

Lactate Racemization and Beyond

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Abstract

Enzymatic racemization of lactate has been reported in several bacterial species, including *Lactobacillus* species. The role of lactate racemase (Lar) is still a matter of debate and is probably dependent if the species in which it is found is a lactate producer, a lactate consumer, or both. A transcriptomic experiment revealed the involvement of two operons of 9 genes in lactate racemization in *L. plantarum*: the lar (MN) QO and the larABCDE operons. The lactate racemase, LarA, has been shown to harbour a tethered nickel pincer complex, which we call Nickel Pincer Nucleotide or NPN in this review. This cofactor seems well adapted to catalyse lactate racemisation by a hydride transfer mechanism. The cofactor is synthesized from nicotinic acid adenine dinucleotide by the NPN biosynthetic enzymes: LarB, LarC, and LarE. LarD is an aquaglyceroporin, LarR a transcriptional regulator, and Lar (MN) QO a three-component nickel transporter. Lactate racemase gene was reported to be widespread in bacterial and archaeal genomes. We suggest that many other enzymatic functions are present in the LarA superfamily of enzymes in addition to lactate racemization.

Keywords: Lactate racemase; Nickel; Pincer complex; *Lactobacillus plantarum*; Aquaglyceroporin; Transcriptional regulator; Cofactor

The Lactate Racemization Operon

Enzymatic racemization of lactate was first reported in 1936 in *Clostridium beijerinckii* (formerly *C. butylicum*) [1] and was then detected in *Staphylococcus aureus* (formerly *S. ureae*), in *Lactobacillus sakei* (formerly *L. sake*) [2], in *L. plantarum* [3], and in several other *Lactobacillus* sp. [4], as well as in the rumen bacteria *Selenomonas ruminantium* and *Megasphaera elsdenii* [5,6] and in halophilic archaea [7].

The role of lactate racemase (Lar) is still a matter of debate and is probably dependent on the species in which it is found. In bacteria consuming lactate, like *M. elsdenii*, Lar enables the consumption of both L- and D-lactate isomers, despite the presence of only one D- or L-lactate dehydrogenase [6]. In lactic acid bacteria, the presence of a lactate racemase is less straightforward, as lactate is a waste product of fermentation and its racemization is not required for growth. In *L. plantarum*, Lar was suggested to be used as a rescue pathway for the formation of D-lactate, which is incorporated in the cell wall and confers to the bacteria a resistance to the vancomycin antibiotic [8,9]. This role can be extended to the growth conditions in which the bacteria perform malolactic fermentation, producing only L-lactate [10]. However, the presence of the lactic acid channel LarD suggests that this is probably not the only role Lar fulfils in lactic acid bacteria. Lactate racemization of externally produced L-lactate in D-lactate consuming bacteria could be a way for the lactate racemizing bacteria to generate a proton gradient across its cell wall, if a hypothetical transporter of L-lactate was present in these species [10].

The identity of the Lar enzyme remained unknown until a transcriptomic experiment performed in 2014 revealed the involvement of two operons of 9 genes in lactate racemization in *L. plantarum* [9,11]. These genes were shown to encode a nickel transport

system Lar(MN)QO, a nickel-dependent lactate racemase LarA, a set of three proteins of unknown function LarB, LarC and LarE necessary for the maturation of the racemase, a lactic acid channel LarD [12], and a transcriptional regulator LarR responding to the enantiomeric excess of L-lactate [13]. These proteins constitute the lactate racemase system found in *L. plantarum* and in many other *Lactobacillus* species (Figure 1) [10].

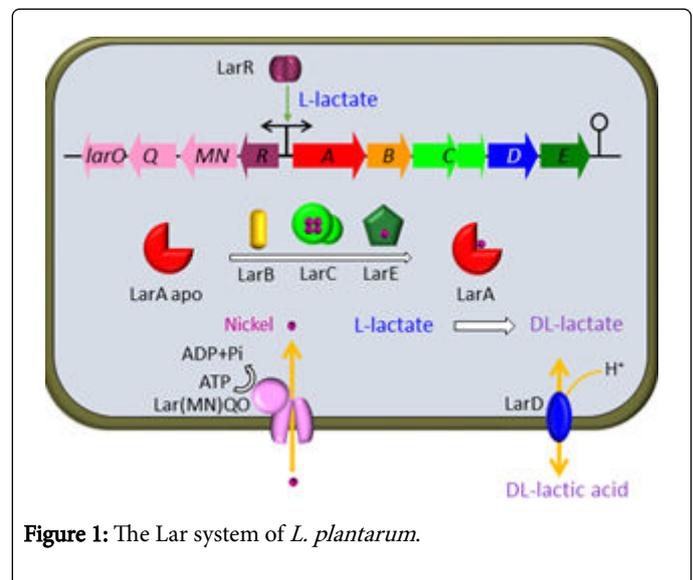


Figure 1: The Lar system of *L. plantarum*.

An Intriguing Cofactor in Lactate Racemase

In 2015, LarA was shown to contain a covalently tethered (SCS) Ni (II) pincer complex, which catalyses lactate racemization (Figure 2) [14]. Pincer complexes are well known in organometallic chemistry but have never been observed in any wild type enzyme before. For simplicity, we call it the nickel pincer nucleotide or NPN. This cofactor

exhibits many original features never previously reported in any biological molecule, such as a stable nickel-carbon bond and the presence of a unique pincer structure made of a pyridinium ring

substituted by two sulfur-containing groups (Figure 2). Both the mechanism of action of NPN in this reaction and the reason why a nickel ion is required are still unknown.

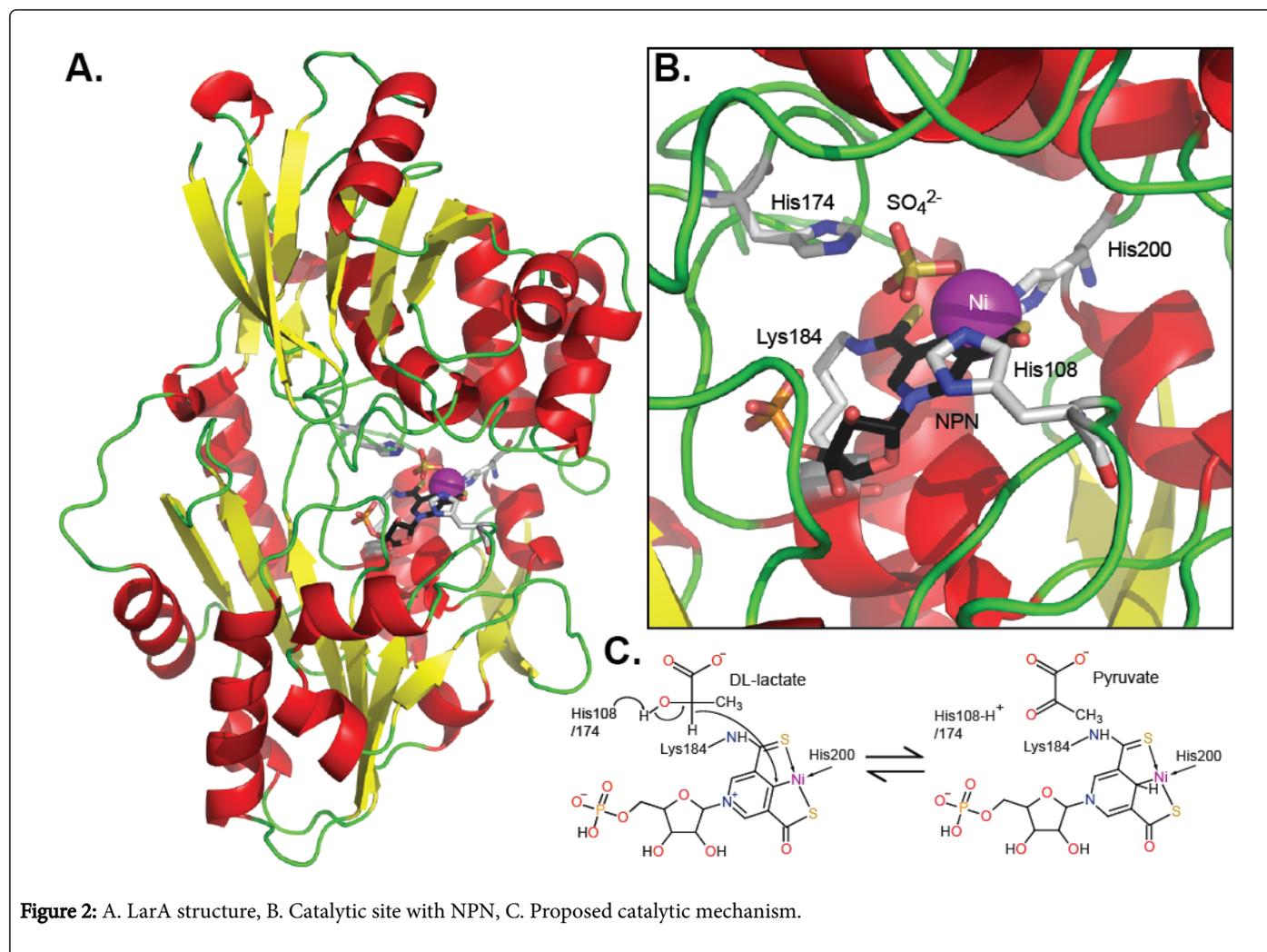


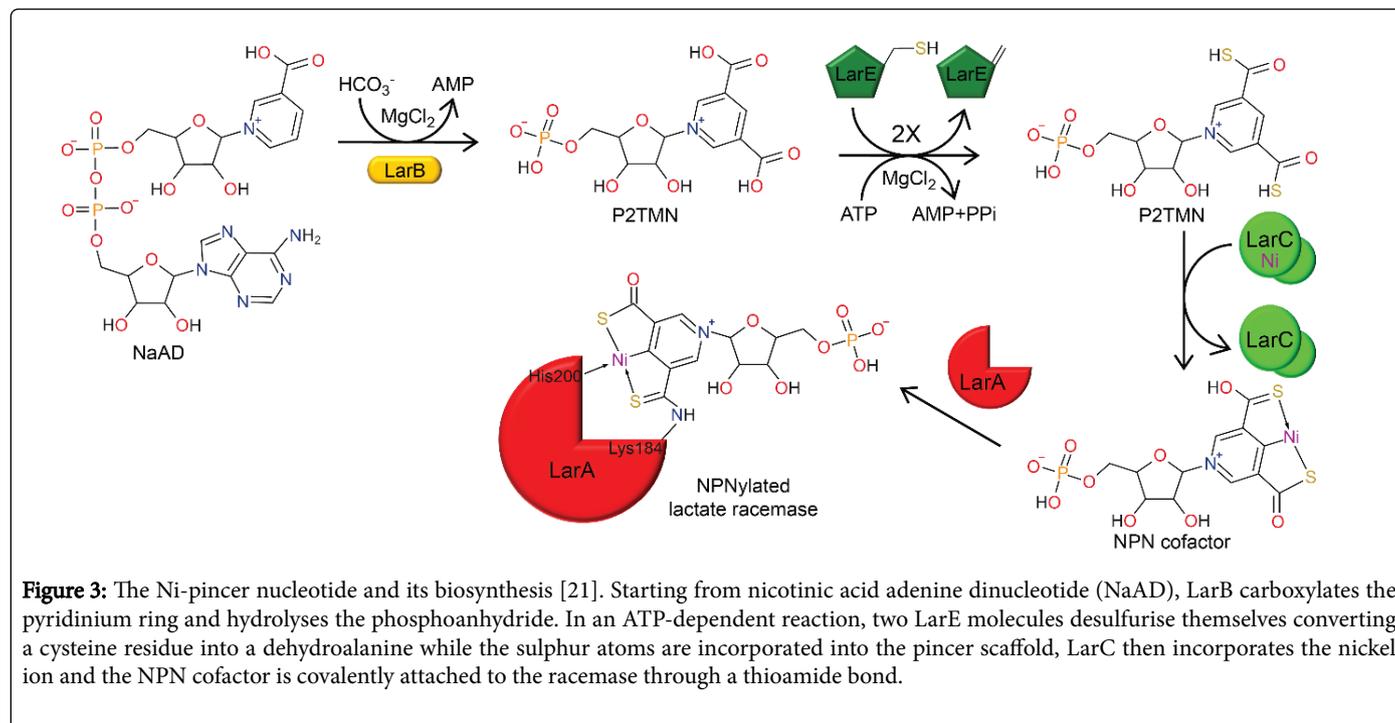
Figure 2: A. LarA structure, B. Catalytic site with NPN, C. Proposed catalytic mechanism.

Past reports suggested that there is a hydride transfer mechanism for lactate racemisation [15,16]. In light of the new cofactor structure, the existence of such a mechanism seems plausible since NPN appears well adapted for hydride transfer chemistry that would generate a pyruvate/NPNH intermediate. This hypothesis has been confirmed by recent DFT calculations, which suggest that the role of NPN is either to destabilize the intermediate [17] or to provide greater hydride-addition reactivity [18]. Following the discovery of NPN in lactate racemase, another mechanism based on proton coupled single electron transfer (PCET) and cleavage of the C-C bond between the alpha carbon and the carboxylic group was proposed based on quantum mechanics/molecular mechanics calculations [19]. In this case, a paramagnetic cofactor, with Ni(III), is predicted to be present in the ground state of the enzyme, and a single acidic-basic residue (His108) is predicted to perform the deprotonation of both lactate isomers, with the reaction then proceeding through different tightly bound intermediates, i.e. acetaldehyde and carbon dioxide radical anion (CO₂⁻). Even though this hypothetical mechanism is plausible, we consider that it is very unlikely since it is not compatible with old kinetic isotope effect (KIE) experiments that indicated a primary isotope effect for lactate racemization in *C. beijerinckii* [16], meaning that the C-H bond is

cleaved during catalysis, and it is very likely that the *C. beijerinckii* racemase is a member of the LarA family. Interestingly, in 2016, a nickel pincer complex featuring some similarities with NPN was synthesized and shown to mediate dehydrogenation of alcohols [20].

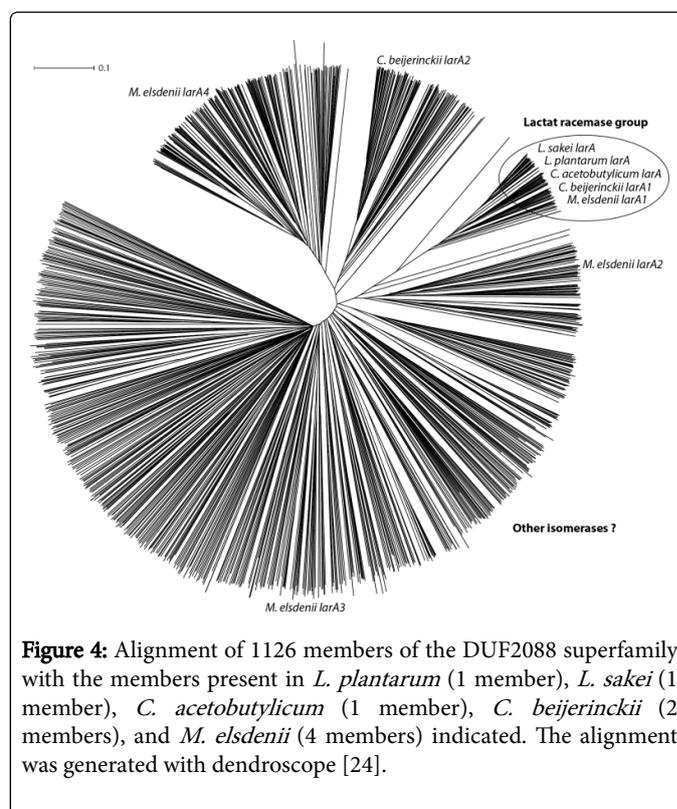
Shortly after the discovery of NPN, its biosynthesis was shown to involve LarB, LarC and LarE and nicotinic acid adenine dinucleotide (NaAD) as a precursor [21]. LarB starts by catalysing the carboxylation of the nicotinic ring that is accompanied by hydrolysis of the phosphoanhydride bond, forming pyridinium biscalboxylic acid mononucleotide (P2CMN). The energy generated by the hydrolysis of the phosphoanhydride bond probably facilitates the carboxylation reaction, although this coupling remains mysterious. LarE converts both the carboxylate groups of P2CMN into thiocarboxylate groups by an ATP-dependent sacrificial sulphur insertion, forming pyridinium bithiocarboxylic acid mononucleotide (P2TMN). As the two sulphur atoms inserted into P2CMN are derived from one cysteine of LarE, two LarE proteins are required for the synthesis of one P2TMN [22]. Finally, nickel-containing LarC catalyses the nickel insertion between the two sulphur atoms and the pyridinium carbon 4 of P2TMN,

generating NPN [21]. Finally, in case of covalent NPNylation, NPN reacts with a lysine of LarA, forming a thioamide bond (Figure 3).



Beyond Lactate Racemisation

A bioinformatic analysis of over 1000 prokaryotic genomes revealed the presence of the genes coding for NPN-biosynthetic enzymes and LarA homologs in 10% of the studied genomes [11] suggesting a wide distribution of lactate racemization in the prokaryotic world. Nevertheless, very few organisms have been shown to actually racemize lactate [1-7]. Furthermore, it has been hypothesized that some LarA homologs likely do not racemize lactate but catalyze some other, probably similar, reaction [10]. LarA is indeed part of a large superfamily, the superfamily of unknown enzymes DUF2088 (or PF09861), which comprises 1126 identified sequences in 662 species [23]. The majority of members of this superfamily are found in bacteria, mostly in Firmicutes (270 species), in Actinobacteria (74) and in Proteobacteria (83 species), yet some members have been found in Archaea (63 species) and even in Eucaryota (17 species, mostly green algae and oomycetes). In this diversity of members, the lactate racemase compose only a fraction of the sequences, 1 in 6 approximately, the vast majority of the members of this family code for enzymes of unknown function (Figure 4). As these enzymes are homologs of LarA, they probably also catalyse epimerization or racemization reactions, which in both cases involve the inversion of a chiral center. Nevertheless, other types of isomerization could also be catalysed by hydride transfer and may also be considered, like cis-trans isomerization or intramolecular oxidoreductions. The observation that some species harbor several LarA paralogs, e.g. 4 paralogs for *M. elsdenii* (Figure 4), strengthens the hypothesis that several other reactions are catalyzed by the members of this superfamily.



In some cases, the reaction catalysed by the LarA homolog can be hypothesized based on its genetic context. In Lactobacillales, some LarA homologs were shown to be associated with citrate lyase genes or with glycolysis preparatory phase genes [10]. This suggests that

isocitrate or metabolites of the glycolysis preparatory phases could be substrates for these LarA homologs. In *Thermotoga maritima*, a LarA homolog (termed GntE) is associated with genes of the hexuronate catabolic pathway and has been suggested to epimerise D-mannanate to D-gluconate [25]. As this reaction involves the inversion of a stereocenter in a position to a carboxylic acid, as lactate racemization, this reaction is very likely catalysed by this LarA homolog.

Furthermore, the genes coding for NPN-biosynthesis enzymes were also identified in another 15% of the studied genomes that lack larA [11], suggesting that enzymes that are not homologous to LarA use NPN as well. These enzymes could catalyse many other reactions requiring NPN, not necessarily involving an epimerization or racemization reaction and not necessarily involving a hydride transfer mechanism.

Conclusion

As a conclusion, we can say the lactate racemization is probably just the tip of the iceberg of all NPN-dependent enzymatic reactions, there is still a lot to discover on the subject.

References

1. Tatum EL, Peterson WH, Fred EB (1936) Enzymic racemization of optically active lactic acid. *Biochem J* 30: 1892-1897.
2. Katagiri H, Kitahara K (1937) Racemase, an enzyme which catalyses racemization of lactic acids. *Biochem J* 31: 909-914.
3. Kitahara K, Obayashi A, Fukui S (1952) Racemase. I. Cell-free racemase. *Enzymologia* 15: 259-266.
4. Stetter KO, Kandler O (1973) Formation of DL-lactic acid by lactobacilli and characterization of a lactic acid racemase from several streptobacteria (author's transl). *Arch Mikrobiol* 94: 221-247.
5. Melville SB, Michel TA, Macy JM (1988) Pathway and sites for energy conservation in the metabolism of glucose by *Selenomonas ruminantium*. *J Bacteriol* 170: 5298-5304.
6. Hino T, Kuroda S (1993) Presence of lactate dehydrogenase and lactate racemase in *Megasphaera elsdenii* grown on glucose or lactate. *Appl Environ Microbiol* 59: 255-259.
7. Oren A, Gurevich P (1995) Diversity of lactate metabolism in halophilic archaea. *Can J Microbiol* 41: 302-307.
8. Ferain T, Hobbs JN, Richardson J, Bernard N, Garmyn D, et al. (1996) Knockout of the two *ldh* genes has a major impact on peptidoglycan precursor synthesis in *Lactobacillus plantarum*. *J Bacteriol* 178: 5431-5437.
9. Goffin P, Deghorain M, Mainardi JL, Tytgat I, Champomier-Vergès MC, 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.
10. Desguin B, Soumillion P, Hausinger RP, Hols P (2017) Unexpected complexity in the lactate racemization system of lactic acid bacteria. *FEMS Microbiol Rev* 41: S71-S83.
11. Desguin B, Goffin P, Viaene E, Kleerebezem M, Diaconescu VM, et al. (2014) Lactate racemase is a nickel-dependent enzyme activated by a widespread maturation system. *Nat Commun* 5: 3615.
12. Bienert GP, Desguin B, Chaumont F, Hols P (2013) Channel-mediated lactic acid transport: a novel function for aquaglyceroporins in bacteria. *Biochem J* 454: 559-570.
13. Desguin B, Goffin P, Bakouche N, Diman A, Viaene E, et al. (2015) Enantioselective regulation of lactate racemization by LarR in *Lactobacillus plantarum*. *J Bacteriol* 197: 219-230.
14. Desguin B, Zhang T, Soumillion P, Hols P, Hu J, et al. (2015) A tethered niacin-derived pincer complex with a nickel-carbon bond in lactate racemase. *Science* 349: 66-69.
15. Cantwell A, Dennis D (1974) Lactate racemase. Direct evidence for an alpha-carbonyl intermediate. *Biochemistry* 13: 287-291.
16. Shapiro SS, Dennis D (1965) Lactic Acid Racemization in *Clostridium butylicum*. Evidence for a Direct Internal Hydride Shift*. *Biochemistry* 4: 2283-2288.
17. Zhang X, Chung LW (2017) Alternative Mechanistic Strategy for Enzyme Catalysis in a Ni-Dependent Lactate Racemase (LarA): Intermediate Destabilization by the Cofactor. *Chemistry* 23: 3623-3630.
18. Yu MJ, Chen SL (2017) From NAD+ to Nickel Pincer Complex: A Significant Cofactor Evolution Presented by Lactate Racemase. *Chemistry* 23: 7545-7557.
19. Wang B, Shaik S (2017) The Nickel-Pincer Complex in Lactate Racemase Is an Electron Relay and Sink that acts through Proton-Coupled Electron Transfer. *Angew Chem Int Ed Engl* 56: 10098-10102.
20. Xu T, Wodrich MD, Scopelliti R, Corminboeuf C, Hu X (2017) Nickel pincer model of the active site of lactate racemase involves ligand participation in hydride transfer. *Proc Natl Acad Sci USA* 114: 1242-1245.
21. Desguin B, Soumillion P, Hols P, Hausinger RP (2016) Nickel-pincer cofactor biosynthesis involves LarB-catalyzed pyridinium carboxylation and LarE-dependent sacrificial sulfur insertion. *Proc Natl Acad Sci USA* 113: 5598-5603.
22. Fellner M, Desguin B, Hausinger RP, Hu J (2017) Structural insights into the catalytic mechanism of a sacrificial sulfur insertase of the N-type ATP pyrophosphatase family, LarE. *Proc Natl Acad Sci USA* 114: 9074-9079.
23. Finn RD, Cogill P, Eberhardt RY, Eddy SR, Mistry J, et al. (2016) The Pfam protein families database: towards a more sustainable future. *Nucleic Acids Res* 44: 279-285.
24. Huson DH, Scornavacca C (2012) Dendroscope 3: an interactive tool for rooted phylogenetic trees and networks. *Syst Biol* 61: 1061-1067.
25. Rodionova IA, Scott DA, Grishin NV, Osterman AL, Rodionov DA (2012) Tagaturonate-fructuronate epimerase UxaE, a novel enzyme in the hexuronate catabolic network in *Thermotoga maritima*. *Environ Microbiol* 14: 2920-2934.