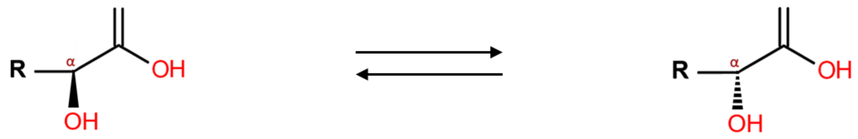
UNCOVERING A SUPERFAMILY OF α-HYDROXY ACID RACEMASES AND EPIMERASES

Julian Urdiain-Arraiza, Benoît Desguin
UCLouvain, LIBST, BGM
julian.urdiain@uclouvain.be

1. Racemases and epimerases of α-hydroxy acids

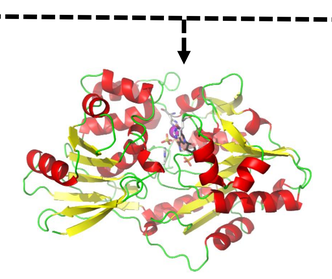
- Organic carboxylic acids
- Omnipresent in the living world
- Building blocks in organic synthesis

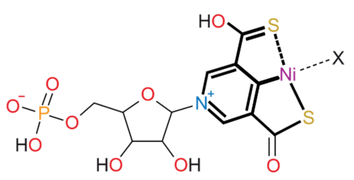
- If the α-hydroxy acid has only one chiral center: racemase
- If the α-hydroxy acid has several chiral centers: epimerase

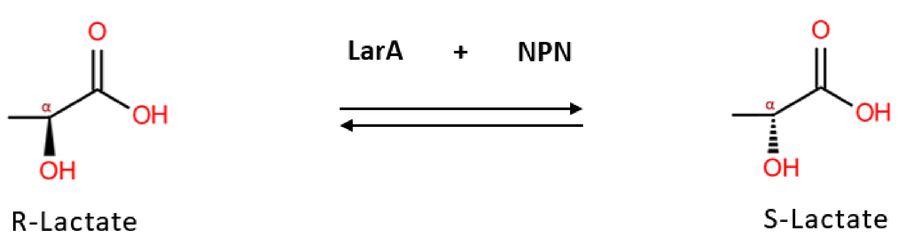


R-α-hydroxy acid S-α-hydroxy acid

2. Lactate racemase (LarA) uses the NPN cofactor







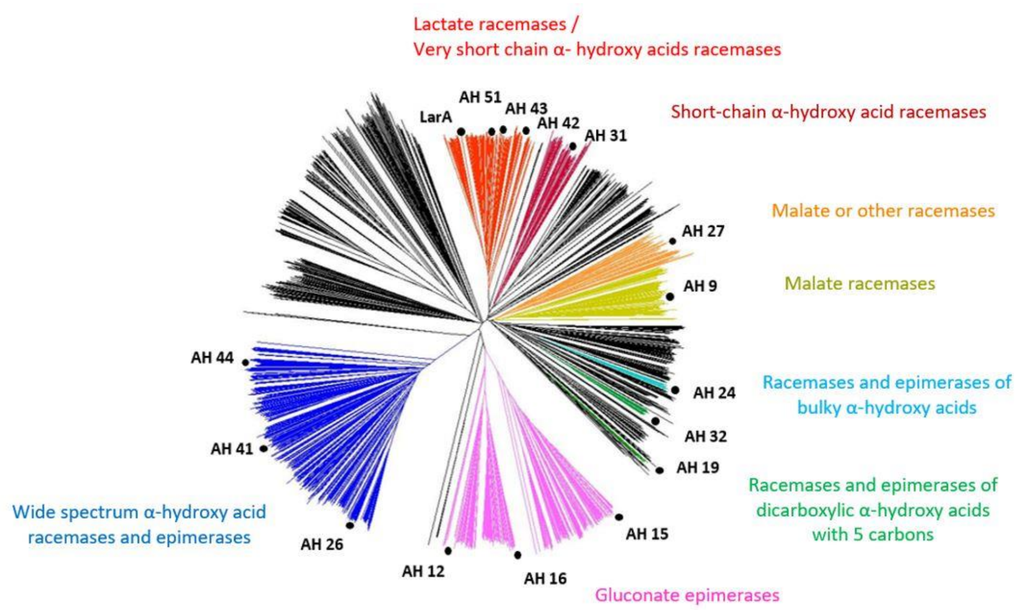
R-Lactate S-Lactate

3. The LarA superfamily

Interestingly, the genes coding for the NPN-biosynthetic enzymes and **LarA homologs (LarAHs)** are present in 12 % of bacterial and archaeal genomes and they are even present in some unicellular eukaryotes. An alignment of 2121 LarAHs sequences from 1103 species showed the diversity of sequences in this superfamily, suggesting that some LarAHs could racemize or epimerize other substrates than lactate.

Our goal is to explore the variety of reactions catalyzed by the LarA superfamily. For this purpose, we heterologously expressed LarAHs of various phylogenetic groups of the LarA superfamily and assayed the activity of these LarAHs in presence of in vitro synthesized NPN and a variety of α-hydroxy acids by enzymatic assay or by capillary electrophoresis.

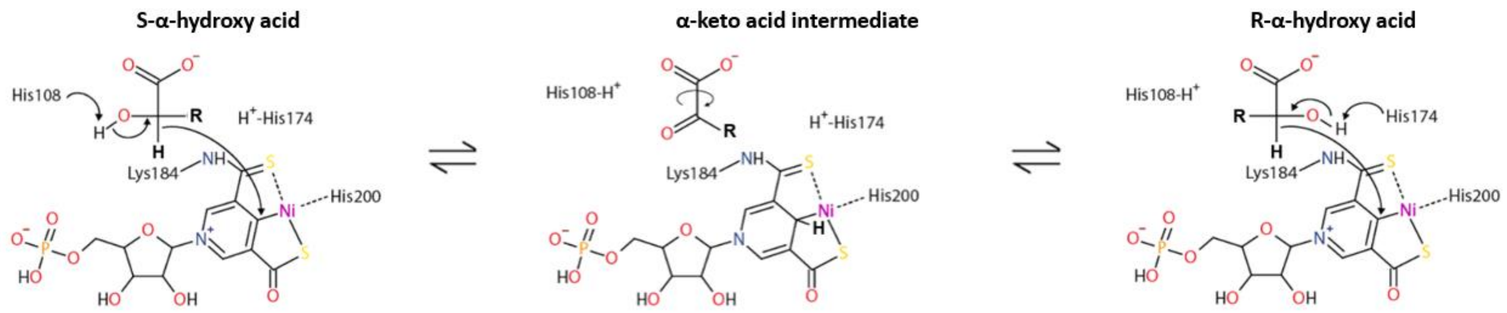
Indeed, we discovered that LarA was active on very short chain α-hydroxy acids in addition to lactate. Other phylogenetic groups of LarAH were active on α-hydroxy acids with short side chains, on malate, on α-hydroxyglutarate, on bulky α-hydroxy acids or on gluconate. We also discovered a large phylogenetic group of wide spectrum α-hydroxy acid racemases and epimerases that are active on 10 to 20 different α-hydroxy acids. Some of these LarAHs could have industrial applications for the synthesis of rare α-hydroxy acids isomers.



Phylogenetic tree of the LarA superfamily. All LarAHs with an identified reaction are indicated as "AH X". The colors of the phylogenetic groups correspond to the different reactions identified in these groups.

4. The LarA superfamily uses the NPN cofactor

NPN cofactor and its catalytic mechanism. The NPN cofactor facilitates a proton-coupled hydride transfer mechanism. During the reaction, the α chiral carbon of an α-hydroxy acid is transiently oxidized, generating an α-keto acid intermediate. The residues numbers correspond to the residues of *L. plantarum* LarA (PDB code 5HUQ or 6C1W).

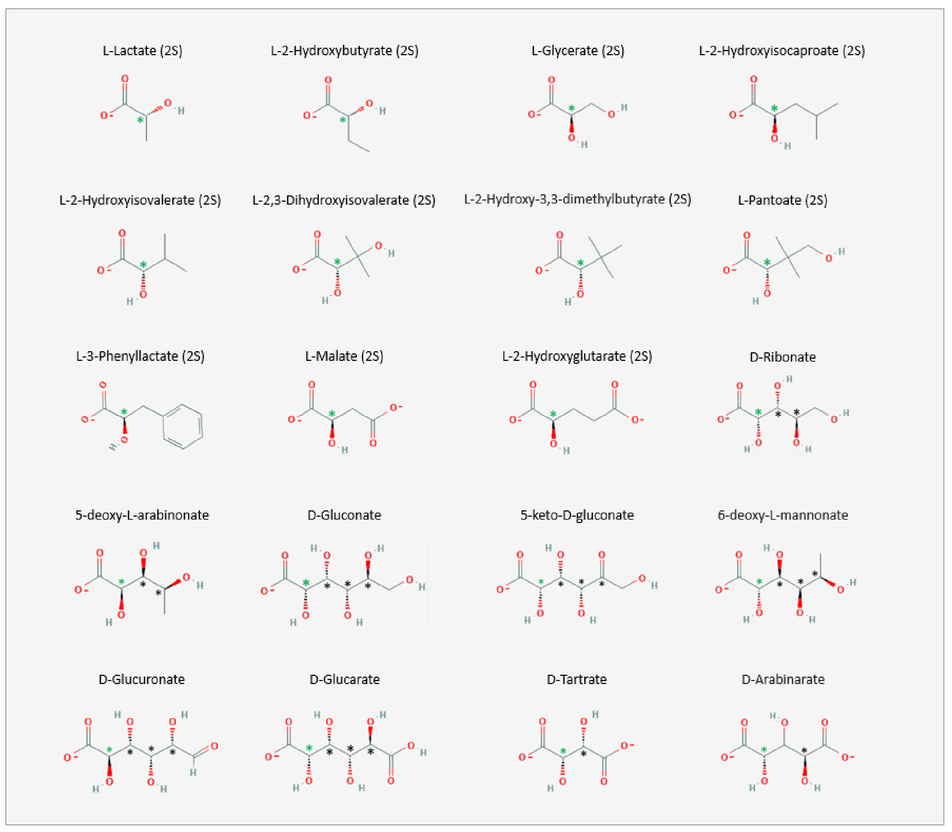


5. LarA and LarAHs form a superfamily of α-hydroxy acid racemases and epimerases with many promiscuous activities

α-hydroxy acids racemization and epimerization reactions catalyzed by LarAHs.

α-hydroxy acids	LarAHs																	
α-monohydroxy acids	LarA	AH9	AH12	AH15	AH16	AH19	AH24	AH26	AH27	AH31	AH32	AH41	AH42	AH43	AH44	AH49	AH51	AH52
Alkyl α-monohydroxy acids																		
L-Lactate ↔ D-Lactate																		
L-2-Hydroxybutyrate ↔ D-2-hydroxybutyrate																		
L-2-OH-3,3-Diméthylbutyrate ↔ D-2-OH-3,3-Diméthylbutyrate																		
L-2-Hydroxyisovalérate ↔ D-2-Hydroxyisovalérate																		
L-2-Hydroxyisocaproate ↔ D-2-Hydroxyisocaproate																		
Polycarboxy α-monohydroxy acids																		
L-Malate ↔ D-Malate																		
L-2-Hydroxyglutarate ↔ D-2-hydroxyglutarate																		
Arylalkyl α-monohydroxy acids																		
L-Phenyllactate ↔ D-Phenyllactate																		
Poly-hydroxy acids																		
L-Pantoate ↔ D-Pantoate																		
L-2,3-Dihydroxyisovalérate ↔ D-2,3-Dihydroxyisovalérate																		
Aldonic acids																		
L-Glycérate ↔ D-Glycérate																		
D-Erythronate ↔ D-Threonate																		
4-Deoxy-L-erythronate ↔ 4-Deoxy-L-threonate																		
D-Ribonate ↔ D-Arabinonate																		
L-Ribonate ↔ L-Arabinonate																		
D-Xylonate ↔ D-Lyxonate																		
L-Xylonate ↔ L-Lyxonate																		
5-deoxy-L-arabinonate ↔ 5-deoxy-L-ribonate																		
D-Gluconate ↔ D-Mannonate																		
D-Gulonate ↔ D-Idonate																		
D-Galactonate ↔ D-Talonate																		
5-keto-D-gluconate ↔ 5-keto-D-fructuronate																		
6-deoxy-L-galactonate ↔ 6-deoxy-L-talonate																		
6-deoxy-D-galactonate ↔ 6-deoxy-D-talonate																		
6-deoxy-L-mannonate ↔ 6-deoxy-L-gluconate																		
Uronic acids																		
D-Glucuronate ↔ L-Iduronate																		
D-Galacturonate ↔ L-Alturonate																		
D-Mannuronate ↔ L-guluronate																		
Aldaric acids																		
D-Tartrate ↔ Meso-Tartrate																		
D-Arabinarate ↔ D-Ribarate																		
D-Glucarate ↔ D-Mannarate																		

Green boxes: activity detected; Yellow boxes: slight activity detected; Red boxes: activity not detected.



Structure of substrates racemized or epimerized by LarAHs. For each substrate with one chiral center, only one of the two enantiomers is represented. For each substrate with multiple chiral centers, only one of the diastereomers is represented. Green asterisks indicate stereoinversion sites, black asterisks indicate other chiral centers of the molecules.

6. Summary

- Lactate racemase (LarA) uses the NPN cofactor to invert the stereochemistry of lactate enantiomers.
- LarA homologs (LarAHs) are NPN-dependent racemases and epimerases of α-hydroxy acid.
- LarAHs seems to form a superfamily of α-hydroxy acid racemases and epimerases with many promiscuous activities.
- Some LarAHs are active on 10 to 20 different α-hydroxy acids.
- LarAHs could be used as tools to provide new routes for the synthesis of rare α-hydroxy acid isomers.
- Many LarAHs are still to be discovered.