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Practical Biocatalytic Synthesis of Aromatic Nitriles

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Abstract

Aromatic nitriles are important building blocks with wide applications in pharmaceutical and agrochemical industries as well as materials science. In a recent *Chem Catalysis* paper, Gröger and coworkers engineered aliphatic aldoxime dehydratases (Oxds) for the scalable synthesis of aromatic nitriles, delivering a sustainable and energy-efficient technology for the manufacturing of aromatic nitriles.

Aromatic nitriles constitute ubiquitous structural elements of pharmaceuticals, agrochemicals, and specialty chemicals.[1–3] In industry, large-scale manufacturing of aryl nitriles is usually carried out though the catalytic ammoxidation of toluene derivatives.[4] Despite the immense synthetic utility of this chemistry, ammoxidation processes are energy intensive and environmentally unfriendly. To reduce the ecological footprint of industrial aromatic nitrile manufacturing, biocatalysis represents an appealing alternative, due to its ability to circumvent the use of toxic organic solvents, exceptionally mild reaction conditions, and excellent turnover efficiency.

Recently, the Gröger group developed an efficient biocatalytic protocol for the large-scale synthesis of aromatic nitriles through engineered aldoxime dehydratases (Oxds) (Fig 1A), further demonstrating the potential of Oxds for practical aromatic nitrile synthesis.[5] Previously, elegant work from Gröger and coworkers has led to a set of aldoxime dehydratases allowing for the efficient and stereoselective synthesis of aliphatic nitriles using a cyanide-free approach.[6–10] However, the utility of these enzymes in the preparation of aromatic nitriles was not established. In the present study, Gröger and coworkers examined wild-type aliphatic aldoxime dehydratase from *Rhodococcu*s sp. N-771 (OxdRE-wt) using a panel of *ortho*-, *meta*-, and *para*-substituted benzaldoximes. While the OxdRE-wt exhibited good activity toward benzaldoxime derivatives with a small *ortho*-substituent such as methyl and a halogen group, this enzyme showed low activity toward *meta*- and *para*-substituted substrates as well as those bearing a larger *ortho*-substituents. Together, these results demonstrated that the steric profile of the benzaldoxime substrate plays an essential role in controlling its activity in biotransformations using OxdRE-wt.

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To expand the substrate compatibility of biocatalysts, the authors next evaluated a library of OxdRE active-site mutants derived from their previous work.[10] Among these enzyme variants, the M29G mutant with a broadened active site exhibited substantially improved activities toward *meta*-substituted substrates. In an effort to identify OxdRE variants for the more efficient biotransformation of *para*-substituted substrates, a representative model substrate was docked into the active site of OxdRE, revealing the proximity of residues M29 and F306 to the *para*-substituent of the benzaldoxime substrate (Figure 1B). With this insight from docking studies, OxdRE F306A was generated by site-directed mutagenesis and tested with a set of *para*-substituted aromatic substrates. Indeed, this F306A mutant was found to be reactive toward a range of *para*-substituted substrates. For example, it afforded 4-phenylbenzaldoxime in 17% yield, which represented a more than 17-fold improvement over the wild type aldoxime dehydratase.

With a set of tailored enzyme variants in hand, Gröger and coworkers next set out to develop a practical enzyme technology relevant to industrial applications by improving the stability and expression level of the recombinant biocatalyst. First, in light of the enhanced stability of aldoxime hydratases in the cellular environment, biocatalysts in intact *E. coli* cells were used for large-scale processes. Second, based on the author's prior study, leaky expression using a pET-28a(+) vector-based expression system with the *E. coli* BL21(DE3)-STAR bacterial strain furnished significantly improved enzyme activities. Further lowering the reaction temperature to 10 °C eventually led to optimal conditions for this whole-cell transformation. Under the optimized process conditions, wild-type enzyme OxdRE-Wt demonstrated high activities for benzaldoximes possessing a small *ortho*-substituent with activities ranging from 122 to 170 mU/mg_{bww}. In addition, OxdRE M29G displayed good activity for *meta*-substituted benzaldoximes with an activity of up to 3.6 mU/mg_{bww}. Furthermore, OxdRE F306A permitted the conversion of *para*-substituted benzaldoximes (2.1 to 4.4 mU/mg_{bww}).

To demonstrate the potential of this optimized biotransformation in large-scale processes, a 10 mL-scale reaction was performed. With substrate concentrations of up to 500 mM (i.e., 79 g/L substrate loading), 2-chlorobenzaloxime was converted to the corresponding acrylonitrile in 24 h in 91% yield. Similar results were also obtained with other substrates, demonstrating the compatibility of this technology with high substrate loadings, a key criterion for industrial biocatalysis.

In summary, Gröger and coworkers engineered a set of *Rhodococcus sp.* aldoxime dehydratase variants, allowing the efficient biocatalytic synthesis of various substituted aromatic nitriles from the corresponding benzaldoximes under mild reaction conditions. Importantly, through further optimization of enzyme expression and reaction conditions, the authors developed a practical biocatalytic dehydration protocol that operates under high substrate loadings, underscoring the potential of biotransformations in the industrial production of useful chemicals.

Acknowledgments

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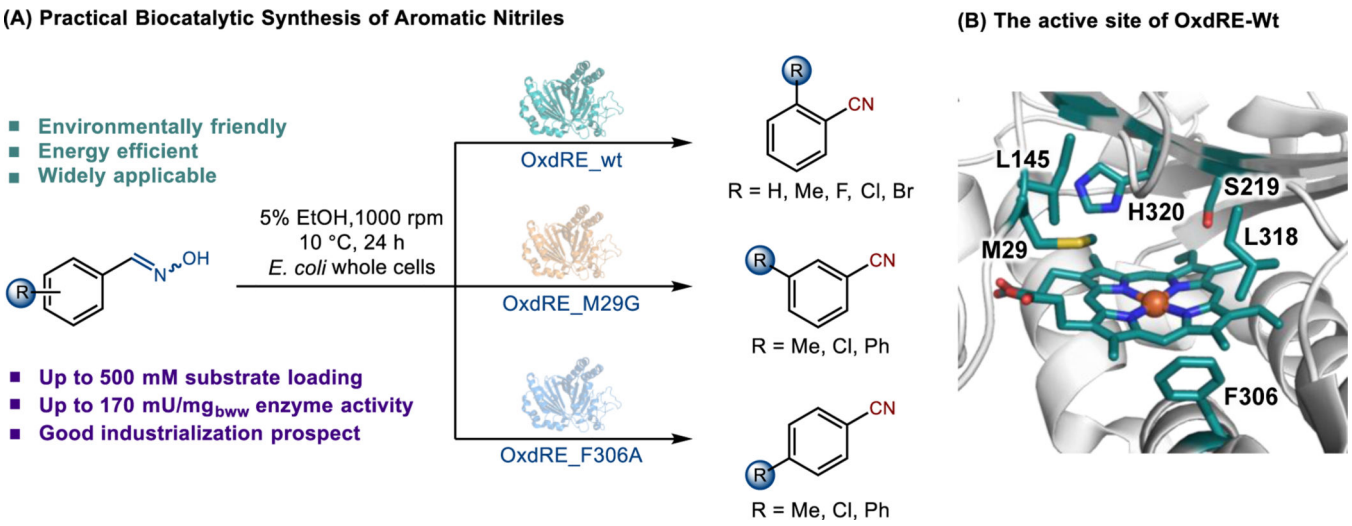

Fig 1.

(**A**) The overview of biocatalytic synthesis of aromatic nitriles; (**B**) The active site of OxdRE-Wt (PDB ID: 3A16).