NITRILASE FROM *Rhodococcus rhodochrous* ATCC BAA-870: FIBRE FORMATION OVER TIME

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Introduction

A versatile nitrile-degrading bacterium, *Rhodococcus rhodochrous* ATCC BAA-870, was isolated through enrichment culturing of soil samples from Johannesburg, South Africa[1]. We have previously shown that *Rhodococcus rhodochrous* ATCC BAA-870 possesses nitrile-hydrolysing enzymes capable of metabolising a wide range of aliphatic and aromatic nitriles and amides[2]. The biocatalyst expressed a two enzyme system with sequential nitrile-converting activity (**Figure 1**).

Nitrilase enzymes catalyse the hydrolysis of a nitrile into its corresponding carboxylic acid and ammonia, and have become important industrial enzymes as a result of the products they afford (**Figure 2**). Successful commercial examples of nitrile bioconversion include production of nicotinic acid and acrylic acid[3].

The nitrilase from *Rhodococcus rhodochrous* J1 , which shows similarity to *Rhodococcus rhodochrous* ATCC BAA-870, was previously shown to undergo oligomerization into stable active helices[4]. The wide substrate profile of nitrilase from *Rhodococcus rhodochrous* ATCC BAA-870 makes it a valuable enzyme for biotechnology, and its ability to form fibres was therefore monitored.

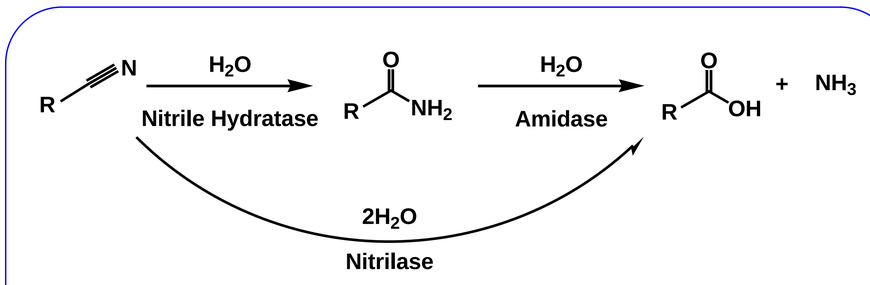
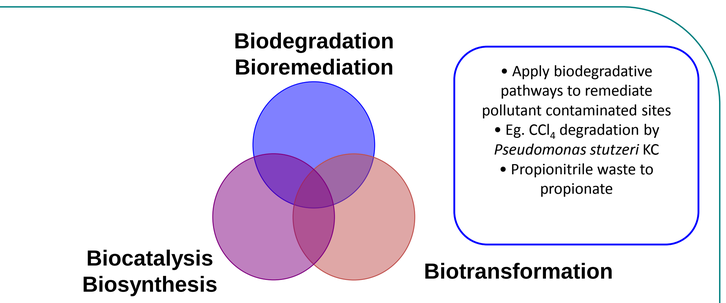


Figure 1: Nitrile hydrolysis catalysed by one of two distinct pathways.

Nitrile-metabolising enzymes are of great interest in the biotechnology arena, as their activities have a myriad of applications in chemical and pharmaceutical industries. This biocatalytic nitrile hydrolysis affords valuable applications in industry, including production of solvents, extractants, pharmaceuticals, drug intermediates (chiral synthons), and pesticides, as well as in the organic synthesis of amines, amides, esters, carboxylic acids, aldehydes, ketones and heterocyclic compounds.



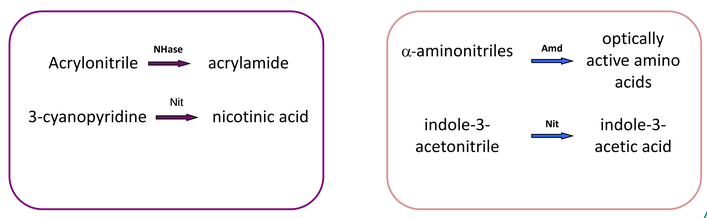


Figure 2: The three main applications of nitrile hydrolysis.

Experimental and Results

Protein Purification

- Organism growth and enzyme induction in benzonitrile
- Cell sonication and isolation of protein extract
- Ion exchange chromatography
- Active fractions pooled for gel filtration chromatography

Enzyme Verification

- Nitrilase-containing fractions analysed by SDS-PAGE
- MALDI-TOF mass spectrometry of appropriate band
- Daughter peptide fragments BLASTed against protein databases

Electron Microscopy

- TEM grids of purified nitrilase prepared by negative staining with uranyl acetate
- Purified nitrilase was stored at 4 °C and electron micrographs taken over a period of time to monitor fibre self-assembly

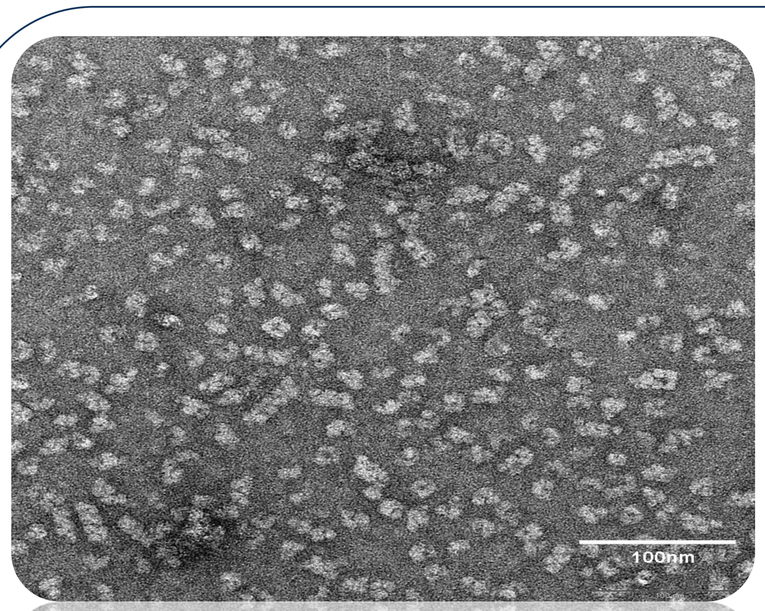


Figure 3: Nitrilase purified from *Rhodococcus rhodochrous* ATCC BAA-870. Micrographs were recorded at 50 000X magnification using a Leo 912 operating at 120 kV. Energy filtering was coupled to a CCD camera and the film digitized at 5.2 Å/pixel.

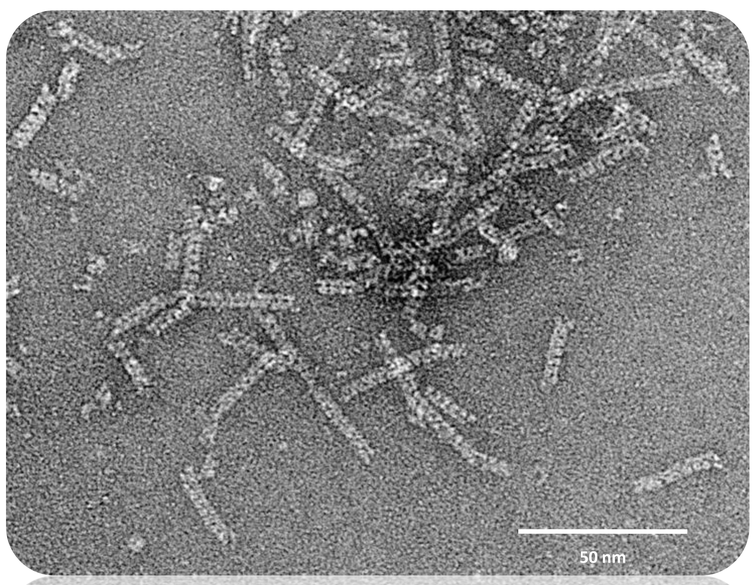


Figure 4: Nitrilase purified from *Rhodococcus rhodochrous* ATCC BAA-870 stored at 4 °C for 20 days. Micrographs were recorded using a JeoL 1200 EXII under low-dose conditions at 50 000X magnification, using a scale of 2 Å/pixel.

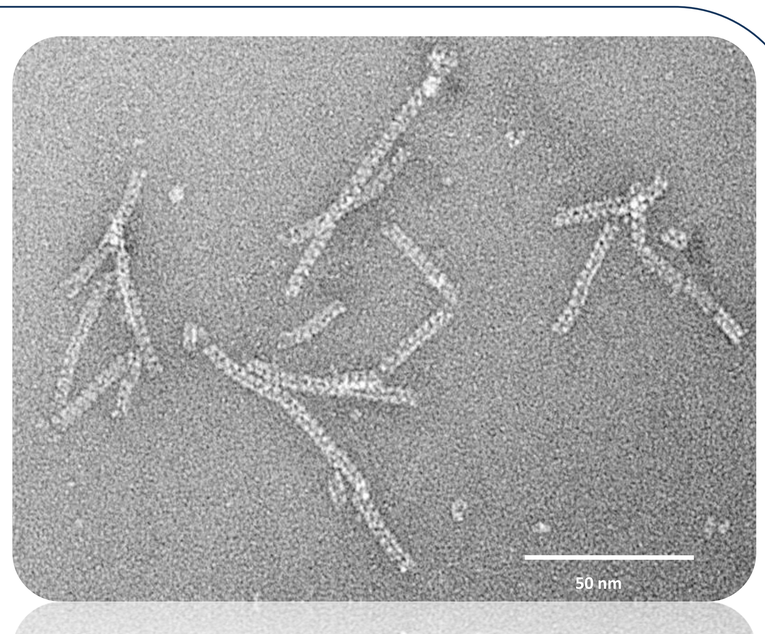


Figure 5: Low-dose electron micrograph of nitrilase fibres from *Rhodococcus rhodochrous* ATCC BAA-870. Nitrilase was purified and stored at 4 °C for 35 days. Micrographs were recorded using a JeoL 1200 EXII under low-dose conditions at 50 000X magnification, using a scale of 2 Å/pixel.

Conclusions

- Benzonitrile activity in the gel filtration eluted fractions coincided with protein at size ~608 KDa
- Nitrilase from *Rhodococcus rhodochrous* ATCC BAA-870 is a ~96% match to nitrilase from *Rhodococcus rhodochrous* J1
- Freshly purified nitrilase was observed to be a mixed species of mostly small subunit complexes, with very few long assemblies (Figure 3)
- Nitrilase shows assembly of subunits into fibres of variable length over time. Most, if not all, nitrilase particles assembled into long fibres after 35 days of cool storage (Figure 4 and 5)
- The measured diameter of the nitrilase fibres are ~130 Å
- Negative stain EM shows nitrilase from *Rhodococcus rhodochrous* ATCC BAA-870 forms homooligomeric fibres of a range of sizes
- Ability of nitrilase from *Rhodococcus rhodochrous* ATCC BAA-870 to form stable enzymatic complexes makes the enzyme a potentially useful tool for biotransformation and a worthwhile candidate for future applications in biotechnology

References

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4. Thuku, R.N., Weber, B.W., Varsani, A. and Sewell, B.T. (2007) Post-translational cleavage of recombinantly expressed nitrilase from Rhodococcus rhodochrous J1 yields a stable, active helical form. FEBS J. **274**, 2099-2108.

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