalyses the first step in respiratory utilization of lactate by *Lactococcus lactis*. *FEBS Open Bio* 3, 379–386
11 Fuller, J.R. *et al.* (2011) Identification of a lactate-quinone oxidoreductase in *Staphylococcus aureus* that is essential for virulence. *Front. Cell. Infect. Microbiol.* 1, 19
12 Chai, Y. *et al.* (2009) A widely conserved gene cluster required for lactate utilization in *Bacillus subtilis* and its involvement in biofilm formation. *J. Bacteriol.* 191, 2423–2430
13 Thomas, M.T. *et al.* (2011) Two respiratory enzyme systems in *Campylobacter jejuni* NCTC 11168 contribute to growth on L-lactate. *Environ. Microbiol.* 13, 48–61
14 Cristescu, M.E. and Egbosimba, E.E. (2009) Evolutionary history of D-lactate dehydrogenases: a phylogenomic perspective on functional diversity in the FAD binding oxidoreductase/transferase type 4 family. *J. Mol. Evol.* 69, 276–287
15 Fraaije, M.W. *et al.* (1998) A novel oxidoreductase family sharing a conserved FAD-binding domain. *Trends Biochem. Sci.* 23, 206–207
16 Dym, O. *et al.* (2000) The crystal structure of D-lactate dehydrogenase, a peripheral membrane respiratory enzyme. *Proc. Natl. Acad. Sci. U.S.A.* 97, 9413–9418
17 Kato, O. *et al.* (2010) Quinone-dependent D-lactate dehydrogenase Dld (Cg1027) is essential for growth of *Corynebacterium glutamicum* on D-lactate. *BMC Microbiol.* 10, 321
18 Pallotta, M.L. *et al.* (2004) Two separate pathways for D-lactate oxidation by *Saccharomyces cerevisiae* mitochondria which differ in energy production and carrier involvement. *Biochim. Biophys. Acta* 1608, 104–113
19 Reed, D.W. and Hartzell, P.L. (1999) The *Archaeoglobus fulgidus* D-lactate dehydrogenase is a Zn^{2+} flavoprotein. *J. Bacteriol.* 181, 7580–7587
20 Pinchuk, G.E. *et al.* (2011) Pyruvate and lactate metabolism by *Shewanella oneidensis* MR-1 under fermentation, oxygen limitation, and fumarate respiration conditions. *Appl. Environ. Microbiol.* 77, 8234–8240
21 Mowat, C.G. *et al.* (2004) Altered substrate specificity in flavocytochrome b_2: structural insights into the mechanism of L-lactate dehydrogenation. *Biochemistry* 43, 9519–9526
22 Tegoni, M. *et al.* (1995) X-ray structure of two complexes of the Y143F flavocytochrome b_2 mutant crystallized in the presence of lactate or phenyl lactate. *Biochemistry* 34, 9840–9850
23 Tsai, C.L. *et al.* (2007) Mechanistic and structural studies of H373Q flavocytochrome b_2: effects of mutating the active site base. *Biochemistry* 46, 7844–7851
24 Cunane, L.M. *et al.* (2002) Crystallographic study of the recombinant flavin-binding domain of baker's yeast flavocytochrome b_2: comparison with the intact wild-type enzyme. *Biochemistry* 41, 4264–4272
25 Tegoni, M. and Cambillau, C. (1994) The 2.6-Å refined structure of the *Escherichia coli* recombinant *Saccharomyces cerevisiae* flavocytochrome b_2-sulfite complex. *Protein Sci.* 3, 303–313
26 Fitzpatrick, P.F. (2004) Carbanion versus hydride transfer mechanisms in flavoprotein-catalyzed dehydrogenations. *Bioorg. Chem.* 32, 125–139
27 Daff, S. *et al.* (1994) Strategic manipulation of the substrate specificity of *Saccharomyces cerevisiae* flavocytochrome b_2. *Biochem. J.* 301, 829–834
28 Sinclair, R. *et al.* (1998) Re-design of *Saccharomyces cerevisiae* flavocytochrome b_2: introduction of L-mandelate dehydrogenase activity. *Biochem. J.* 333, 117–120
29 Diep Le, K.H. *et al.* (2010) Structural evidence for the functional importance of the heme domain mobility in flavocytochrome b_2. *J. Mol. Biol.* 400, 518–530
30 Leiros, I. *et al.* (2006) The 2.1 Å structure of *Aerococcus viridans* L-lactate oxidase (LOX). *Acta Crystallogr. Sect. F: Struct. Biol. Cryst. Commun.* 62, 1185–1190
31 Umena, Y. *et al.* (2006) The crystal structure of L-lactate oxidase from *Aerococcus viridans* at 2.1 Å resolution reveals the mechanism of strict substrate recognition. *Biochem. Biophys. Res. Commun.* 350, 249–256
32 Li, S.J. *et al.* (2007) Crystallographic study on the interaction of L-lactate oxidase with pyruvate at 1.9 Angstrom resolution. *Biochem. Biophys. Res. Commun.* 358, 1002–1007
33 Dym, O. and Eisenberg, D. (2001) Sequence-structure analysis of FAD-containing proteins. *Protein Sci.* 10, 1712–1728
34 Nikolaus, N. and Strehlitz, B. (2007) Amperometric lactate biosensors and their application in (sports) medicine, for life quality and wellbeing. *Microchim. Acta* 160, 15–55
35 Smutok, O. *et al.* (2005) A novel L-lactate-selective biosensor based on flavocytochrome b_2 from methylotrophic yeast *Hansenula polymorpha*. *Biosens. Bioelectron.* 20, 1285–1290
36 Goriushkina, T. (2009) Application of L-lactate-cytochrome *c*-oxidoreductase for development of amperometric biosensor for L-lactate determination. *Biopolym. Cell* 25, 194–203
37 Nguyen-Boisse, T.T. *et al.* (2013) Highly sensitive conductometric biosensors for total lactate, D- and L-lactate determination in dairy products. *Sens. Actuat. B: Chem.* 179, 232–239
38 Aydın, G. *et al.* (2002) Amperometric enzyme electrode for L(+)-lactate determination using immobilized L(+)-lactate oxidase in poly(vinylferrocenium) film. *Sens. Actuat. B: Chem.* 87, 8–12
39 Banica, F.G. (2012) *Chemical Sensors and Biosensors: Fundamentals and Applications*, John Wiley & Sons
40 Pereira, A.C. *et al.* (2011) Development of a carbon paste electrode for lactate detection based on Meldola's Blue adsorbed on silica gel modified with niobium oxide and lactate oxidase. *Electroanalysis* 23, 1470–1477
41 Yashina, E.I. *et al.* (2010) Sol-gel immobilization of lactate oxidase from organic solvent: toward the advanced lactate biosensor. *Anal. Chem.* 82, 1601–1604
42 Zanini, V.P. *et al.* (2010) An L-lactate amperometric enzyme electrode based on L-lactate oxidase immobilized in a laponite gel on a glassy carbon electrode. application to dairy products and red wine. *Electroanalysis* 22, 946–954
43 Garjonyte, R. *et al.* (2006) Mediated amperometric biosensors for lactic acid based on carbon paste electrodes modified with baker's yeast *Saccharomyces cerevisiae*. *Bioelectrochemistry* 68, 191–196
44 Pohanka, M. and Zbořil, P. (2008) Amperometric biosensor for D-lactate. *Food Technol. Biotechnol.* 46, 107–110
45 Gao, C. *et al.* (2010) Pyruvate producing biocatalyst with constitutive NAD-independent lactate dehydrogenases. *Process Biochem.* 45, 1912–1915
46 Xu, P. *et al.* (2008) Biotechnological routes to pyruvate production. *J. Biosci. Bioeng.* 105, 169–175
47 Gao, C. *et al.* (2011) Biotechnological routes based on lactic acid production from biomass. *Biotechnol. Adv.* 29, 930–939
48 Ma, C. *et al.* (2007) Membrane-bound L- and D-lactate dehydrogenase activities of a newly isolated *Pseudomonas stutzeri* strain. *Appl. Microbiol. Biotechnol.* 77, 91–98
49 Ma, C.Q. *et al.* (2003) Highly efficient conversion of lactate to pyruvate using whole cells of *Acinetobacter* sp. *Biotechnol. Prog.* 19, 1672–1676
50 Ma, C.Q. *et al.* (2004) An enzymatic route to produce pyruvate from lactate. *Appl. Microbiol. Biotechnol.* 66, 34–39
51 Burdick, B.A. and Schaeffer, J.R. (1987) Co-immobilized coupled enzyme systems on nylon mesh capable of gluconic and pyruvic acid production. *Biotechnol. Lett.* 9, 253–258
52 Hekmat, D. *et al.* (1999) Production of pyruvate from (R)-lactate in an enzyme-membrane reactor with coupled electrochemical regeneration of the artificial mediator anthraquinone-2,6-disulfonate. *Enzyme Microb. Technol.* 24, 471–479
53 Gao, C. *et al.* (2010) Efficient production of 2-oxobutyrate from 2-hydroxybutyrate by using whole cells of *Pseudomonas stutzeri* strain SDM. *Appl. Environ. Microbiol.* 76, 1679–1682
54 Futai, M. and Kimura, H. (1977) Inducible membrane-bound L-lactate dehydrogenase from *Escherichia coli*. Purification and properties. *J. Biol. Chem.* 252, 5820–5827

55 Gao, C. *et al.* (2012) NAD-independent L-lactate dehydrogenase is required for L-lactate utilization in *Pseudomonas stutzeri* SDM. *PLoS ONE* 7, e36519
56 Gao, C. *et al.* (2009) Enantioselective oxidation of racemic lactic acid to D-lactic acid and pyruvic acid by *Pseudomonas stutzeri* SDM. *Bioresour. Technol.* 100, 1878–1880
57 Gao, C. *et al.* (2011) Kinetic resolution of 2-hydroxybutanoate racemic mixtures by NAD-independent L-lactate dehydrogenase. *Bioresour. Technol.* 102, 4595–4599
58 Jiang, T. *et al.* (2012) Rationally re-designed mutation of NAD-independent L-lactate dehydrogenase: high optical resolution of racemic mandelic acid by the engineered *Escherichia coli*. *Microb. Cell Fact.* 11, 151
59 Hao, J. *et al.* (2006) *Pseudomonas stutzeri* as a novel biocatalyst for pyruvate production from DL-lactate. *Biotechnol. Lett.* 29, 105–110
60 Smith, H. *et al.* (2007) Effect of host lactate on gonococci and meningococci: new concepts on the role of metabolites in pathogenicity. *Infect. Immun.* 75, 4190–4198
61 Smith, H. *et al.* (2001) Lactate stimulation of gonococcal metabolism in media containing glucose: mechanism, impact on pathogenicity, and wider implications for other pathogens. *Infect. Immun.* 69, 6565–6572
62 Britigan, B.E. *et al.* (1988) Phagocyte-derived lactate stimulates oxygen consumption by *Neisseria gonorrhoeae*. An unrecognized aspect of the oxygen metabolism of phagocytosis. *J. Clin. Invest.* 81, 318–324
63 Fu, H.S. *et al.* (1989) Oxidant stress in *Neisseria gonorrhoeae*: adaptation and effects on L-(+)-lactate dehydrogenase activity. *Infect. Immun.* 57, 2173–2178
64 Exley, R.M. *et al.* (2005) *Neisseria meningitidis* lactate permease is required for nasopharyngeal colonization. *Infect. Immun.* 73, 5762–5766
65 Exley, R.M. *et al.* (2005) Available carbon source influences the resistance of *Neisseria meningitidis* against complement. *J. Exp. Med.* 201, 1637–1645
66 Exley, R.M. *et al.* (2007) Lactate acquisition promotes successful colonization of the murine genital tract by *Neisseria gonorrhoeae*. *Infect. Immun.* 75, 1318–1324
67 Herbert, M.A. *et al.* (2002) Signature Tagged Mutagenesis of *Haemophilus influenzae* identifies genes required for *in vivo* survival. *Microb. Pathog.* 33, 211–223
68 Richardson, A.R. *et al.* (2008) A nitric oxide-inducible lactate dehydrogenase enables *Staphylococcus aureus* to resist innate immunity. *Science* 319, 1672–1676
69 Brutinel, E.D. and Gralnick, J.A. (2012) Preferential utilization of D-lactate by *Shewanella oneidensis*. *Appl. Environ. Microbiol.* 78, 8474–8476
70 Erwin, A.L. and Gotschlich, E.C. (1993) Oxidation of D-lactate and L-lactate by *Neisseria meningitidis*: purification and cloning of meningococcal D-lactate dehydrogenase. *J. Bacteriol.* 175, 6382–6391
71 Gao, C. *et al.* (2012) Lactate utilization is regulated by the FadR-type regulator LldR in *Pseudomonas aeruginosa*. *J. Bacteriol.* 194, 2687–2692
72 Aguilera, L. *et al.* (2008) Dual role of LldR in regulation of the *lldPRD* operon, involved in L-lactate metabolism in *Escherichia coli*. *J. Bacteriol.* 190, 2997–3005
73 Georgi, T. *et al.* (2008) Regulation of L-lactate utilization by the FadR-type regulator LldR of *Corynebacterium glutamicum*. *J. Bacteriol.* 190, 963–971
74 Jiang, T. *et al.* (2012) Genome sequence of *Pseudomonas stutzeri* SDM-LAC, a typical strain for studying the molecular mechanism of lactate utilization. *J. Bacteriol.* 194, 894–895
75 Rigali, S. *et al.* (2002) Subdivision of the helix-turn-helix GntR family of bacterial regulators in the FadR, HutC, MocR, and YtrA subfamilies. *J. Biol. Chem.* 277, 12507–12515
76 Gao, Y.G. *et al.* (2008) Structural and functional characterization of the LldR from *Corynebacterium glutamicum*: a transcriptional repressor involved in L-lactate and sugar utilization. *Nucleic Acids Res.* 36, 7110–7123
77 Huffman, J.L. and Brennan, R.G. (2002) Prokaryotic transcription regulators: more than just the helix-turn-helix motif. *Curr. Opin. Struct. Biol.* 12, 98–106
78 San Martin, A. *et al.* (2013) A genetically encoded FRET lactate sensor and its use to detect the Warburg effect in single cancer cells. *PLoS ONE* 8, e57712
79 Sukumar, N. *et al.* (2001) Structure of an active soluble mutant of the membrane-associated (S)-mandelate dehydrogenase. *Biochemistry* 40, 9870–9878
80 van Aalten, D.M. *et al.* (2000) Crystal structure of FadR, a fatty acid-responsive transcription factor with a novel acyl coenzyme A-binding fold. *EMBO J.* 19, 5167–5177