sschi SLOVAK SOCIETY OF CHEMICAL ENGINEERING

Slovak Society of Chemical Engineering
Institute of Chemical and Environmental Engineering
Slovak University of Technology in Bratislava

PROCEEDINGS

52nd International Conference of the Slovak Society of Chemical Engineering SSCHE 2026

Hotel SOREA TRIGAN
Štrbské Pleso, Slovakia
May 26 - 29, 2026

Editors: Assoc. prof. Mário Mihaľ

ISBN: 978-80-8208-177-3, EAN: 9788082081773

Published by the Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava in Slovak Chemistry Library for the Institute of Chemical and Environmental Engineering; Radlinského 9, 812 37 Bratislava, 2026

Hambalko, J., Ali, Y., Kuzmiaková, M., Dobiašová, H., Kozmon, S., Both, P.: In Search of Suitable Amine Dehydrogenase: Identification and Development of AmDHs with Industrially Relevant Substrate Scope, Editors: Mihaľ, M., In *52nd International Conference of the Slovak Society of Chemical Engineering SSCHE 2026*, Štrbské Pleso, Slovakia, 2026.

In Search of Suitable Amine Dehydrogenase: Identification and Development of AmDHs with Industrially Relevant Substrate Scope

Jaroslav Hambalko[1], Yasir Ali[2], Michaela Kuzmiakova[1], Hana Dobiašova[1], Stanislav Kozmon[2], Peter Both[1]

[1]Axxence Slovakia s.r.o, Mickiewiczova 9, 811 07 Bratislava, Slovakia
[2]Chemický Ústav, Slovenská Akadémia Vied, Dúbravská cesta 9, 84538 Bratislava, Slovakia
e-mail: jaroslav.hambalko@axxence.sk

Key words: amine dehydrogenase, biocatalysis, chiral amines, homology, molecular dynamics, phylogeny, flavor and fragrance

Abstract:
Amine dehydrogenases (AmDHs) are NAD(P)H-dependent oxidoreductases catalyzing the reductive amination of carbonyl compounds into amines. Due to their excellent stereoselectivity, simplified reaction stoichiometry, and environmentally favorable reaction conditions, AmDHs represent attractive biocatalysts for pharmaceutical, fine chemical, and flavor and fragrance industries. However, only a limited number of naturally occurring AmDHs active toward short-chain aliphatic and hydroxy-functionalized substrates have been described.
The aim of this work is to identify and characterize novel AmDH candidates with substrate specificity relevant for industrial applications. A substrate panel consisting of 15 structurally diverse aldehydes, ketones, hydroxyaldehydes, and hydroxyketones was designed to guide enzyme selection and evaluation. A comprehensive literature survey covering 120 scientific publications resulted in a curated database containing information on 218 AmDH enzymes and variants, including substrate scope, catalytic activity, cofactor preference, and reaction conditions.
Based on their diverse substrate specificity, 53 enzymes were selected for phylogenetic analysis using the iTOL platform. Ten representative enzymes with distinct phylogenetic distribution and substrate preference were further analyzed by homology searches against publicly available genomic databases. Structural analysis involving molecular docking, molecular dynamics simulations, and SmarTSzyme analysis was subsequently performed for selected candidates to identify structural determinants affecting substrate specificity and to propose potentially beneficial mutations.
The combined bioinformatic workflow enabled the identification of several promising AmDH candidates suitable for heterologous expression and experimental validation. The presented approach provides an effective strategy for the discovery and engineering of novel AmDHs with potential applications in sustainable synthesis of chiral amines and amino alcohols.

Acknowledgements : Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I01-03-V04-00072.

In Search of Suitable Amine Dehydrogenase: Identification and Development of AmDHs with Industrially Relevant Substrate Scope

Jaroslav Hambalko[1], Yasir Ali[2], Michaela Kuzmiakova[1], Hana Dobiašova[1], Stanislav Kozmon[2], Peter Both[1]

[1]*Axxence Slovakia s.r.o, Mickiewiczova 9, 811 07 Bratislava, Slovakia*
[2]*Chemický Ústav, Slovenská Akadémia Vied, Dúbravská cesta 9, 84538 Bratislava, Slovakia*

e-mail: jaroslav.hambalko@axxence.sk

Key words: amine dehydrogenase, biocatalysis, chiral amines, homology, molecular dynamics, phylogeny, flavor and fragrance

Abstract

Amine dehydrogenases (AmDHs) are NAD(P)H-dependent oxidoreductases catalyzing the reductive amination of carbonyl compounds into amines. Due to their excellent stereoselectivity, simplified reaction stoichiometry, and environmentally favorable reaction conditions, AmDHs represent attractive biocatalysts for pharmaceutical, fine chemical, and flavor and fragrance industries. However, only a limited number of naturally occurring AmDHs active toward short-chain aliphatic and hydroxy-functionalized substrates have been described.

The aim of this work is to identify and characterize novel AmDH candidates with substrate specificity relevant for industrial applications. A substrate panel consisting of 15 structurally diverse aldehydes, ketones, hydroxyaldehydes, and hydroxyketones was designed to guide enzyme selection and evaluation. A comprehensive literature survey covering 120 scientific publications resulted in a curated database containing information on 218 AmDH enzymes and variants, including substrate scope, catalytic activity, cofactor preference, and reaction conditions.

Based on their diverse substrate specificity, 53 enzymes were selected for phylogenetic analysis using the iTOL platform. Ten representative enzymes with distinct phylogenetic distribution and substrate preference were further analyzed by homology searches against publicly available genomic databases. Structural analysis involving molecular docking, molecular dynamics simulations, and SmarTSzyme analysis was subsequently performed for selected candidates to identify structural determinants affecting substrate specificity and to propose potentially beneficial mutations.

The combined bioinformatic workflow enabled the identification of several promising AmDH candidates suitable for heterologous expression and experimental validation. The presented

approach provides an effective strategy for the discovery and engineering of novel AmDHs with potential applications in sustainable synthesis of chiral amines and amino alcohols.

1. Introduction

Amine dehydrogenases (AmDHs) are oxidoreductases catalyzing the reversible reductive amination of carbonyl compounds, such as aldehydes and ketones, using ammonia and the reduced cofactors NADH or NADPH. Due to their high stereoselectivity and ability to directly produce chiral amines, AmDHs have attracted significant attention in biocatalysis and industrial biotechnology.

Compared to frequently used transaminases, AmDHs provide several practical advantages. While transaminases require amino donors and pyridoxal phosphate-dependent transamination systems, AmDHs catalyze direct reductive amination using inexpensive ammonia as the nitrogen source. This simplifies reaction stoichiometry, improves atom economy, and reduces the formation of side products. In addition, many AmDHs exhibit excellent enantioselectivity, making them attractive biocatalysts for the synthesis of optically pure amines.

Chiral amines represent important building blocks in the pharmaceutical, agrochemical, and fine chemical industries. Their significance is particularly evident in the synthesis of active pharmaceutical ingredients and flavor and fragrance compounds, where stereochemistry strongly influences biological activity, odor perception, and sensory properties. In recent years, increasing attention has been focused on the development of sustainable enzymatic routes for the synthesis of amino alcohols and related compounds from hydroxyketones and hydroxyaldehydes.

Despite significant advances in enzyme engineering and directed evolution, relatively few naturally occurring AmDHs exhibiting activity toward short-chain aliphatic and hydroxy-functionalized substrates have been described. To our knowledge, systematic exploration of AmDHs active toward such substrates remains limited. Therefore, the discovery and development of novel AmDHs with broader substrate scope and improved catalytic properties represent important objectives in industrial biotechnology.

The aim of this work is the identification, development and characterization of novel AmDH candidates with substrate specificity relevant for industrial applications and their subsequent prioritization for experimental validation.

2. Experimental Section

2.1.Selection of Substrate Panel and Literature Survey

A substrate panel of 15 structurally diverse compounds was designed for this study. The panel included aldehydes, ketones, dialdehydes, diketones, hydroxyaldehydes, and hydroxyketones with varying carbon chain lengths and structural properties. The selected substrates were chosen to represent industrially attractive target structures relevant for industrial, especially flavor and fragrance applications.

A comprehensive literature survey focusing on published AmDHs, their substrate specificity, catalytic activity, and reaction conditions was subsequently performed. The resulting database

contained information on 218 AmDH enzymes and variants, including protein sequences, mutations, organism origin, reaction conditions, cofactor specificity, substrate scope, and reported catalytic activities or conversions. Enzymes active toward substrates from the panel designed for this project were prioritized for further analysis. Special attention was dedicated to reaction conditions affecting enzymatic activity, including pH, temperature, buffer composition, substrate concentration, cofactor preference, and cofactor regeneration systems.

2.2.Phylogenetic and Homology Analysis

Based on substrate specificity, 53 enzymes exhibiting activity toward at least one substrate from the selected substrate panel were chosen for further bioinformatic analysis. A phylogenetic tree was constructed using the iTOL (Interactive Tree Of Life) platform to evaluate evolutionary relationships and functional diversity among identified enzymes.

The phylogenetic analysis enabled identification of distinct enzyme clusters associated with specific substrate preferences. Enzymes located in distant phylogenetic branches were considered particularly attractive due to their potential functional diversity and unexplored catalytic properties.

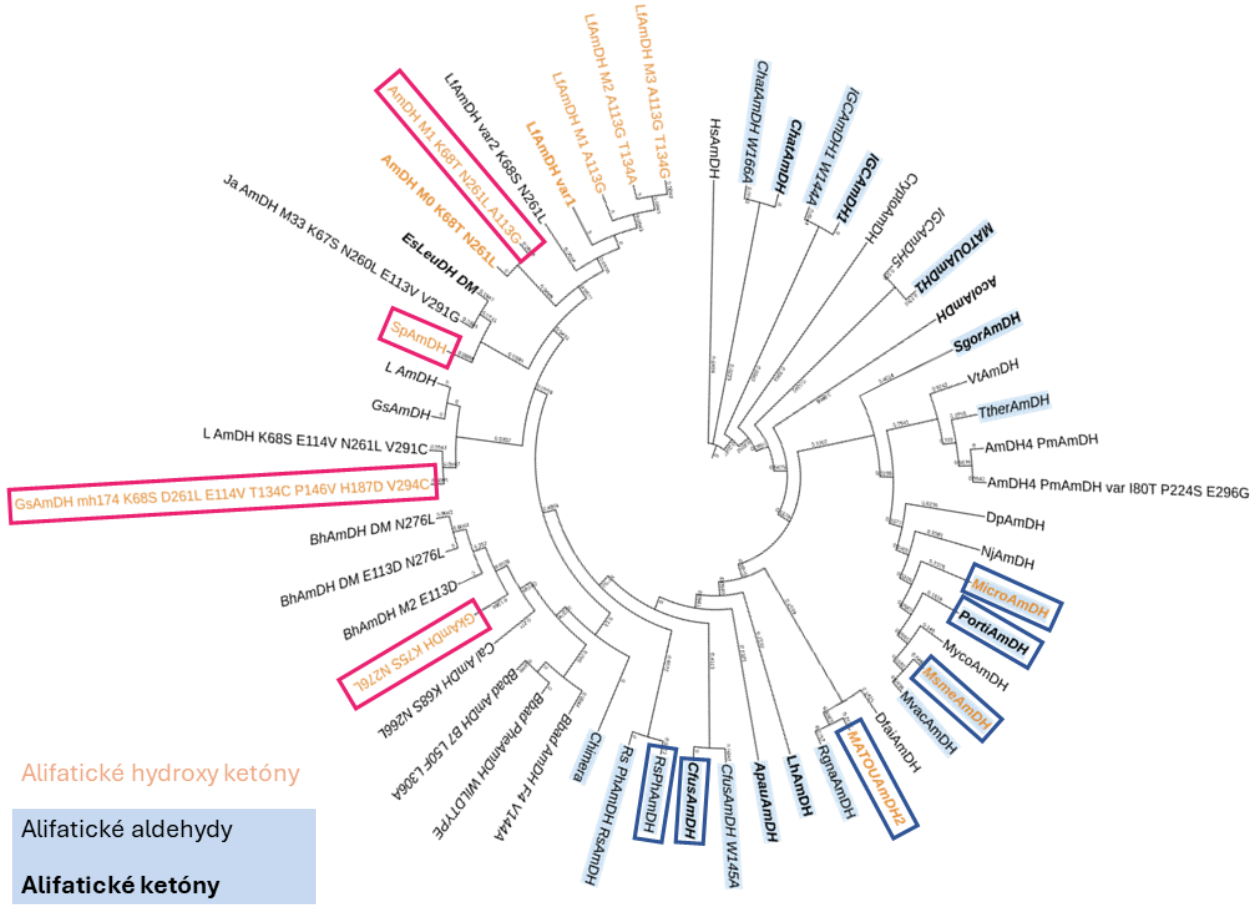

Figure 1. A phylogenetic tree of AmDHs with described activity constructed using the iTOL platform. The 10 selected AmDHs are highlighted in boxes.

Ten representative enzymes with distinct phylogenetic distribution and catalytic profiles were selected for detailed analysis:

- AmDH M1 (*Lysinibacillus sphaericus*)
- SpAmDH (*Sporosarcina psychrophila*)
- GsAmDH (*Geobacillus stearothermophilus*)
- GkAmDH (*Geobacillus kaustophilus*)
- RsPhAmDH (*Rhodococcus* sp.)
- Cfus AmDH (*Cystobacter fuscus*)
- MATOUAmDH2 (*Anaerosphaera multitolerans*)
- MsmeAmDH (*Mycolicibacterium smegmatis*)
- PortiAmDH (*Porticoccus* sp.)
- MicroAmDH (*Microbacterium* sp. MA1)

These enzymes represented well-characterized AmDHs with industrially relevant scopes suitable for subsequent homology mining and structural analysis.

Protein sequences of selected enzymes were subsequently used for homology searches using pBLAST against publicly available genomic databases including GenBank, EMBL, DDBJ, PDB, and RefSeq. Sequence similarity thresholds ranged from 45–80 %, depending on the availability of homologous sequences for individual enzymes.

The resulting homologous sequence libraries were further analyzed using phylogenetic methods to identify previously uncharacterized putative AmDH candidates. Three promising candidates identified through homology analysis included:

- leucine dehydrogenase from *Sporosarcina* sp. Te-1 (A0A975C8J4),
- dihydrodipicolinate reductase from *Mycolicibacterium* sp. CBMA 247 (A0A7K1K7V5),
- leucine dehydrogenase from *Heyndrickxia camelliae* (A0A2N3LNA2).

The phylogenetic workflow enabled efficient prioritization of enzymes for downstream computational and experimental studies.

2.3.Structural Analysis and Molecular Dynamics

Three enzymes — AmDH M1, SpAmDH, and MsmeAmDH — were selected for detailed structural analysis involving molecular docking and molecular dynamics simulations. The objective was to identify structural determinants responsible for substrate specificity and to propose mutations potentially improving catalytic activity toward selected hydroxyketones and hydroxyaldehydes.

In parallel, SmarTSzyme analysis was employed to evaluate active-site characteristics and structural variability among selected enzymes. Comparative heat map analyses were performed separately for hydroxyaldehyde- and hydroxyketone-active enzymes to identify conserved structural trends associated with substrate preference.

The obtained structural data provided a basis for rational enzyme engineering and candidate prioritization for experimental validation. First candidate chosen for experimental validation in laboratory conditions was MsmeAmDH and its Top3 mutants designed for substrate specificity enhancement towards chosen hydroxyaldehyde substrate.

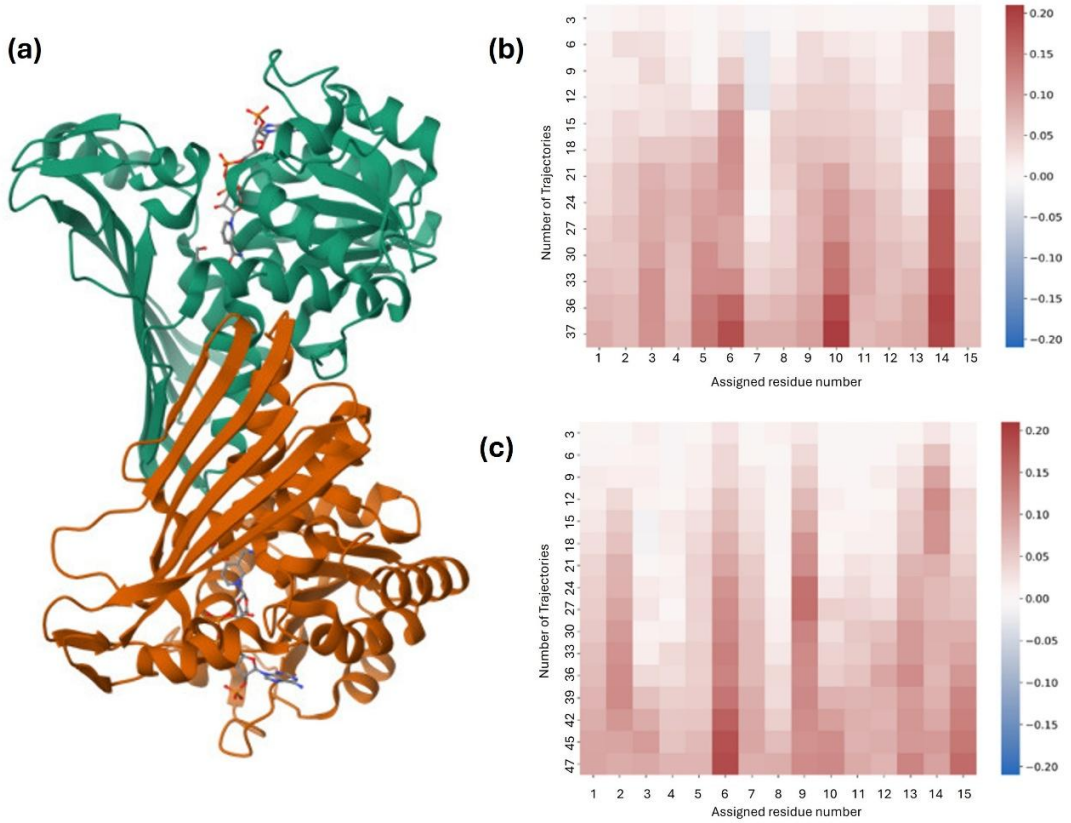

Figure 2. (a) Structure of Amine Dehydrogenase from Mycobacterium smegmatis (b) Top 15 residues with non-favorable coupling to the reaction coordinate and active site across 37 sMD trajectories for a hydroxyaldehyde substrate (c) Top 15 residues identified for a hydroxyketone substrate using the same analysis originated from 47 sMD simulations. Positions are anonymized to protect company IP.

2.4.Selection of Candidate Enzymes from Literature Research

Additional candidate enzymes were selected from the large-scale bioinformatic study published by Elisée et al. (2024), which focused on discovery of natural AmDHs from large genomic datasets.

The selected candidates included:

- METDB-00128-1-DN9853
- T2D-106A GL0090439
- V1.UC17-2 GL0137933

These enzymes were selected based on predicted catalytic diversity and potential activity toward industrially relevant aliphatic substrates.

2.5.Experimental Screening and Validation

Selected AmDH candidates, including enzymes identified through literature research, homology analysis, and structure-guided engineering, were prioritized for heterologous expression and biochemical characterization.

Initial experimental work focused primarily on optimization of reaction conditions and development of analytical methods suitable for screening multiple enzyme–substrate combinations. Different reaction parameters, including enzyme concentration, substrate concentration, buffer composition, cofactor type, cofactor concentration, and solubility conditions, were systematically evaluated.

An important part of the project was dedicated to development of analytical methods for monitoring substrate conversion and product formation. Both gas chromatography (GC) and high-performance liquid chromatography (HPLC) methods were investigated together with different extraction and derivatization procedures. The aim was to establish robust analytical conditions enabling simultaneous detection of substrates and corresponding amine products.

The developed workflow provides a foundation for future quantitative activity measurements, conversion analysis, and detailed biochemical characterization of newly identified AmDH candidates.

3. Conclusion

This work presents a systematic bioinformatic and experimental workflow for the identification and development of novel amine dehydrogenases with industrially relevant substrate specificity.

By combining literature mining, phylogenetic analysis, homology searches, molecular docking, molecular dynamics simulations, and structural bioinformatics, several promising AmDH candidates were identified and prioritized for experimental validation. In addition, analytical workflows suitable for qualitative screening of enzyme activity across a structurally diverse substrate panel were successfully established.

The presented approach provides an effective platform for discovery and engineering of novel AmDHs suitable for sustainable synthesis of chiral amines and amino alcohols relevant to pharmaceutical, fine chemical, and flavor and fragrance industries.

Future work will focus on quantitative biochemical characterization, determination of substrate conversions and catalytic activities, and further structure-guided engineering of selected enzyme candidates. Preliminary results based on qualitative analysis uncovered four new enzymes with activities towards the most interesting substrates.

4. Acknowledgements

Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I01-03-V04-00072.