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A novel bacterial short-chain dehydrogenase/reductase (SDR) active on guaiacyl, p-hydroxyphenyl and syringyl monolignols

AUTHORS
Anna BRILHANTE / BRAZILIAN BIORENEWABLES NATIONAL LABORATORY, R. GIUSEPPE MÁXIMO SCOLFARO, 10000 - POLO II DE ALTA TECNOLOGIA, CAMPINAS, SP
Priscila GIUSEPPE / BRAZILIAN BIORENEWABLES NATIONAL LABORATORY, R. GIUSEPPE MÁXIMO SCOLFARO, 10000 - POLO II DE ALTA TECNOLOGIA, CAMPINAS, SP
Lúcia WOLF / BRAZILIAN BIORENEWABLES NATIONAL LABORATORY, R. GIUSEPPE MÁXIMO SCOLFARO, 10000 - POLO II DE ALTA TECNOLOGIA, CAMPINAS, SP

PURPOSE OF THE ABSTRACT
The conversion of lignin-derived aromatics (LDA) into value-added chemicals is a renewable alternative to support the transition from a fossil-based industry to a sustainable economy based on renewable sources. Given the heterogeneity of lignin depolymerization products, convergent biocatalysis performed by microorganisms has emerged as a promising approach to funneling LDA into target molecules. Biological pathways and enzymes for the catabolism of aromatic compounds derived from the syringyl (S), guaiacyl (G), and p -hydroxyphenyl (H) subunits of lignin (e.g. syringate, vanilate, and p-coumarate, respectively) have been described for some bacterial strains [1] . Despite these advances, there is still little information regarding the enzymatic strategies for the catabolism of the monolignols coniferyl alcohol (G), p-coumaryl alcohol (H) and sinapyl alcohol (S). In this work, we identified an aryl-alcohol dehydrogenase (XarA) from Xanthomonas citri pv. citri 306 that belongs to the short-chain dehydrogenase/reductases (SDR) superfamily. Its gene (XAC0353) is located close to the operon of protocatechuate catabolism, a key intermediate in the metabolism of H and G lignin-derived aromatic compounds. XarA has about 60% sequence identity with a coniferyl alcohol dehydrogenase from Pseudomonas sp. HR199 (CalAHR199), the only bacterial SDR active on coniferyl alcohol with experimental data available so far (U.S. Patent Application Publication No. 2003/0228670 A1). However, the substrate range of CalAHR199 was poorly investigated and the structural properties defining substrate recognition mechanisms of bacterial SDRs able to oxidize monolignols are still elusive. Therefore, our group aims to investigate XarA substrate profile and structure-function relationships using as main approaches biochemical and biophysical assays in vitro, X-ray diffraction crystallography, cryo-EM and molecular dynamic simulations. Here, we highlight the main results obtained so far. Biochemical assays demonstrated that XarA is a NAD+-dependent dehydrogenase, with optimal activity at 30 °C (pH 10). XarA specific activity on several aryl-alcohols, evaluated by NADH quantification using HPLC, showed the following profile (relative activity): p-coumaryl alcohol (100%) > cinnamyl alcohol (83%) > coniferyl alcohol (48%) > sinapyl alcohol (34%) > 4-hydroxybenzyl alcohol (10%) > vanillyl alcohol (3%) > benzyl alcohol (2%), indicating that XarA has greater activity on aryl alcohols containing longer (propenol) side chains, with a decrease in specific activity as the degree of methoxylation in the aromatic ring increases, and only a residual activity on aryl alcohols with shorter (methanol) side chains. XarA formed crystals in complex with NAD+, which were soaked with coniferyl alcohol and diffracted to 2.9 Å resolution. The crystal structure was solved by Molecular Replacement, using an AlphaFold-generated model structure as template, and model refinement is in progress. SEC-RALS/LALS assays indicate that XarA forms dodecamers in solution that can dissociate into smaller oligomers depending on the medium composition. The mechanisms underlying this oligomeric state transition will be further investigated by cryo-EM analyses. In summary, to the best of our knowledge, this is the first report of a bacterial SDR family member able to convert the three main precursors of lignin (G, H and S monolignols) into their respective aldehydes, the first step required for their entry into catabolic funneling pathways. By combining structural

analyses, molecular dynamics simulations and kinetic studies, we expect to provide a deeper understanding on the substrate recognition mechanisms that determine the substrate preference of XarA, thus contributing to the knowledge regarding the structure-function relationships of SDRs active on monolignols and supporting the development of biotechnologies for the sustainable production of industrially relevant bioproducts.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS
alcohol dehydrogenase | aromatic compounds | lignin | biotransformation

BIBLIOGRAPHY

[1] Ravi, K. et al, 2019, bioresour. technol., 285, 121327.