Lactate racemase is a nickel-dependent enzyme activated by a widespread maturation system

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Racemases catalyse the inversion of stereochemistry in biological molecules, giving the organism the ability to use both isomers. Among them, lactate racemase remains unexplored due to its intrinsic instability and lack of molecular characterization. Here we determine the genetic basis of lactate racemization in *Lactobacillus plantarum*. We show that, unexpectedly, the racemase is a nickel-dependent enzyme with a novel α/β fold. In addition, we decipher the process leading to an active enzyme, which involves the activation of the apo-enzyme by a single nickel-containing maturation protein that requires preactivation by two other accessory proteins. Genomic investigations reveal the wide distribution of the lactate racemase system among prokaryotes, showing the high significance of both lactate enantiomers in carbon metabolism. The even broader distribution of the nickel-based maturation system suggests a function beyond activation of the lactate racemase and possibly linked with other undiscovered nickel-dependent enzymes.

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Lactic acid (L- and D-isomers) is an important and versatile compound produced by microbial fermentation. It is used in a range of applications in the agro-food, pharmaceutical and chemical sectors where optical purity is of tremendous importance. Lactic acid is also common in numerous ecosystems and is involved in the energy metabolism of many prokaryotic species, as a product of sugar fermentation or as a carbon and electron source to sustain growth. It can even be a component of the bacterial cell wall in order to confer resistance to the vancomycin antibiotic. Micro-organisms have the remarkable ability to metabolize both lactic acid isomers via stereospecific lactate dehydrogenases. However, when only one stereospecific lactate dehydrogenase is present, production or utilization of the other isomer may proceed by lactate isomerization involving a specific lactate racemase (Lar). This activity was first reported in *Clostridium beijerinckii* (formerly *C. butylicum*) and, since then, it has been identified in several species including *Lactobacillus*.

Besides Lar, the only known α-hydroxyacid racemase is the mandelate racemase, which is a Mg-dependent enzyme of the enolase superfamily. The majority of racemases are amino acid racemases, which are either pyridoxal 5'-phosphate (PLP)-dependent or PLP-independent enzymes. Both mandelate racemase and PLP-independent racemases rely on intramolecular stabilization of a deprotonated reaction intermediate for their catalysis. Concerning the Lar, another mechanism is probably involved in lactate racemization, a transcriptomic approach was used based on the induction of Lar activity by L-lactate but not by D-lactate. Besides the previously reported *larA–E* operon, we identified a second operon consisting of four genes (*lp_0103 to lp_0100*) as positively induced by L-lactate (Supplementary Table 1). This operon is located upstream of the *larA–E* operon in an opposite orientation (Fig. 1a). The first gene of the operon, named *larR*, codes for a transcriptional regulator of the Crp-Fnr family while the other three genes, *lar(MN)QO*, encode a three-component ATP-binding cassette (ABC) transporter (Fig. 1a). Intriguingly, this ABC transporter is homologous to high-affinity Ni transporters, which are generally associated with Ni-dependent enzymes, although no such enzyme is known or predicted in *L. plantarum*. We investigated the contribution of this transporter to the Lar activity by marker-less gene

**Results**

Four proteins and Ni are required for *in vivo* Lar activity. In order to investigate whether the previously identified *larA–E* operon of *L. plantarum* is sufficient to confer Lar activity, it was cloned on a multicopy plasmid and expressed in the heterologous host *Lactococcus lactis*—a lactic acid bacterium with no Lar activity—under the control of a nisin-inducible promoter. Although Lar proteins could easily be detected from cell extracts of nisin-induced cultures after gel separation, no Lar activity could be measured. As the *larA–E* operon was not sufficient to confer Lar activity, we hypothesized that additional genes were required.

To obtain an extended view of potential *L. plantarum* genes involved in lactate racemization, a transcriptomic approach was used based on the induction of Lar activity by L-lactate but not by D-lactate. Besides the previously reported *larA–E* operon, we identified a second operon consisting of four genes (*lp_0103 to lp_0100*) as positively induced by L-lactate (Supplementary Table 1). This operon is located upstream of the *larA–E* operon in an opposite orientation (Fig. 1a). The first gene of the operon, named *larR*, codes for a transcriptional regulator of the Crp-Fnr family while the other three genes, *lar(MN)QO*, encode a three-component ATP-binding cassette (ABC) transporter (Fig. 1a). Intriguingly, this ABC transporter is homologous to high-affinity Ni transporters, which are generally associated with Ni-dependent enzymes, although no such enzyme is known or predicted in *L. plantarum*. We investigated the contribution of this transporter to the Lar activity by marker-less gene

**Figure 1 | Analysis of the *lar* gene cluster and its encoded Lar proteins.** (a) *lar* locus of *L. plantarum* with the two operons *lar(MN)QO* and *larA–E*. (B) Effect of *larA–E* and *larQO* deletions in *L. plantarum* on specific Lar activity in crude extracts. Supplementation assays of the Δ*larQO* mutant with CoCl$_2$ (1 mM) and NiCl$_2$ (0.2, 0.4, 0.8 and 1.5 mM). (C) Specific Lar activity in crude extracts after the expression of *lar* genes in *Lc. lactis* *larA–E* operon ± NiCl$_2$ (1 mM), in frame deletions of individual genes (Δ) in the *larA–E* operon with NiCl$_2$ supplementation (1 mM). Data in b and c are average of quadruplicates from one representative experiment of three independent experiments showing similar results. The error bars represent the 95% confidence interval (Student’s t-test).
LarA is the Ni-dependent Lar. To evaluate which protein is catalytically active as Lar, each protein of the operon was purified in the presence of Ni in the culture medium. For purification needs, each one was individually fused to a StrepII-tag at either its N- or C terminus and expressed in Lc. lactis using the larA–E expression vector. Compatibility of the inserted StrepII-tags with Lar activity was verified before purification (Fig. 2a). Purification of LarA and LarE could readily be achieved from the strain expressing the entire operon, whereas tagged LarB and LarC could only be purified when the corresponding genes were individually subcloned (LarA-Lp, LarB, LarC and LarE; Fig. 2b). For crystallographic needs, we also purified a LarA ortholog belonging to the family of Ni-dependent metalloenzymes. To validate the importance of Ni, each one was individually fused to a StrepII-tag at either its N- or C terminus and expressed in Lc. lactis harboring a 1-bp insertion at the end of larC (C-fused), with the in-frame deletions of larC2 and expressing the artificial operon larATtBCDE in which LarATt has been fused to a StrepII-tag at the C terminus (A-TtST). NiCl2 was added in all cases (1 mM). Data are average of quadruplicates from one representative experiment of two independent experiments showing similar results. The error bars represent the 95% confidence interval (Student’s t-test). (b) SDS-PAGE of purified StrepII-tagged LarA-Lp, LarA-Tt, LarB, LarC (LarC1 and LarC2), LarE and LarC-fused (only LarC1C2).

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similarly reported for the activation of three other nickel-dependent enzymes.\textsuperscript{24}

**LarA shows a novel \(\alpha/\beta\) fold.** In order to identify the Lar catalytic site, the crystal structure of Lar\textsubscript{ATt} was determined. Lar\textsubscript{ATt} crystals were obtained after 2 months of crystallization. The structure was solved by multi-wavelength anomalous dispersion and the structure was refined to 1.8 Å (Table 2). As expected, given the spontaneous Ni leakage from the enzyme, no Ni was present in the crystals. Crystals were soaked with NiCl\textsubscript{2} in presence or absence of lactate but no Ni incorporation was observed. The enzyme crystallized as a dimer with both monomers showing nearly identical structures (Fig. 3a). LarA contains 18 \(\beta\)-strands and 16 \(\alpha\)-helices arranged in a novel fold composed of two domains of similar size, connected by two hinges (Fig. 3b and Supplementary Fig. 3 for a stereo view). As the strand order 162345, observed in domain A, was not found in any fold of the SCOP database,\textsuperscript{25} we hypothesize that LarA shows a new fold of the \(\alpha/\beta\) class.

**LarA Ni centre is coordinated by His residues.** In order to characterize the binding site of Ni in the LarA structure, X-ray absorption spectroscopy (XAS) experiments were conducted on

**Table 1 | Properties of LarA from *L. plantarum* (LarA\textsubscript{LP}) and *T. thermosaccharolyticum* (LarA\textsubscript{TT}).**

| Proteins | L \(\rightarrow\) D-Lac | D \(\rightarrow\) L-Lac | Nickel content (%) |
|----------|----------------|----------------|------------------|
|          | \(k_{cat}\) (s\(^{-1}\)) | \(K_m\) (mM) | \(k_{cat}\) (s\(^{-1}\)) | \(K_m\) (mM) | ICP-AES | PAR |
| LarA\textsubscript{LP} | 4,745 ± 544 | 46 ± 20 | 1,333 ± 131 | 11 ± 4 | 21.6 ± 0.8 | 19 ± 6 |
| LarA\textsubscript{TT} | 986 ± 76 | 8 ± 3 | 551 ± 102 | 3 ± 2 | 15.3 ± 1.0 | 11 ± 3 |

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**Figure 3** | **3D structure and topology of LarA.** (a) 3D dimeric structure of LarA1 (RSCB Protein Data Bank (PDB) accession code 2YJG). α-Helices are in red, β-sheets in yellow, and loops in green. Conserved residues are shown in stick representation and ethylene glycol, sulphate and Mg(II) are in sphere representation with C in white, N in blue, O in red, Mg in cyan and S in yellow. Surface representation is shown at the background. (b) The Lar fold. α-Helices are in red, β-strands in yellow and loops are in black. The topology of the β-sheet of domain A is six parallel β-strands in the order 162345. The topology of the β-sheet of domain B is six β-strands in the order 321456 with the last β-strand antiparallel to the rest. The numbers indicate the position of the two hinges.

**Figure 4** | **LarA catalytic site.** (a) Catalytic site of the first monomer (b) Catalytic site of the second monomer. Sulphates and ethylene glycol are displayed. Colour code as in Fig. 3a.
consistent with the strong over-representation of His residues in LarC in a ratio of about 1.5:1 (Fig. 2b). This observation is protein, considering that purified LarC is a mixture of LarC1 and Ni content among all Lar proteins with 7–10 mol Ni per mol of LarE, albeit at different levels. Purified LarC displayed the highest (FT) of LarATt (Fig. 5b,c) is dominated by an intense feature at X-ray absorption fine structure). The Fourier-transformed spectra transition (Fig. 5a). LarA was also analysed by EXAFS (extended spectroscopy using PAR (Fig. 6a)22. Ni was detected in LarC and assayed in purified LarB, LarC and LarE by ICP-AES and visible systems) that inserts Ni into the catalytic site24. Therefore, Ni was provided by LarC that could act as a Ni carrier/storage protein. Not co-expressed (Fig. 6a). This shows the requirement of LarC to undetectable levels when Ni(II) was present but LarBC were culture medium during LarE expression, and went further down 0.08 mol Ni per mol of protein when no Ni(II) was present in the presence of Ni and LarBC. The amount of Ni decreased to of protein were found when the protein was expressed in the presence of a His-rich region. As for LarE, 0.8 mol Ni per mol of protein were found when the protein was expressed in the presence of Ni and LarBC. The amount of Ni decreased to 0.08 mol Ni per mol of protein when no Ni(II) was present in the culture medium during LarE expression, and went further down to undetectable levels when Ni(II) was present but LarBC were not co-expressed (Fig. 6a). This shows the requirement of LarC and/or LarB for the Ni loading of LarE, the Ni probably being provided by LarC that could act as a Ni carrier/storage protein.

**LarC and LarE are Ni-containing proteins.** The assembly of nickel metalcenters by maturases usually requires the presence of at least one nickel carrier (for example, UreE in the urease system) that inserts Ni into the catalytic site24. Therefore, Ni was assayed in purified LarB, LarC and LarE by ICP-AES and visible spectroscopy using PAR (Fig. 6a)22. Ni was detected in LarC and LarE, albeit at different levels. Purified LarC displayed the highest Ni content among all Lar proteins with 7–10 mol Ni per mol of protein, considering that purified LarC is a mixture of LarC1 and LarC in a ratio of about 1.5:1 (Fig. 2b). This observation is consistent with the strong over-representation of His residues in LarC (8.0 versus 1.7% in average *L. plantarum* proteins25) and the presence of a His-rich region. As for LarE, 0.8 mol Ni per mol of protein were found when the protein was expressed in the presence of Ni and LarBC. The amount of Ni decreased to 0.08 mol Ni per mol of protein when no Ni(II) was present in the culture medium during LarE expression, and went further down to undetectable levels when Ni(II) was present but LarBC were not co-expressed (Fig. 6a). This shows the requirement of LarC and/or LarB for the Ni loading of LarE, the Ni probably being provided by LarC that could act as a Ni carrier/storage protein.

**In vitro activation of LarA by a LarBC-activated LarE.** To investigate LarA activation by the putative Lar accessory proteins, *in vitro* experiments were performed. For this purpose, an inactive version of LarA (apoprotein, apo-LarA) was purified from a strain lacking larBCE (ALpNi, Fig. 5b). The ability of purified LarB, LarC and LarE to activate apo-LarA was then evaluated in

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**Figure 5 | XANES and EXAFS spectra.** (a) Ni K-edge XANES spectra of LarATt and LarALp showing a four-square planar or five-square pyramidal coordination geometry for nickel binding; 10 mM Tris buffer, pH 7.5, 20% glycerol. (b) Ni K-edge EXAFS spectra of LarATt and fit for N2H1H1S1. Fourier-transformed EXAFS spectra (no phase correction, FT window = 2-12.5 Å⁻¹). Inset: k³-weighted unfiltered EXAFS spectra; data (circles), best fit (line). (c) Ni K-edge EXAFS spectra of LarATt and fit for N2H2H1S1. Fourier-transformed EXAFS spectra (no phase correction, FT window = 2-12.5 Å⁻¹). Inset: k³-weighted unfiltered EXAFS spectra; data (circles), best fit (line). N: N/O scatterers, H: histidine scatterer, S: S/Cl scatterers and G: glycerol scatterer.
Lar accessory proteins. (a) Ni content of Lar proteins as measured by PAR assays and ICP-AES. PAR data are average of two independent experiments (triplicates in each experiment). ICP-AES data are average of duplicates from one experiment. (b) Specific Lar activity of purified proteins and in vitro activation of apo-LarA. A\textsuperscript{NiBCE} LarA purified from a Lc. lactis strain expressing LarBCE and cultivated in the presence of Ni(II); A\textsuperscript{NiBCE} LarA purified from a Lc. lactis strain not expressing LarBCE and cultivated in the presence of Ni(II); + E\textsuperscript{NiBCE}, assay performed in the presence of an excess of LarE purified from a Lc. lactis strain expressing LarBCE and cultivated in the presence of Ni(II); + E\textsuperscript{BCE}, assay performed in the presence of an excess of LarE purified from a Lc. lactis strain not expressing LarBCE and cultivated in the absence of Ni(II); + E\textsuperscript{NiBC}, assay performed in the presence of an excess of LarE purified from a Lc. lactis strain not expressing LarBCE and cultivated in the presence of Ni(II); + E\textsuperscript{Ni}, LarB purified from a Lc. lactis strain cultivated in the presence of Ni(II); and C\textsuperscript{BC}, LarC purified from a Lc. lactis strain cultivated in the presence of Ni(II). Data are average of quadruplicates from one representative experiment of two independent experiments showing similar results. L-lactate was used as a substrate for all Lar activity measurements. The error bars represent the 95% confidence interval (Student’s t-test) in a and b.

Figure 6 | Lar accessory proteins.

LarA and its maturation system are widespread in prokaryotes. In order to get an overview of the role of Lar proteins in the microbial world, we analysed the distribution of lar genes from the larA–E operon in prokaryotic genomes. The lar genes encoding putative nickel transport and regulation proteins (cluster larR(MN)QO) were not considered in this in silico analysis since nickel transport may be achieved by a wide variety of transporters\textsuperscript{19} and transcriptional regulation of lactate racemization is not a prerequisite for this function. BlastP searches were performed against all complete prokaryotic genomes of the NCBI database (1,087 bacterial and archaeal genomes) using the different Lar proteins of \textit{L. plantarum} WCFS1 as query sequences. This search revealed the presence of at least one homologue of larA, larB, larC, larD and larE, in 111, 260, 263, 9 and 259 species, respectively (Fig. 7a). The larA gene appears to be present in most bacterial classes and in archaea. The largest number of larA homologues were found in clostridia (26 out of 78 species) and in δ proteobacteria (19 out of 39 species), some species bearing up to 4 larA paralogues (Supplementary Tables 5, 6 and 7). This suggests that lactate racemization may not only be useful to lactic acid producers such as lactic acid bacteria but also to a wide variety of species with different metabolisms, including aceticogenic, sulphate-reducing, metal-reducing, fumarate-reducing and butyrate-producing bacteria (Supplementary Table 6). These bacterial taxa are indeed documented to utilize lactate as a carbon and/or electron source\textsuperscript{28–31}.

Ninety-two per cent of the genomes bearing a larA homologue (102 out of 111 genomes) also contained the genes for the Lar accessory proteins (larBCE), further reinforcing the necessity of these proteins for LarA activation (Fig. 7a). Strikingly, the accessory protein-encoding genes were also found in 153 species with no larA homologues (Fig. 7a). To get a better view of the relationship between these genes, their clustering within each genome was examined (Fig. 7b). The presence of a complete cluster including larABCDE seems to be restricted to only six species, all of them belonging to the lactobacillaceae family (Supplementary Table 6). As LarD was shown to be a lactic acid channel\textsuperscript{18}, the expression of the whole cluster is expected to

Figure 7 | In silico analysis of lar genes. (a) Distribution of the lar genes in 1,087 sequenced bacterial and archaeal genomes. The Venn diagram illustrates the occurrence and overlap of predicted larA (red), larB (blue), larC (green), larD (blue) and larE genes (olive green). (b) Gene clustering of predicted larA (A), larB (B), larC (C), larD (D) and larE (E) genes in 1,087 bacterial and archaeal genomes. The numbers indicate the total number of clusters of each type. Non-clustered representatives are not included in b.

enhance the lactic acid transport, besides the racemization of lactate. Twelve species harbour a larABCE cluster, but the most recurrent cluster only includes larBCE, which appears in 69 species, among which only 23 species also possess a larA homologue (Fig. 7b). Such putative operonic structures suggest that these genes likely participate in a common function that is not necessarily linked with lactate racemization.

Discussion
Nickel is an essential component of eight metalloenzymes involved in energy (for example, hydrogenases) and nitrogen (urease) metabolism and is used by 80% of the archaea and 60% involved in energy (for example, hydrogenases) and nitrogen metabolism and is used by 80% of the archaea and 60% of the eubacteria. As we showed that the Lar is Ni-dependent, the number of Ni enzymes is now brought to nine. In this study, we characterized the Lar, LarA, and determined its 3D structure, which shows a novel multidomain fold of the αβ class. When comparing LarA structure with all known folds using the VAST algorithm, very few similarities with known structure could be identified (best score of 12.3 with E value of 1.45E-02, supplementary Table 8). Nevertheless, domain A was found to be weakly similar to the small domain of trimethylamine dehydrogenase, whose function is unknown, and domain B was found to share some similarities with S-adenosyl methionine (SAM)-dependent methyltransferases, although the SAM-binding site is neither conserved in the LarA structure nor in its primary sequence (Fig. 3a and Supplementary Fig. 5). The catalytic site was predicted to be composed of 7 conserved residues (3 His, 2 Lys, 1 Asp and 1 Arg). XAS analyses suggest that at least two histidines are involved in Ni coordination. The conserved residues His108, His174 and His200 are good candidates for this function. A lactate molecule, showing the same O-C-C-O connectivity as glycerol and binding in a bidentate fashion, would also coordinate Ni. Finally, a yet undefined ligand would complete the coordination sphere, forming the predicted five coordinate square pyramidal site.

Although nickel is absolutely required as a central component of the catalytic machinery of Ni-dependent enzymes, it can only be found in trace amounts in the environment. Therefore, sufficient nickel acquisition by these enzyme systems is a consequential process that can also be complicated by the expression of several nickel enzymes in the same organism. Specific nickel-trafficking proteins are necessary to meet the distinct cellular demands for nickel. Those accessory proteins that are responsible for shuttling the nickel are thought to transfer nickel to the enzyme precursors through protein–protein interactions in a complex stepwise process. However, Ni-binding accessory proteins were only identified in three of the eight Ni-dependent enzymes, that is, urease, [NiFe]-hydrogenase and carbon monoxide dehydrogenase (illustrated for the urease at Fig. 8a). In this study, we identified three new accessory proteins, LarB, LarC and LarE, which are required for the Lar activity. These accessory proteins participate in the incorporation of Ni in the Lar apoprotein, as their absence leads to an inactive Ni-less enzyme (Fig. 6). In addition, this mechanism is flexible and conserved among Lars, as L. plantarum accessory proteins are able to activate the orthologous LarA2 enzyme (Table 1).

The incorporation of Ni in the apoprotein usually requires several accessory proteins and the hydrolysis of GTP (Fig. 8a). Yet, only one accessory protein, LarE, is required in the Lar system and ATP or GTP addition had no effect (Supplementary Table 4). As LarE primary sequence shows similarities with ATP-utilizing enzymes of the PP-loop superfamily, it is tempting to propose that the hydrolysis of ATP in AMP is taking place during the activation cascade, but more likely for the activation of LarE itself rather than for the activation of LarA. Furthermore, an excess of Ni can generally overcome the loss of one or several accessory proteins in other nickel-based systems, whereas here Ni supplementation could neither complement the absence of any Lar accessory protein in vivo nor activate LarA apoprotein in vitro (Fig. 1c and Supplementary Table 4). This suggests that the metalcenter of the Lar contains one or more ligand(s) in addition to Ni. In this case, LarE would serve as a scaffold protein for the synthesis of the Ni-containing metalcenter, which is then transferred into the catalytic site of LarA in one step. The synthesis of this metalcenter on LarE would require LarB and LarC. As LarC purified from L. lactis cells grown in the presence of Ni was shown to contain nickel independently of LarE and/or LarB, LarC is probably the Ni carrier of the Lar system (see Fig. 8b for a model). This activation mechanism is completely different from other maturase-activated Ni-dependent enzymes, where the assembly of the metalcenter takes place on the apoprotein and the Ni carrier transfers its Ni directly into the catalytic site (Fig. 8a). Yet, some similarities may be found with the activation mechanism described for [FeFe]-hydrogenases. This mechanism also involves one accessory protein (HydF), able to activate the apoprotein only when purified in presence of two other accessory proteins (HydG and HydE) (Fig. 8c), but these similarities refer only to the overall sequence of the activation cascade.

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**Figure 8 | Model of assembly of Lar metallocenter.** (a) Assembly of urease metallocenter: one triangle represents one urease trimer of UreABC. UreD, UreE, UreF and UreG are the urease accessory proteins. (b) Proposed model for the assembly of Lar metallocenter. LarA is the Lar, while LarB, LarC and LarE are the Lar accessory proteins. (c) Assembly of [FeFe]-hydrogenase metallocenter. HydA is the [FeFe]-hydrogenase. HydE and HydG are the [FeFe]-hydrogenase accessory proteins. For clarity, the Fe5–clusters of [FeFe]-hydrogenase have been omitted. The purple balls represent Ni or a Ni-containing centre in (a,b). The dark red balls represent the H-cluster in (c). ATP, adenosine triphosphate; AMP, adenosine monophosphate; GTP, guanosine triphosphate; GDP, guanosine diphosphate; Pi, phosphate; SAM, S-adenosyl methionine.
To conclude, this work reports the first molecular characterization of a Lar that is a novel maturase-activated Ni-dependent enzyme. The requirement for Ni is a novelty among racemases but the absence of an electron-withdrawing group on lactate may explain its use for the catalysis of lactate racemization by a postulated hydride transfer mechanism. This hypothesis is supported by the identification of a similar catalytic mechanism in [NiFe]-hydrogenases. In addition, the proposed assembly of the metalcenter on a single pre-activated maturation protein is novel and has not been described so far for any other maturase-activated Ni-dependent enzyme. Finally, the occurrence of the genes encoding the Lar maturation machinery in many bacterial and archaeal genomes containing or not a Lar-encoding gene shows the broad importance of this novel Ni-based system and also suggests that this machinery might have been recruited for another function probably linked with the activation of one or more Ni-dependent enzyme(s).

**Methods**

**Biological material and growth conditions.** Bacterial strains and plasmids used in the present study are listed in Supplementary Table 9. All plasmid constructions were performed in *Escherichia coli*. DH10B was used as template and in *L. lactis* NZ9000 for pNZ8048 derivatives. *L. plantarum* was grown in De Man–Rogosa–Sharpe (MRS) broth at 28 °C without shaking. *L. lactis* was grown in M17 broth supplemented with 0.5% glucose at 28 °C at 120 rpm. When appropriate, chloramphenicol and kanamycin were used at concentrations of 10 μg/ml and 10 μg/ml, respectively.

**Construction of the _larQ* mutant.** The *larQ* deletion vector pGIR001 was constructed in two steps. Initially, a 1.62-kb fragment located downstream of *larQ* was amplified by PCR with primers LP096A1 and LP0101B1. This fragment was digested with ClaI and self-ligated, generating an in-frame deletion of the selected gene. The ligation mixture was digested with DpnI before transformation in *L. lactis* in order to digest the original pGIR001 plasmid used as template. The sequence of the expression cassettes was verified by sequencing with primers UP_pNZ8048 and 632SEQA4 to 632SEQA14.

**Construction of plasmids for the purification of Lar proteins.** Plasmids for expression of selected Strepti-tagged Lar proteins together with expression of all other Lar proteins were derived from pGIR100 (containing the entire *larA–E* operon): pGIR112 (LarA-Strep-tag), pGIR122 (LarB-Strep-tag), pGIR131 (LarC-Strep-tag) and pGIR172 (LarD-Strep-tag). A fragment comprising the whole pGIR112 plasmid was amplified by PCR using primer pairs LarStrep_A/PNZ8048 and LarStrep_B/pNZ8048. The PCR fragments were digested with BstB1 and self-ligated. The ligation mixtures were digested with DpnI and transformed into *L. lactis* for expression of the desired protein.

**Construction of plasmids for the modification of LarC**

The intermediate plasmid pGEM_larABCDE was constructed by subcloning of a DNA fragment comprising the whole *larABCDE* operon from *L. plantarum* NCIMB88826, which was amplified by PCR with primers StreptBZ_A2 and StreptB_B2, digested with PciI and SacI, and then ligated in the pNZ8048 plasmid digested with NcoI and SacI. The resulting plasmid was transfected into *L. lactis*.

**Mircravy experiments.** A culture of *L. plantarum* TF101 (Adh+)3 was grown to an *OD*~600~ of 0.75 and divided into three sub-cultures. Pure L-lactate (200 mM final concentration) was added to one of the sub-cultures. An equimolar mixture of D- and L-lactate (100 mM final concentration for each isomer) was added to a second sub-culture. The third subculture was not treated. The three sub-cultures were further incubated for 90 min, before harvesting by centrifugation (5,000×g, 10 min). Cell pellets were stored at −20 °C until RNA extraction. Cells were disrupted with four subsequent 40-s treatments in a Fastprep cell disrupter, interspaced by 1 min on ice (Qbiogene Inc., Illkirch, France)44. After disruption, the plasmid sequences were confirmed by sequencing with primers UP_pNZ8048 and 632SEQA4 to 632SEQA14.

**Conflict of interest.** The authors declare no conflict of interest.

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RNA was isolated with a High Pure RNA Isolation Kit, which included 1 h of treatment with DNase I (Roche Diagnostics, Mannheim, Germany)43. The RNA quality was assessed using the RNA 6000 Nano Assay in an Agilent 2100 Bioanalyzer (Agilent technologies, Palo Alto, CA, USA) following the manufacturer’s instructions. cDNA synthesis was carried out by the CyScire Post-Labeling and Purification kit (Amersham Biosciences, Buckinghamshire, UK) following the manufacturer’s instructions. Hybridization was performed on custom designed Lactobacillus plantarum WCFS1 11 K Agilent oligo microarrays using the Agilent hybridization protocol (version 5.5). These microarrays contained an average of three probes per gene. The hybridization scheme contained the following cDNA comparisons: (a) untreated culture versus L- lactate-treated culture and (b) untreated crude extract (L. plantarum) was collected by centrifugation at 13,000 g for 10 min at 4 °C. After lysis was performed by running the microtubes two times for 2 min at 60 °C in a water bath. Microtubes were cooled for 5 min on ice between the runs. After lysis, the soluble fraction (referred to as the crude extract) was collected by centrifugation at 13,000g for 15 min (4 °C). When larger volumes of culture were used, cells were resuspended and lysed in 50 ml Falcon tubes (BD, NJ, USA) using the same protocol.

Routine protein content was measured with the Bradford assay45. Since the Bradford assay is highly variable from one protein to another, the NanoOrange protein quantification kit (Invitrogen) was used for a lower protein-to-protein variability46. The conversion from g l−1 to mol l−1 was calculated with the theoretical molecular weight of the proteins, assuming they were 100% pure. The weight ratio of LarCl/LarC was estimated to be 1.5/1, yielding a molecular ratio of 2.4/1. Weedh thickening lactic acid production (PAGE) was performed with 10% acrylamide gels47. The proteins were stained with Coomassie Brilliant Blue R and the protein mass ladder used was the PageRuler Prestained Protein Ladder (Fermentas, France). For protein identification, the protein band was cut off the gel, digested and analysed on an Applied Biosystems 4800 MALDI TOF/TOF Analyser48. The MALDI/TOF data have been deposited in the PRoteomics IDENTifications (PRIDE) database under accession code PXD000775.

Protein purification. Affinity chromatography was performed with Gravity flow Strep-Tactin Superflow high capacity columns of 1 ml or 5 ml (ref. 49), with the theoretical molecular weight of the proteins, assuming they were 100% pure. The column, equilibrated at pH 7.5 (using 500 mM Tris at pH 10) before loading; LarB, 300 mM NaCl instead of 150 mM NaCl, pH 7.5; LarE, 220 mM NaCl, pH 7.5; LarA, 250 mM NaCl, pH 7.5; LarC, 300 mM NaCl, pH 7.5; LarENi, 250 mM NaCl, pH 7.5; LarNC, 200 mM NaCl, pH 7.5; LarAF, 150 mM NaCl, pH 7.5; LarAENi, 220 mM NaCl, pH 7.5; LarAEG, 250 mM NaCl, pH 7.5; LarAF, 200 mM NaCl, pH 7.5; Lc. Lactis LarC, 300 mM NaCl, pH 7.5; L. lactis LarC, 300 mM NaCl, pH 7.5; L. lactis LarE, 200 mM NaCl, pH 7.5; L. lactis LarA, 250 mM NaCl, pH 7.5; L. lactis LarA, 200 mM NaCl, pH 7.5; L. lactis LarCL, 250 mM NaCl, pH 7.5; L. lactis LarC, 200 mM NaCl, pH 7.5; L. lactis LarC, 150 mM NaCl, pH 7.5; L. lactis LarC, 100 mM NaCl, pH 7.5; and L. lactis LarC, 50 mM NaCl, pH 7.5.

In vitro Lar activation assays. The effect of accessory Lar proteins and cofactors on the in vitro activation of LarA_Ni(II) (apo-LarA) by LarE_Ni(II) was assayed by incubating 2 pmol of LarA_Ni(II) at room temperature in a 50 ml solution of 60 mM MES buffer (pH 6.5) supplemented with 20 pmol LarE, LarC or LarE and various concentrations of NiCl2. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C. The assay was performed in a 1 ml reaction volume with 25 pmol of LarA, 100 pmol of LarE and 100 mM MES buffer (pH 6.5) at 25 °C.
molecule and 1 supposed Mg2+ ion located on the non-crystallographic two-fold axis linking the two protein chains. Atomic coordinates for the LarA of T. thermosaccharolyticum have been deposited in the RSCB Protein Data Bank (PDB) database under accession code 2YIG.

**EXAFS and XANES analyses.** Protein samples from Lc. lactis LarAα (4.5 mM, 0.1 mol Ni per mol protein) and LarAβ (1.2 mM, 0.17 mol Ni per mol protein) were prepared in 10 mM Tris-HCl (pH 7.5), 20% glycerol buffers for XAS. Samples were kept at ~80 °C and transported at liquid nitrogen temperatures until run. X-ray absorption data collection was carried out at SSRl (Stanford Synchrotron Radiation Lightsource, 3 GeV ring) beam line 7-3 equipped with a 13-element Ge detector array with a Si(220) pho = 0° double crystal monochromator and a liquid helium cryostat for the sample chamber. Soller slits were used to reduce scattering and a 3-μm Z-1 element filter was placed between the sample and the detector. Internal energy calibration was performed by collecting spectra simultaneously in transmission on a nickel metal foil.

Data averaging and energy calibration was performed using SixPack64. The first inflection points from the XANES spectral regions were set to 8,331.6 eV for nickel foil. The AUTOBK algorithm available in the Athena software package was employed for data reduction and normalization65. A linear pre-edge function followed by a quadratic polynomial for the post-edge was used for background subtraction followed by normalization of the edge-jump to 1. EXAFS data was extracted using an Rn4 stiff 1 and a spline from k = 1–14 Å−1 with no clamps. The k2-weighted data was fit in R-space over the k = 2–12.5 Å−1 region with E0 for nickel set to 8,340 eV. All data sets were processed using a Kaiser-Bessel window with a cut-off radius of 0.8 Å. Artemis employing the FEFF6 and FEFFIT algorithms was used to generate and fit scattering paths to data62,64. Single scatter and multiple scatter fits were performed as described above. Average values and bond lengths obtained from crystallographic data were used to construct initial fitting models for multiple scatter analysis65. The paths from a particular multiple scatter model were generally afforded two degrees of freedom and were fit in terms of the distance from the first ligand atom–metal bond and a ligand-specific sigma square component of the Debye–Waller factor66–68. To assess the goodness of fit from different fitting models, the goodness of fit (% w) were minimized. Increasing the number of adjustable parameters is generally expected to improve the % w; however, C may go through a minimum then increase indicating the model is over-fitting the data.

**Bioinformatic analyses of Lar proteins.** To identify conserved residues in LarA proteins, 148 LarA homologues were aligned with clustalWX2 (ref. 69). Some of them were identified manually by looking at the annotation of some species were available, the genome containing the highest content in archaeal genomes, BlastP searches were performed using

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Additional information
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Supplementary Information
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