Nitrilases are enzymes which catalyze the hydrolysis of a nitrile into the corresponding carboxylic acid and ammonia. These enzymes are potentially applicable in biocatalysis and bioremediation because of their advantages over the conventional (chemical) methods of nitrile hydrolysis (lower demand for energy, safety, simplicity, high yields, selectivity).

In this work, genome mining was used to search for the sequences of hypothetical nitrilases from filamentous fungi. The amino acid sequences of previously characterized fungal nitrilases were used as the templates. Then the new synthetic genes together with other genes from our nitrilase library were expressed in *E. coli* and the substrate specificities of the enzymes thus produced were compared. Significant attention was focused on the relationships between the sequence of the enzyme and its substrate specificity.

The arylacetonitrilases from *Arthroderma benhamiae* (NitAb) and *Nectria haematococca* (NitNh) were purified and characterized. Their substrate specificities, kinetic parameters, pH and temperature profiles and subunit and holoenzyme size were assessed.

NitAb and NitNh together with other recombinant fungal nitrilases were employed in the hydrolysis of high concentrations of (*R,S*)-mandelonitrile in a batch or fed-batch mode. Nitrilase from *Aspergillus niger* displayed the best results in enantioselectivity, enabling to prepare (*R*)-mandelic acid with 97.6 % e.e., and in catalyst productivity of 40 g of the product per g of dry cell weight. NitAb displayed a moderate enantioselectivity, which, together with its stability at low pH, make it applicable in the production of (*S*)-mandelic acid from (*S*)-mandelonitrile.

A set of recombinant fungal arylacetonitrilases was tested in hydrolysis of (±)-*trans*-2,4-diphenyl-4,5-dihydrooxazole-5-carbonitrile. The corresponding carboxylic acid is a precursor of taxol, an anti-cancer drug. All tested enzymes displayed a complete conversion of 1 mM substrate within 1-22 hours. Nitrilase from *Neurospora crassa* was the most active and was thus used for the preparative-scale synthesis of (±)-*trans*-2,4-diphenyl-4,5-dihydrooxazole-5-carboxylic acid.