Volume 48(1-4):65, 2004
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

DISSERTATION SUMMARY

Characterization of the biodegradation patways of sulfanilic acid and catechol derviatives in *Sphingomonas subarctica*

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The appearance of man-made chemicals in the world are generally incompatible with the life and their natural degradation is very slow or it does not take place at all. Sulfanilic acid (or p-amino-benzenesulfonate), a typical representative of aromatic sulfonated amines, is widely used as an important intermediate in production of azo-dyes, plant protectives and pharmaceuticals.

So far, efficient decomposition of sulfanilic acid has been reported for a bacterial consortium consisted of a *Hydrogenophaga palleronii* and an *Agrobacterium radiobacter* strain (Dangmann et al. 1996). A dioxygenase, capable to oxidize sulfocatechuate was characterized from both strains (Hammer et al. 1996), but the other components involved in the sulfanilic acid degradation are still unknown.

Recently, a single strain capable to use sulfanilic acid as sole carbon, nitrogen and sulfur source has been isolated (Perei et al. 2001). The isolate was identified taxonomically as *Sphingomonas subarctica*.

Screening the substrate specificity of our strain disclosed its capability to completely degrade six analogue aromatic compounds: sulfanilic acid, protocatechuate, p-aminobenzoic acid, 4-sulfocatechuate, 4-hydroxybenzoate, 3,5-dihydroxybenzoate and oil contaminations.

S. subarctica seemed to express distinct enzyme cascades to utilize these molecules, since alternative enzymes were induced in cells grown on various substrates. Similar protein patterns were observed in the case of sulfanilic acid and sulfocatechol indicating that the latter compound was an intermedier in the degradation of sulfanilic acid.

The sulfanilic acid could only be converted with either intact cells or spheroplasts, but the disrupted cells were unable to convert sulfanilic acid. Hence, we analyzed proteins exclusively expressed in cells grown in the presence of the substrates. Two such proteins were sequenced and the corresponding genes were identified. We found a locus containing genes encoding aminotransferase type and ring hydroxylating dioxygenase enzymes, which likely participated in the conversion of sulfanilic acid to sulfocatechol. Other genes encoding proteins possibly involved in the degradation of aromatics were also found, their functional characterization is in progress.

The product of the first step is sulfocatechol, which is further oxidized by a ring cleaving dioxygenase. The sulfocatechol dioxygenase of *S. subarctica* was partially purified and sequenced by mass spectrometry. On the basis of the protein sequence data, a genomic region was isolated, which contained genes coding for sulfocatechol dioxygenase (SCD), sulfomuconate cycloisomerase (SMC), sulfolactone hydrolase (SLH), an oxidoreductase and a permease.

The *S. subarctica* cells have two types of protocatechol dioxygenase enzymes: type I (PCD) degrades to only protocatechol, but type II (SCD) degrades both to sulfo- and protocatechol. Protocatechol and sulfocatechol pathways are overlapped by sulfocatechol dioxygenase, but the next ring connecting steps require different cycloisomerase enzymes.

Finally, three enzymes, SCD, SMC, SLH, were overexpressed in E. coli, and the pathway of sulfanilic acid degradation was demostrated by recombinant proteins.

References

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