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New Hydroxynitrile Lyases

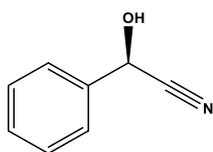
The present invention relates to novel hydroxynitrile lyase enzymes for the preparation of enantiopure cyanohydrins. For the application of HNL enzymes on scale, they should be I) **easily available** in high amounts, II) **highly active**, III) **highly selective** and IV) **stable** at process conditions. The new HNL from the fern *Davallia teyermannii* fulfills all these criteria.

BACKGROUND

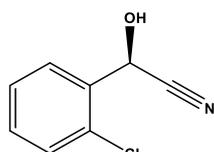
Hydroxynitrile Lyases (HNLs) are valuable tools for selective enzymatic synthesis of chiral building blocks (cyanohydrins). *R*-Selective HNLs are known from different organisms. *Prunus amygdalus* HNL (*Pa*HNL) has been heavily engineered and is used in current processes. However, its production is only possible in *P. pastoris* with moderate yields, limiting broad applicability and other *R*-selective HNL suffer from similar drawbacks including low stability and limited tolerance for low pH.

TECHNOLOGY

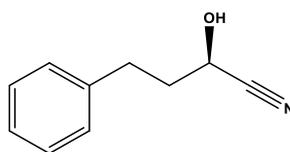
*Dt*HNL is a 20kDa protein which can be produced intracellularly in *E. coli* or in *P. pastoris* in up to 80% of the total protein in the cell free extract, producing preparations with specific activities of up to 300 U/mg. Compared to other patented technology, the efficiency of *Dt*HNL production in *P. pastoris* is >800 times higher. The specific activity of recombinant *Dt*HNL is exceptionally high (350 U/mg) and strict *R*-selectivity allows the production of enantiopure cyanohydrin under optimized conditions. *Dt*HNL isoenzymes are stable in aqueous solutions at pH 4.5 and retain activity even at pH 2.5. *Dt*HNL can be applied for the production of pharma intermediates and fine chemicals such as *R*-mandelic acid and its derivatives, or also for valorization of cyanide waste streams. Here are some examples of *Dt*HNL conversions:



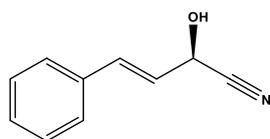
(*R*)-mandelonitrile
time: 30 min
conv.: 95.1%
ee: 99.0%



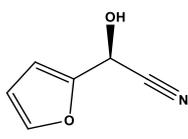
(*R*)-2-chloromandelonitrile
time: 2 h
conv.: >99%
ee: 97.5%



(2*R*)-2-hydroxy-4-phenylbutanenitrile
time: 6 h
conv.: 94.5%
ee: 48.1.4%



(2*R*)-2-hydroxy-4-phenylbutanenitrile
time: 6 h
conv.: 88.2%
ee: 94.5%



(2*S*)-furan-2-ylhydroxyacetonitrile
time: 30 min
conv.: 95.3.2%
ee: 99.3%

BENEFITS

- + Outstanding specific activity
- + Excellent *R*-selectivity
- + *Dt*HNL produced efficiently in pro- and eukaryotic host
- + Stable at low pH
- + Crystal structure available



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KEYWORDS:

- (*R*)-cyanohydrin
- hydroxynitrile lyase
- chiral building blocks
- enantioselective synthesis
- biocatalysis

INVENTORS:

Elisa Lanfranchi
Kerstin Steiner
Margit Winkler
Anton Glieder
Tea Pavkov-Keller
Matthias Diepold

AVAILABLE FOR:

- License agreement
- Collaboration

IPR:

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CONTACT:

Dr. Martin Trinker

ACIB - Austrian Centre of Industrial
Biotechnology
Director Business Development
Petersgasse 14/V
8010 Graz
T: +43 316 873 9316
martin.trinker@acib.at