

**Comparison of the abundance of the
different benzoate degradation pathways
and short stories about enrichments on
Isopropanol, Mandelonitrile, and Boc-
Methionine**

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Distribution of the β -Ketoadipate-pathway in comparison to a new pathway of benzoate degradation

Introduction

Aromatic compounds constitute the second most important class of natural organic compounds, next to carbohydrates. Benzoate is a very simple aromatic model substance. Two strategies of aerobic benzoate degradation are known (1, 2, 3) the β -ketoadipate-pathway and a chimeric pathway. The metabolic strategy of this pathway is between the known aerobic and anaerobic strategies (Fig.1).

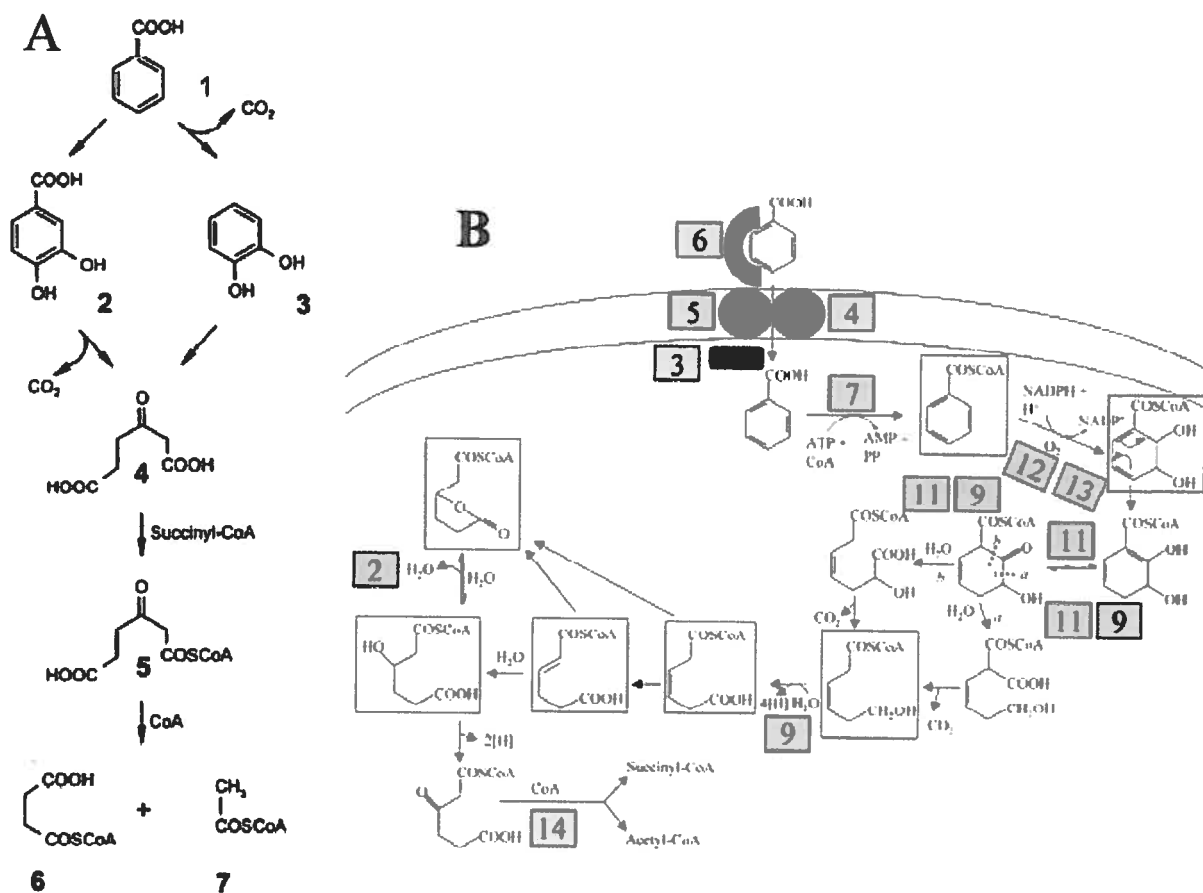


FIG. 1. A: Conventional routes of microbial benzoate oxidation via *ortho* cleavage of catechol (in bacteria) or protocatechuate (in fungi) by the β -ketoadipate pathway. 1, benzoate; 2, protocatechuate; 3, catechol; 4, 3-oxoadipate (β -ketoadipate); 5, 3-oxoadipyl-CoA; 6, succinyl-CoA; 7, acetyl-CoA. Not all intermediates are shown. B: Proposed chimerical benzoate pathway and putative function of gene products. Experimentally documented compounds are enclosed in boxes. The numbers in boxes indicate the ORFs that encode proteins that might catalyze the individual steps.

In the β -keto adipate pathway oxygen is used to hydroxylate and open the aromatic ring. The key-enzymes of that pathway are mono- and dioxygenases.

The chimerical pathway activates benzoate to benzoyl-CoA first and opens the ring after hydroxylation hydrolytically. The key genes of the pathway are boxB and boxA (orf 12 and 13) that code for a new type of dioxygenase and an enoyl-CoA-hydratase (orf 11) that catalyzes the ring cleavage. Until now we know about 8 proteobacterial species and one gram positive organism that have the key genes of the new pathway.

The aim of this study was to elucidate how abundant the new pathway is in comparison to the known β -keto adipate pathway and if there is a special ecological niche in which organisms occur that metabolize benzoate via the new pathway.

Methods

Bacterial strains and media. Sediment cores were taken from an oil contaminated salt marsh, the Eel Pond and a fresh water lake (near Nobska lighthouse). The cores were subdivided in layers of 2 cm. These layers were the inoculums for direct plating and liquid cultures. The salt marsh sediment core had a length of 20 cm, the Eel Pond core had a length of 14 cm, and the Freshwater lake core had also a length of 14 cm. Liquid cultures were streaked on plates after they reached an OD₅₇₈ of 0.5. All isolates were restreaked at least twice.

Media. The Freshwater medium (DSMZ Medium 566; www.dsmz.de) used was a basic potassium phosphate buffer. The medium contained 5mM benzoate as sole carbon and energy source.

Solution A:

KH ₂ PO ₄	0.816	g
K ₂ HPO ₄	5.920	g
Distilled water	500.000	ml

Solution B:

NH ₄ Cl	0.530	g
MgSO ₄ x 7 H ₂ O	0.200	g
KNO ₃	2.000	g
CaCl ₂ x 2 H ₂ O	0.025	g
Distilled water	500.000	ml

Tab. 1: Basal Freshwater medium. Solutions A and B were adjusted to a final pH of 7.2 autoclaved separately and combined after cooling. The final concentration of benzoate in the medium was 5 mM. 15g/l bactoagar were added for the production of plates. 1ml of the 1000x trace element solution SL 10 and of the 1000x 7-vitamin solution were added after the medium was cooled down to 60°C.

7-Vitamin solution

Niacin	35 mg
Thiamine dichloride	30 mg
p-Aminobenzoic acid	20 mg
Pyridoxolium HCL	10mg
Ca-pnthothenate	10mg
Vitamin B12	5mg

Tab. 2: Vitamin solution. The vitamins were dissolved in 100ml water and sterilized by filtration. The solution is 1000x concentrated.

Trace element solution SL 10

Distilled water	1 liter
HCl (37%)	8.5 ml
FeCl ₂ 4H ₂ O	1.5 g
H ₃ BO ₃	6 mg
CoCl ₂ 6H ₂ O	190 mg
MnCl ₂ 4H ₂ O	100 mg
ZnCl ₂	70 mg
Na ₂ MoO ₄ 2H ₂ O	36 mg
NiCl ₂ 6H ₂ O	24 mg
CuCl ₂ 2H ₂ O	2 mg

Tab. 3: Trace element solution SL10. The salts were dissolved and the 1000x solution was sterilized by autoclaving.

The Basal marine medium used was prepared according to the course manual (Tab. 4). The medium contained 5mM benzoate as sole carbon and energy source.

Salt water base

NaCl	20g/l
MgCl ₂ 6H ₂ O	3g/l
CaCl ₂ 2H ₂ O	0.15g/l
KH ₂ PO ₄	0.2g/l
KCl	0.5g/l
NH ₄ Cl	0.25g/l

Tab. 4: Basal marine medium. 990ml of the base were mixed with 0.5ml of 1M Na-Sulfate and 10ml of 1M MOPS buffer pH 7.2. For the production of plates 15g/ml agar were added to the medium.

PCR with key enzyme specific primers. The Primers were derived from conserved domains of the catechol dioxygenase gene, the protocatechuate dioxygenase gene and boxB. In this study only the boxB specific primers 570For and 1110Rev and the 16srDNA primers 8F and 1492rev were used. The annealing temperature of the boxB specific primers was 60°C. Table 5 shows the primer sequences.

Name	Sequence
Catechfor1:	ACCATTTATTTCTTCGACCC
Catechrev1:	GTGATAGAAGATCGCCTTGCCC
Catechfor2:	ACGATCTATTTCTTCGATCC
Catechrev2:	GTGATAGAAGATGCCGCTGCCG
Protocatfor1:	TGGCAGGCCAATGCCGCGGCCG
Protocatfor2:	CTGATCACSCAGMTGTACTTC
Protocatrev1:	GAAGTACAKCTGSGTGATCAG
Protocatrev2:	CCGGCGCCAGSACGATDTCG
570FOR:	CGCGTTCAACGAGAAGAC
630FOR:	CCGACCGTGACGGCAAGTACCAGC
1110REV	GTTCCAGCGGTCGACGCC
1350REV	GCGATCCAGTTGGCGAAC
8F	AGAGTTTGATCMTGGCTCAG
1492R	GGTTACCTTGTTACGACTT

Tab. 5: Primers used.

A usual PCR reaction contained 20 μ l of Platinum Supermix (Invitrogen^R), 1 μ l of a 10 μ M forward primer solution, 1 μ l of a 10 μ M reverse primer solution and 1 μ l of template. Genomic DNA of *Azoarcus evansii* was used as a positive control.

DNA isolation. DNA was isolated from all soil layers using the MoBIO Soil DNA Isolation kit (MoBio laboratories, Inc.). In addition to the manufacturers manual the samples were freeze thawed for 5 to 10 times. For a colony PCR of isolated strains a toothpick was used to take material from a single colony. These materials were diluted in 50 μ l of water, freeze thawed and heated for 10 min to 96 $^{\circ}$ C. 1 μ l of these DNA solutions was used as a PCR template.

Preparation of cell extracts. All steps used for preparation of cell extracts were performed at 4 $^{\circ}$ C. Frozen cells were suspended in an equal volume of 20% Glycerol containing 0.1 mg of DNase I ml⁻¹. 0.6 ml of cold cell suspension was disrupted by grinding with 1.2 g of glass beads in a beat beater for 5 min at fast speed.

Enzyme assay for BoxA (1). BoxA enzyme activity was monitored spectrophotometrically at 365 nm by determining benzoyl-CoA-, oxygen-, and FAD-dependent oxidation of NADPH at 37 $^{\circ}$ C. The standard assay mixture (0.5 ml) contained 100 mM Tris-HCl (pH 8.0), 0.1 mM FAD, 0.3 mM NADPH, and cell extract (10 μ l of supernatant). Addition of 0.1 mM benzoyl-CoA started the test.

Results

Strain Isolation. Direct plating and streaks of the liquid cultures led to the isolation of 120 colonies that were restreaked twice before they were used for further experiments. 61 clones grew on saltwater agar plates. 59 clones grew on freshwater plates.

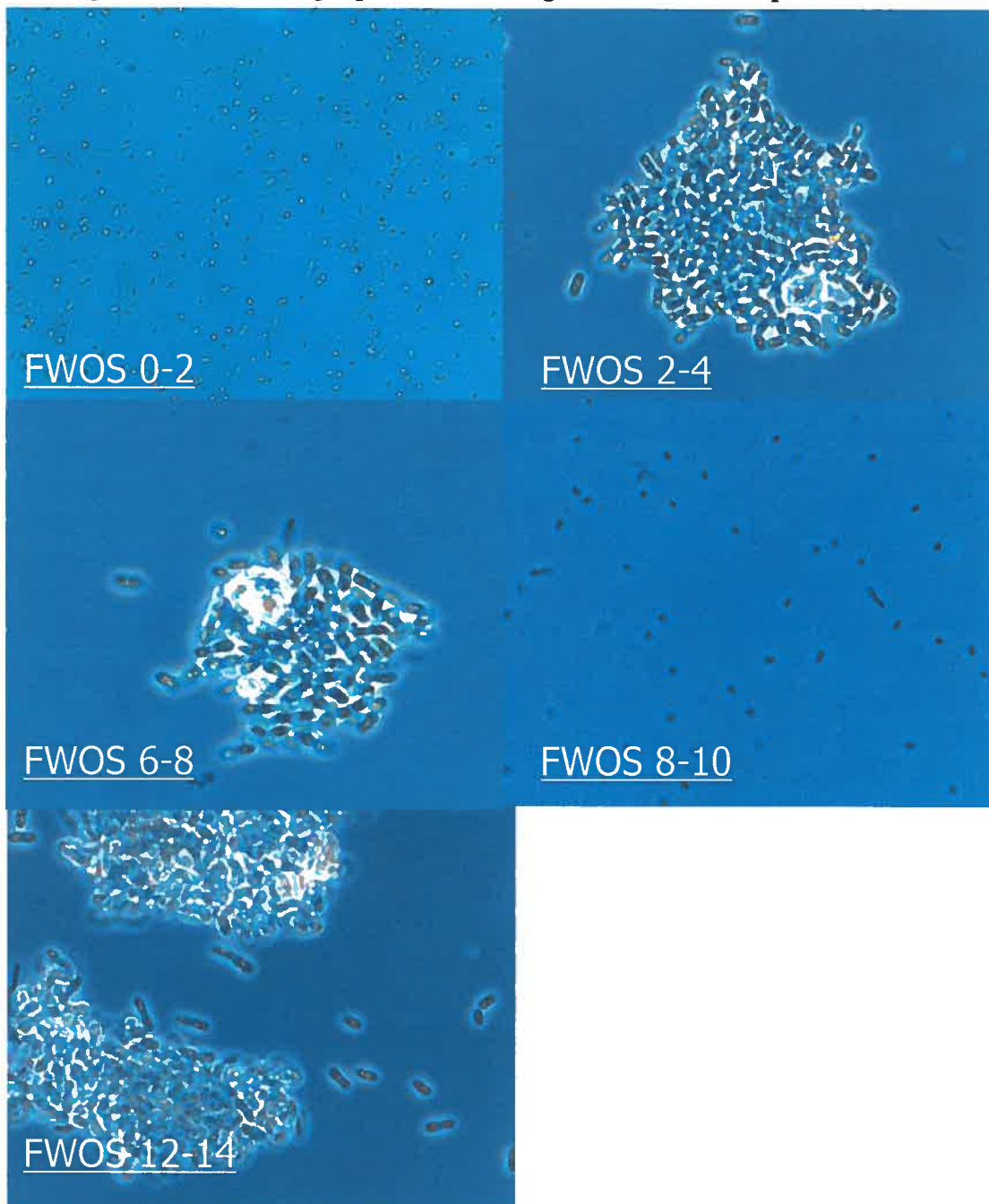


Fig. 2: Freshwater benzoate enrichment cultures of the oil contaminated salt marsh. FW: freshwater medium, OS: Oil contaminated salt marsh. Numbers indicate the cm of the sediment core that were used as an inoculum. The Layers from 4-6 cm, 14-16 cm, 16-18 cm and 18-20 cm did not show growth.

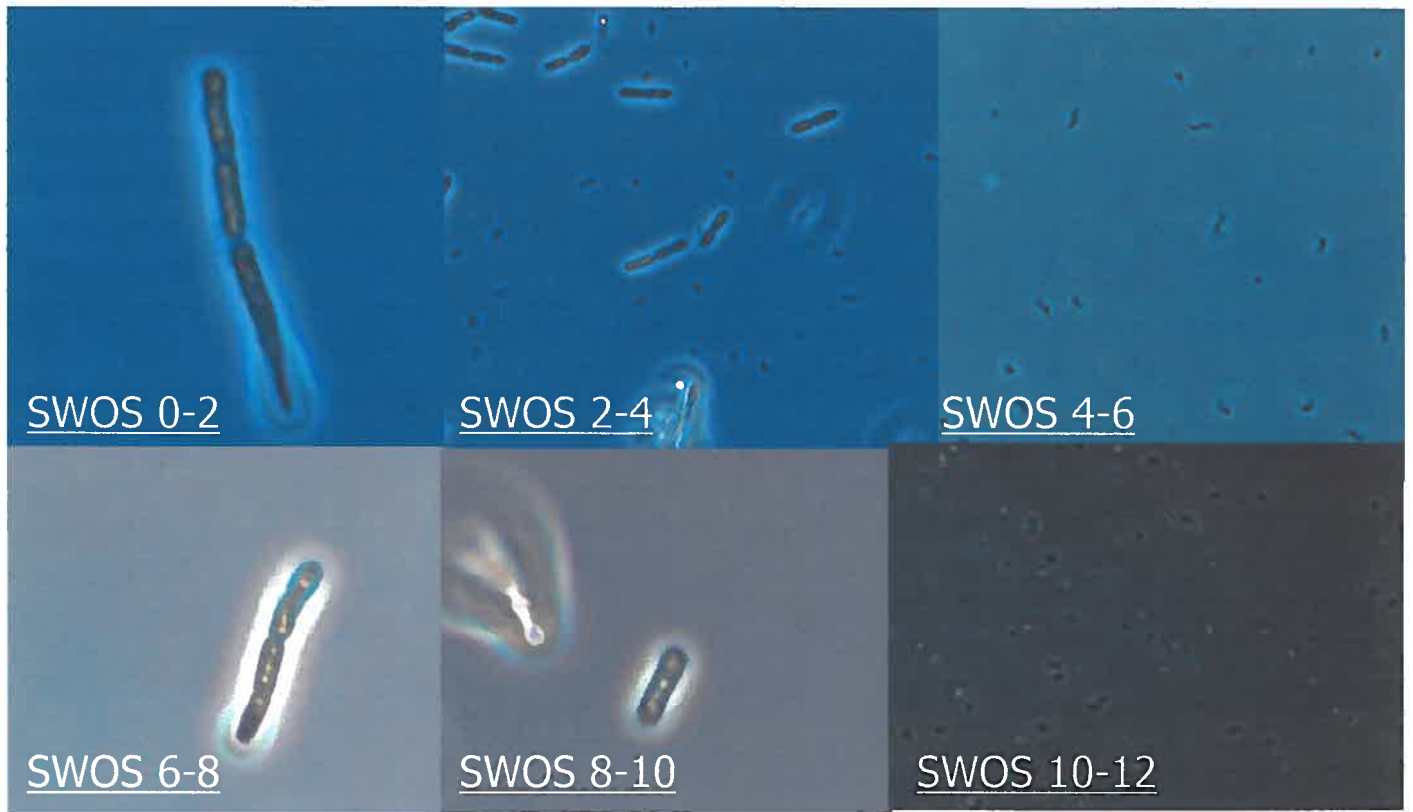


Fig. 3: Saltwater benzoate enrichment cultures of the oil contaminated salt marsh.
SW: saltwater medium, OS: Oil contaminated salt marsh. Numbers indicate the cm of the sediment core that were used as an inoculum. The Layers from 12-14 cm, 14-16 cm, 16-18 cm and 18-20 cm did not show growth.

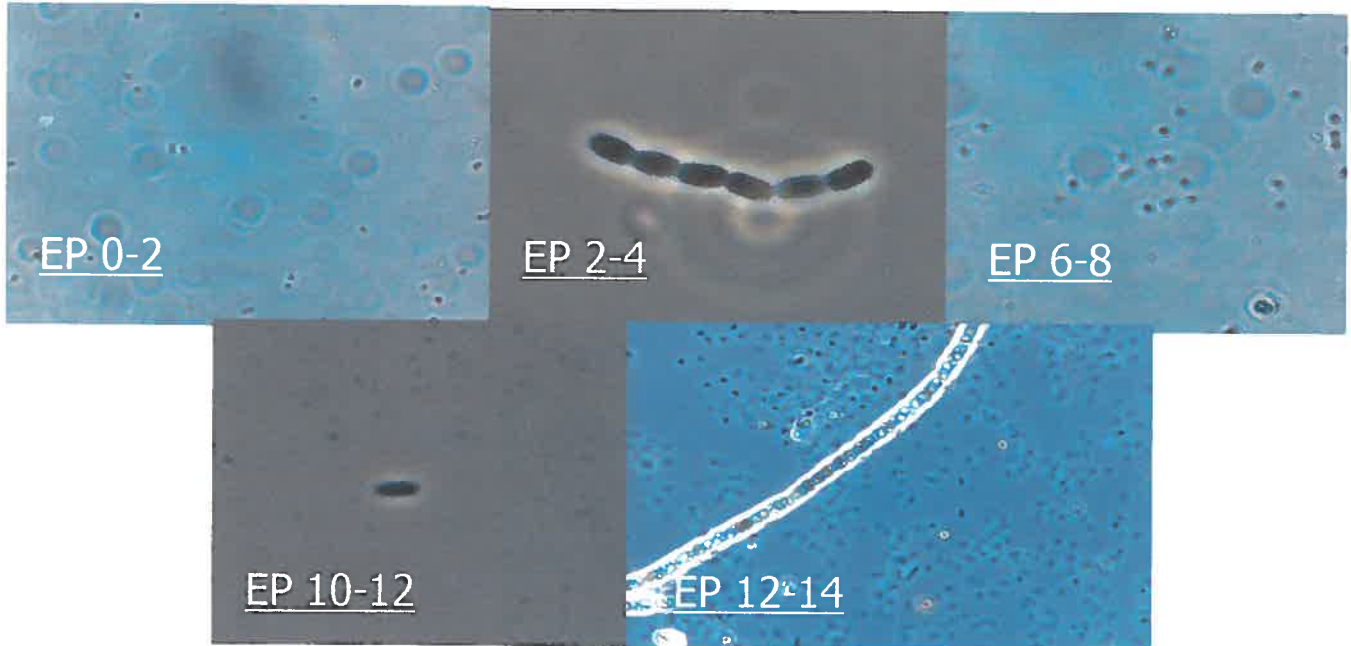


Fig. 4: Saltwater benzoate enrichment cultures of an Eel Pond sediment core.
 EP: Eel Pond sediment core. Numbers indicate the cm of the sediment core that were used as an inoculum.
 The Layer from 4-6 cm did not show growth.

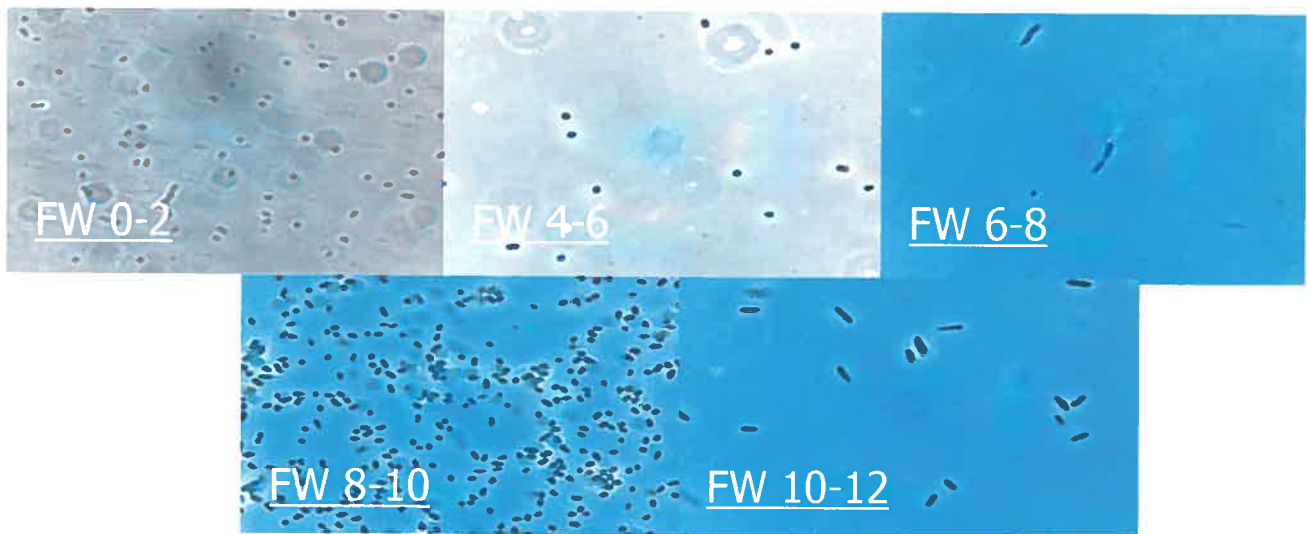


Fig. 5: Freshwater benzoate enrichment cultures of a freshwater sediment core.
 FW: Freshwater sediment core. Numbers indicate the cm of the sediment core that were used as an inoculum. The Layers from 2-4 and 12-14 cm did not show growth.

The Figures 2,3,4 and 5 show pictures of the different liquid cultures. The pictures show a rapid change in the benzoate degrading community within a few centimeters of sediment. One example is the enrichment FW4-6 in comparison to FW6-8. The organisms change here between rods and cocci in 2 cm. Furthermore the comparison of the marsh sediment layer enrichments shows that the resulting community of benzoate degraders does not only depend on the inoculum but also on the buffer system used. The

type of organisms that could be found in the enrichments differs remarkably for example between the enrichments FW2-4 and SW2-4.

Diversity of the isolated benzoate degraders.

An ADRA experiment was undertaken to find the rate of diversity within the 120 clones. The 16srDNA of 70 samples was tried to be amplified via PCR. 40 of the 70 reactions showed a PCR product of the right length. 30 reactions did not show any PCR product. The PCR products were digested with the enzyme MspI and resulting fragments were analyzed on a 1% agarose gel. 11 different fragment patterns could have been found. The macroscopic analyzes showed at least 12 different colony types. Figure 6 shows some of these colony types.



Fig. 6: Different types of colonies under the 120 isolated benzoate degraders.

PCR with specific primers using isolated DNA from soil as template. DNA was isolated from all layers of the sediment cores. The first 4 centimeters of each layer were used as a template in a first test PCR. Genomic DNA of *Azoarcus evansii* was used as positive control. The PCR with the soil DNA samples led to a PCR product after a 10 fold dilution of the template DNA. Only the PCR with the isolated DNA of the first 2 centimeters of marsh sediments as a template did not lead to a PCR product. The DNA of the centimeters 0-8 and 9-14 or 9-20 were pooled. A PCR with these pooled DNAs showed only a very low product formation. For further improvement of the PCR with the aim to build up a clone library was not enough time.

PCR with specific primers using isolated colonies as template. PCR reactions with the isolated benzoate degraders showed 6 positive strains. These organisms were grown in a

larger volume and tested for there BoxA activity. None of the strains showed activity in the assay.

Discussion

PCR of isolated clones. 120 benzoate degrading clones were isolated in this study. They belong to at least 10 different species. The isolated clones were used as templates for a PCR with boxB specific primers. 6 clones showed a positive PCR signal. These clones turned out to be negative in the biochemical assay for BoxA activity. This can have several reasons. First the test is designed for *A. Evansii* if the organisms need different conditions the test would not be positive. Second the test shows only an artificial enzyme activity. BoxA is the reducing component of the benzoyl-CoA-dioxygenase of *A. Evansii*. Under the assay conditions BoxA transfers electrons from NADPH to water. The resulting product is H_2O_2 . If the organism would have a protein similar to BoxA that has a more specific activity no activity would be detectable. Third the organisms might have not only the genes for the chimerical pathway but have also other gene sets for benzoate degradation. If these other sets are active during growth on benzoate the BoxA enzyme assay would not show any activity. Fourth the PCR products could be the result of unspecific primer binding. Sequencing of the fragments is necessary to say whether the amplified PCR products are amplified regions of boxB or not.

PCR with isolated DNA from soil. PCR with the isolated DNA from the first 4 cm of the sediment cores resulted in fragments of the right length. Only the PCR with the cm 0-2 of the marsh sediment did not led to a product. After pooling of the DNA extractions from the layers 0-8 and 9-14 or 9-20 the PCR led only to a very small product formation. A set up of clone libraries and additional sequencing is necessary to see how many different boxB genes you can find at different spots of the environment.

Literature:

1. **Gescher, J., A. Zaar, M. Mohamed, H. Schagger, G. Fuchs.** Genes coding for a new pathway of aerobic benzoate metabolism in *Azoarcus evansii*. 2002. *J Bacteriol.* Nov;184(22):6301-15.
2. **Harwood, C.S., Parales, R. E.** 1996. The beta-ketoadipate pathway and the biology of self-identity. *Annu Rev Microbiol.* 1996;50:553-90
3. **Zaar A, W. Eisenreich, A. Bacher, G. Fuchs.** A novel pathway of aerobic benzoate catabolism in the bacteria *Azoarcus evansii* and *Bacillus stearothermophilus*. 2001. *J Biol Chem.* Jul 6;276(27):24997-5004.

Isopropanol Degradation

Introduction

Isopropanol production worldwide exceeds 1×10^6 tonnes per year. It is used industrially as a solvent, intermediate, de-icer and has many other applications (production of rubber, cosmetics, textiles, pharmaceuticals and fine chemicals) [since it is cheaper than ethanol]. Little is known about the physiology, and subsequent industrial application, of aerobic IPA-degrading microorganisms (most publications describe mixed microbial consortia) (1,2,3). The pathway for Isopropanol degradation seems to be well known (Fig. 1).

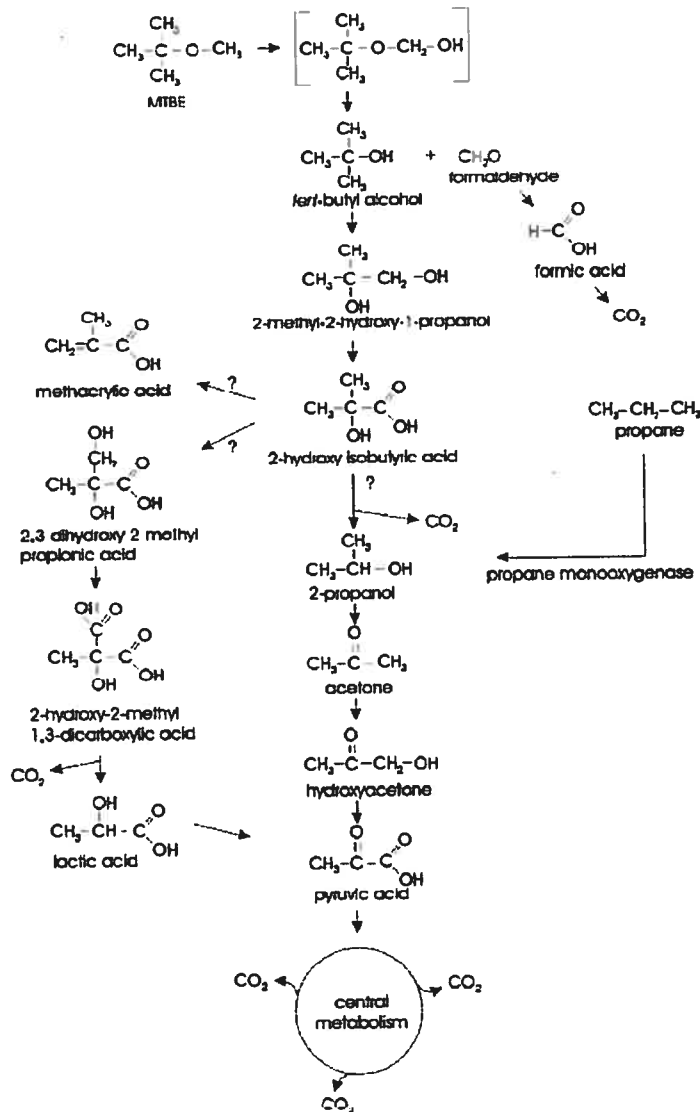


FIG. 1. Proposed pathways for the degradation of MTBE and isopropanol by propane-oxidizing bacteria (3).

The aim of this study was to isolate isopropanol degraders for further research.

Methods

Bacterial strains and media. 300 ml of Eel Pond water and water of the cedar swamp were filtered through a 0,2 μm filter. These filters were used as inoculums for aerobic enrichments on isopropanol as only source of carbon and energy.

Media. The Freshwater medium (DSMZ Medium 566) used was a basic potassium phosphate buffer. The medium contained 10g/l Isopropanol as sole carbon and energy source.

Solution A:

KH_2PO_4	0.816	g
K_2HPO_4	5.920	g
Distilled water	500.000	ml

Solution B:

NH_4Cl	0.530	g
$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$	0.200	g
KNO_3	2.000	g
$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$	0.025	g
Distilled water	500.000	ml

Tab. 1: Basal Freshwater medium. Solutions A and B were adjusted to a final pH of 7.2 autoclaved separately and combined after cooling. 1ml of the 1000x trace element solution SL 10 and of the 1000x 7-vitamin solution were added after the medium was cooled down to 60°C.

7-Vitamin solution

Niacin	35	mg
Thiamine dichloride	30	mg
p-Aminobenzoic acid	20	mg
Pyridoxolium HCL	10	mg
Ca-pnthothenate	10	mg
Vitamin B12	5	mg

Tab. 2: Vitamin solution. The vitamins were dissolved in 100ml water and sterilized by filtration. The solution is 1000x concentrated.

Trace element solution SL 10

Distilled water	1	liter
HCl (37%)	8.5	ml
$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	1.5	g
H_3BO_3	6	mg
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	190	mg
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	100	mg
ZnCl_2	70	mg
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	36	mg
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	24	mg
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	2	mg

Tab. 3: Trace element solution SL10. The salts were dissolved and the 1000x solution was sterilized by autoclaving.

The Basal marine medium used was prepared according to the course manual (Tab. 4). The medium contained 10 g/l isopropanol as sole source of carbon and energy.

Salt water base

NaCl	20g/l
MgCl ₂ 6H ₂ O	3g/l
CaCl ₂ 2H ₂ O	0.15g/l
KH ₂ PO ₄	0.2g/l
KCl	0.5g/l
NH ₄ Cl	0.25g/l

Tab. 4: Basal marine medium. 990ml of the base were mixed with 0.5ml of 1M Na-Sulfate and 10ml of 1M MOPS buffer pH 7.2.

Results

16 days after inoculation the Cedar swamp enrichment became turbid. 2ml of this initial culture were used to inoculate a new flask with 40 ml of sterile medium. This culture reached an OD₅₇₈ of 0.2 in 4 days.

The culture shows two kinds of organisms (Fig. 2). Both rods and cocci seem to occur in a similar number.



Fig. 3: Organisms that grow in the isopropanol enrichment.

Discussion

Further studies are necessary to isolate the two strains and to see if the culture really grows on isopropanol and what the features of the two kinds of organisms are.

Literature

- 1. Bustard M. T., E. M. McEvoy, J. A. S. Goodwin, J. G. Burgess, P. C. Wright.** 2000. Biodegradation of propanol and isopropanol by a mixed microbial consortium. *Appl Microbial Biotechnol*, 54: 424-431
- 2. Bustard M. T, S. Whiting, D. A. Cowan, P. C. Wright.** 2001. Biodegradation of high-concentration isopropanol by a solvent-tolerant thermophile, *Bacillus pallidus*. *Extremophiles*, 6:319 –323
- 3. Steffan RJ, K. McClay, S. Vainberg, C. W. Condee, D. Zhang.** Biodegradation of the gasoline oxygenates methyl tert-butyl ether, ethyl tert-butyl ether, and tert-amyl methyl ether by propane-oxidizing bacteria. 1997. *Appl Environ Microbiol* Nov;63(11):4216-22.

Degradation of Boc-Methionine

Introduction

Tertbutyloxycarbonyl-aminoacids (Boc-aa) are used in solid phase peptide synthesis. The carboxyterminal amino acid is blocked at the amino end by a tertbutyloxycarbonyl (Boc) group and is covalently attached to the resin support as a benzyl ester via the chloromethyl group. Side chain functional groups must also be blocked, usually with benzyl-based derivatives. The Boc group is completely removed with 50%trifluoroacetic acid in dichloromethane, with minimal loss of the anchoring bond or of the other protecting groups (Fig. 1) (1).

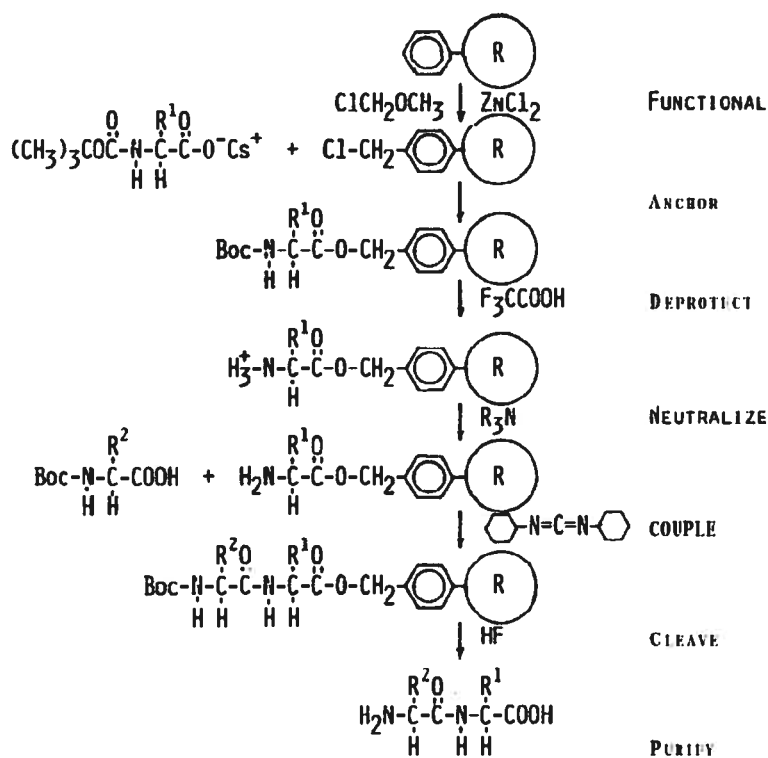


Fig. 1: A scheme for solid phase peptide synthesis

The degradation pathways for the amino acids alone as well as for tert-butyl alcohol are known (2, 3). But nothing is known about the degradation of Boc-aa.

This study aimed in elucidating if a Boc-aa degradation is possible.

Methods

Bacterial strains and media. 300 ml of Eel Pond was filtered through a 0,2 μm filter. This filter was used as inoculum for aerobic enrichments on boc-methionine as the only source of carbon, nitrogen, sulfur and energy.

Media. The Freshwater medium (DSMZ Medium 566) used was a basic potassium phosphate buffer. The medium contained 5mM boc-methionine as the only source of carbon, nitrogen, sulfur and energy.

Solution A:

KH_2PO_4	0.816	g
K_2HPO_4	5.920	g
Distilled water	500.000	ml

Solution B:

MgCl_2	0.076	g
KNO_3	2.000	g
$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$	0.025	g
Distilled water	500.000	ml

Tab. 1: Basal Freshwater medium. Solutions A and B were adjusted to a final pH of 7.2 autoclaved separately and combined after cooling. 1ml of the 1000x trace element solution SL 10 and of the 1000x 7-vitamin solution were added after the medium was cooled down to 60°C.

7-Vitamin solution

Niacin	35	mg
Thiamine dichloride	30	mg
p-Aminobenzoic acid	20	mg
Pyridoxolium HCL	10	mg
Ca-pnthothenate	10	mg
Vitamin B12	5	mg

Tab. 2: Vitamin solution. The vitamins were dissolved in 100ml water and sterilized by filtration. The solution is 1000x concentrated.

Trace element solution SL 10

Distilled water	1	liter
HCl (37%)	8.5	ml
$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	1.5	g
H_3BO_3	6	mg
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	190	mg
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	100	mg
ZnCl_2	70	mg
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	36	mg
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	24	mg
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	2	mg

Tab. 3: Trace element solution SL10. The salts were dissolved and the 1000x solution was sterilized by autoclaving.

The Basal marine medium used was prepared according to the course manual (Tab. 4). The medium contained 5mM boc-methionine as the only source of carbon, nitrogen, sulfur and energy.

Salt water base

NaCl	20g/l
MgCl ₂ 6H ₂ O	3g/l
CaCl ₂ 2H ₂ O	0.15g/l
KH ₂ PO ₄	0.2g/l
KCl	0.5g/l

Tab. 4: Basal marine medium. 990ml of the base were mixed with 10ml of 1M MOPS buffer pH 7.2.

Results

After 21 days of enrichment both enrichment became turbid. The cultures show at least three kinds of organisms (Fig. 2). The shape of the organisms differs between the different cultures.

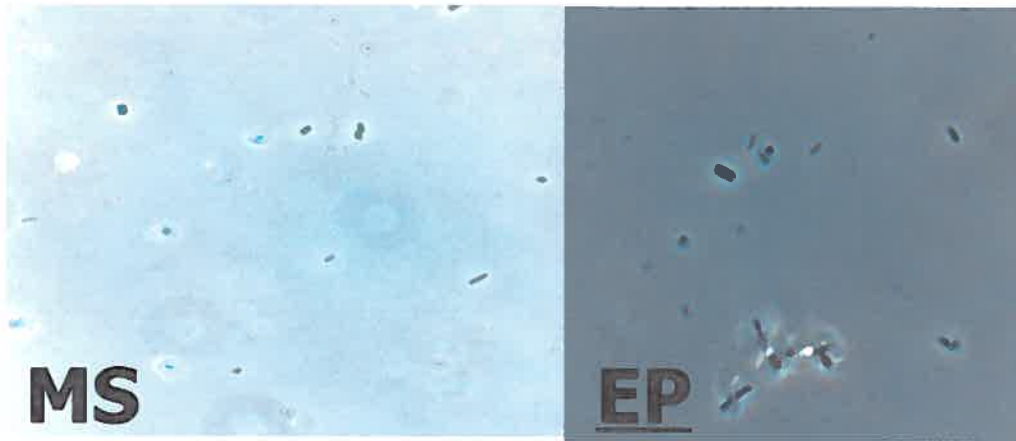


Fig. 3: Organisms that grow in the boc-methionine enrichment. MS: Marsh sediment enrichment; EP: Eel Pond enrichment.

Discussion

Further studies are necessary to isolate the different strains and to see, if the culture really grows on boc-methionine and what the features of the organisms and the key enzymes are.

Literature

1. **Merrifield, B.** Solid Phase Synthesis. 1984 Nobel lecture
<http://www.nobel.se/chemistry/laureates/1984/merrifield-lecture.pdf>
2. **Steffan RJ, K. McClay, S. Vainberg, C. W. Condee, D. Zhang.** Biodegradation of the gasoline oxygenates methyl tert-butyl ether, ethyl tert-butyl ether, and tert-amyl methyl ether by propane-oxidizing bacteria. 1997. *Appl Environ Microbiol* Nov;63(11):4216-22.
3. **Lengeler J. W., Drews, G., Schlegel, H. G. 1999.** *Biology of the Prokaryotes* Blackwell Science, Oxford.

Mandelonitrile Degradation

Introduction

Cyanogenesis is the production of the respiratory poison HCN by biological organisms. It is a defense mechanism of plants against herbivores and pathogens.

The crushing of mature seeds leads to a rapid production of benzaldehyde and HCN. The reaction is catalyzed by the mandelonitrile lyase.

The catalyzed reaction is shown in figure 1.

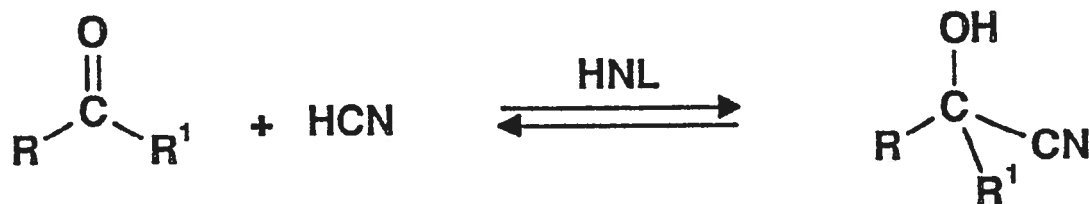


Fig. 1: Reaction catalyzed by mandelonitrile lyase.

HCN is toxic to most aerobic organisms. It inhibits the cytochrome oxidase.

The aim of this enrichment studies was to find an organism that uses mandelonitrile as the sole source of carbon, nitrogen and energy. The hypothesis is that mandelonitrile could be converted to mandelate or to benzaldehyde. The pathway of mandelate metabolism is well known (Fig. 2) (2, 3). One of the intermediates is benzoate.

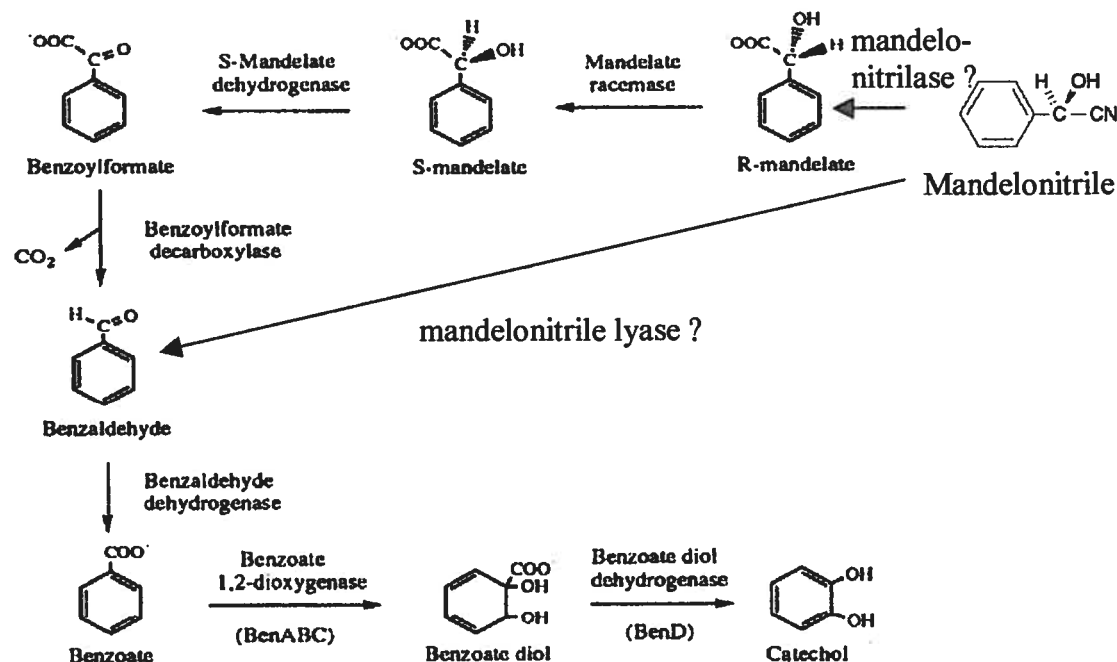


Fig. 2: Proposed pathways for the mandelonitrile degradation (2, 3)

The activity of a mandelonitrile lyase would lead to the production of cyanide that has to be detoxified. Two separate conversions have been described. They are hydrolytic and oxidative conversion, and they yield formic acid and carbon dioxide as reaction by-products, respectively (4).

Methods

Bacterial strains and media. The liquid enrichments for the benzoate degraders were used as inoculums. 50 ml of 8 liquid enrichments were used to inoculate 30ml of liquid media with mandelonitrile as sole source of carbon, energy and nitrogen.

The Freshwater medium (DSMZ Medium 566) used was a basic potassium phosphate buffer. The medium contained 5mM mandelonitrile as sole carbon, nitrogen, and energy source.

Solution A:

KH ₂ PO ₄	0.816	g
K ₂ HPO ₄	5.920	g
Distilled water	500.000	ml

Solution B:

MgSO ₄ x 7 H ₂ O	0.200	g
KNO ₃	2.000	g
CaCl ₂ x 2 H ₂ O	0.025	g
Distilled water	500.000	ml

Tab. 1: Basal Freshwater medium. Solutions A and B were adjusted to a final pH of 7.2 autoclaved separately and combined after cooling. 1ml of the 1000x trace element solution SL 10 and of the 1000x 7-vitamin solution were added after the medium was cooled down to 60°C.

7-Vitamin solution

Niacin	35	mg
Thiamine dichloride	30	mg
p-Aminobenzoic acid	20	mg
Pyridoxolium HCL	10	mg
Ca-pnthothenate	10	mg
Vitamin B12	5	mg

Tab. 2: Vitamin solution. The vitamins were dissolved in 100ml water and sterilized by filtration. The solution is 1000x concentrated.

Trace element solution SL 10

Distilled water	1 liter
HCl (37%)	8.5 ml
FeCl ₂ 4H ₂ O	1.5 g
H ₃ BO ₃	6 mg
CoCl ₂ 6H ₂ O	190 mg
MnCl ₂ 4H ₂ O	100 mg
ZnCl ₂	70 mg
Na ₂ MoO ₄ 2H ₂ O	36 mg
NiCl ₂ 6H ₂ O	24 mg
CuCl ₂ 2H ₂ O	2 mg

Tab. 3: Trace element solution SL10. The salts were dissolved and the 1000x solution was sterilized by autoclaving.

The Basal marine medium used was prepared according to the course manual (Tab. 4). The medium contained 10 g/l isopropanol as sole carbon and energy source.

Salt water base

NaCl	20g/l
MgCl ₂ 6H ₂ O	3g/l
CaCl ₂ 2H ₂ O	0.15g/l
KH ₂ PO ₄	0.2g/l
KCl	0.5g/l
NH ₄ Cl	0.25g/l

Tab. 4: Basal marine medium. 990ml of the base were mixed with 0.5ml of 1M Na-Sulfate and 10ml of 1M MOPS buffer pH 7.2.

Results

After 21 days of enrichment one of the two bottles became turbid. The culture shows only one kind of organism (Fig. 2). It has a bitter almond smell. That indicates that mandelonitrile was degraded to cyanide and another intermediate. If the existence of cyanide is due to a bacterial degradation then benzaldehyde might be another degradation product.



Fig. 3: Organism that grows in the mandelonitrile enrichment.

Discussion

Further isolation and HPLC analyzes is important to answer the question if there is really only one species in the enrichment and if mandelonitrile is degraded via the cyanide benzaldehyde pathway.

Literature

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