Biological conversion of lignin model compounds by isolates from the Baltic Sea

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OVERVIEW AND AIM

Lignin is a heterogeneous alkyl-aromatic polymer found in the plant cell wall, which is under-utilized in the biorefining industry. The monomeric constituents of lignin are potential raw materials for further upgrading by chemical or biological means. In this present study we have selected five bacterial strains which were previously **isolated from sediments of the Baltic Sea.** The isolated organisms were identified tentatively using 16S rRNA sequencing and the species with highest score in BLAST were *Bacillus licheniformis, Bacillus safensis, Rhodococcus erythropolis, Prolinoborus fasciculus* **and** *Pseudomonas deceptionensis*. **Characterization** of these organisms was carried out by shake flask experiments with few selected **lignin model compounds as a sole source of carbon and energy**. The conversion and consumption of these model compounds through the **β-ketoadipate pathway** was observed and the flux through the pathway was calculated.

RESULTS AND DISCUSSION

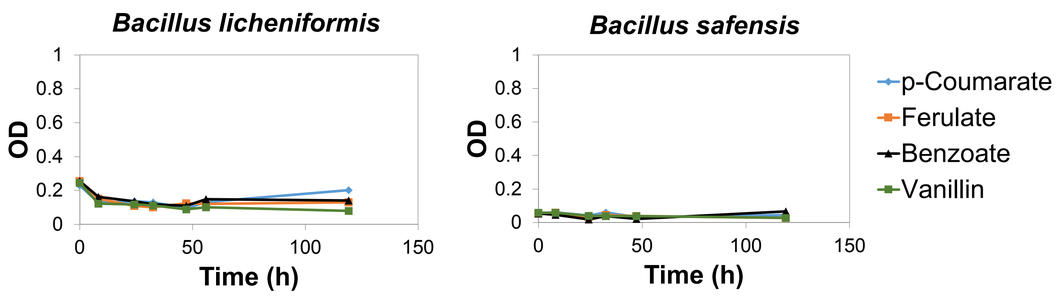

Figure 1: Growth of *B. licheniformis* and *B. safensis* on 5 mM of *p*-coumarate, ferulate, benzoate and vanillin. **No growth was observed until 125 h**. Both organisms seem to lack β-ketoadipate pathway and hence was not interesting for further study.

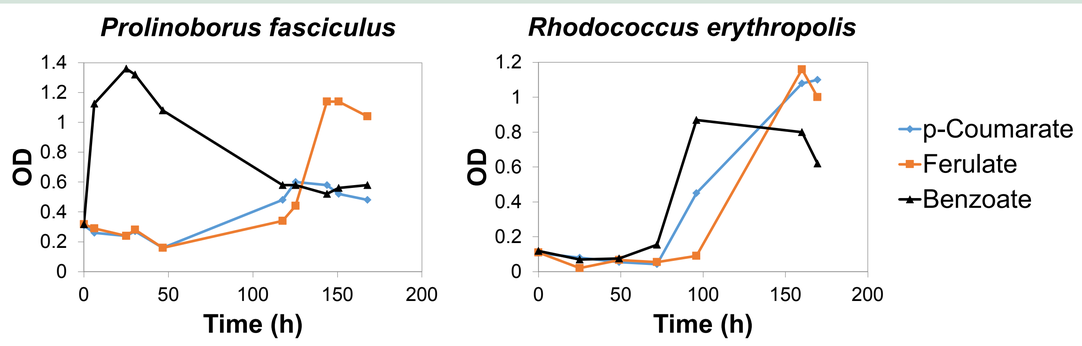

Figure 2: Growth of *P. fasciculus* and *R. erythropolis* on 5 mM of *p*-coumarate, ferulate and benzoate. *P. fasciculus* seems to grow on benzoate without any lag phase. There was a poor growth on *p*-coumarate with the maximum OD of only up to 0.6. The growth on ferulate had a long lag of around 100 h. *P. fasciculus* seems to have most of the branches of the β-ketoadipate pathway, but the ferulate and *p*-coumarate upper funneling braches are quite slow. *R. erythropolis* grew on all three model compounds, but with a lag of around 75 h. This organism seems to exhibit the branches of β-ketoadipate pathway, but was not interesting because of the long lag and slow growth.

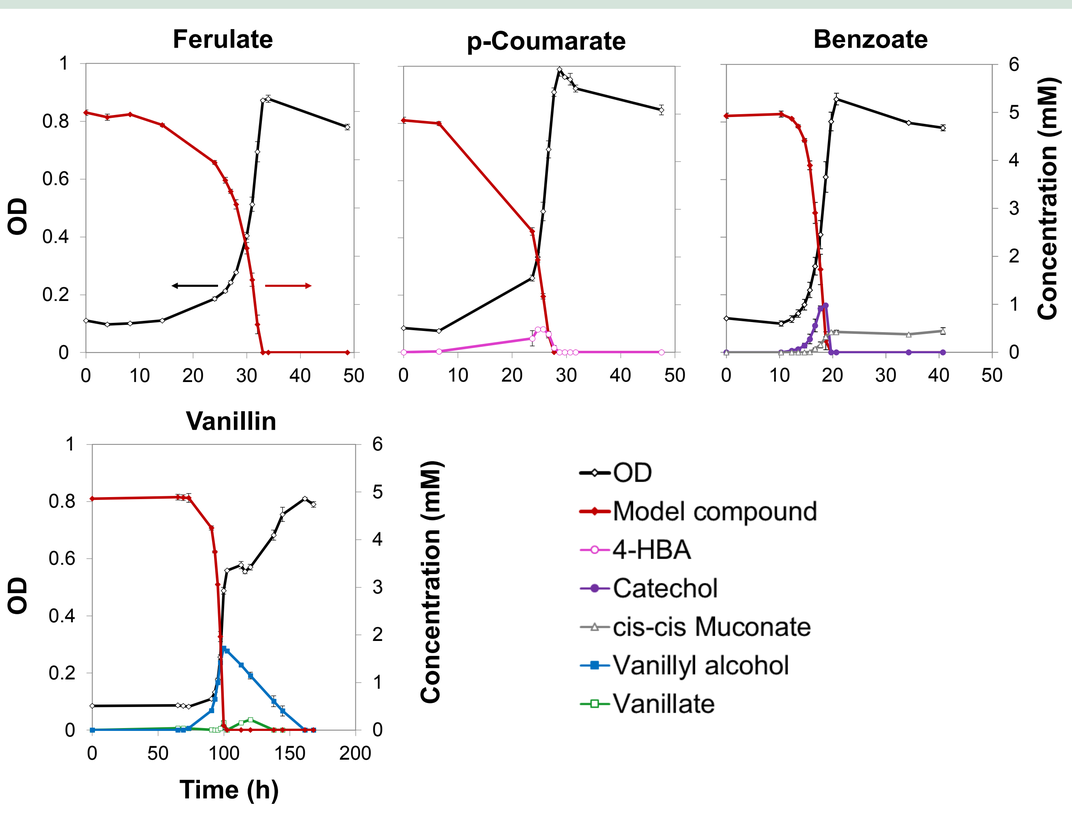

Figure 3: Growth of ***P. deceptionensis*** on 5 mM of ferulate, *p*-coumarate, benzoate, and vanillin. No growth on syringate. (Excreted intermediates – Read from right axis)

There was a clear growth of *P. deceptionensis* on the compounds from the benzoyl, coumaryl and coniferyl branches, which depicts the existence of these branches in the upper funneling β-ketoadipate pathway. The organism seems to excrete several intermediates during growth on some model compounds. Further experiments were carried out with 5 mM of vanillate, 4-hydroxybenzoate (4-HBA), vanillyl alcohol and *cis-cis* muconate.

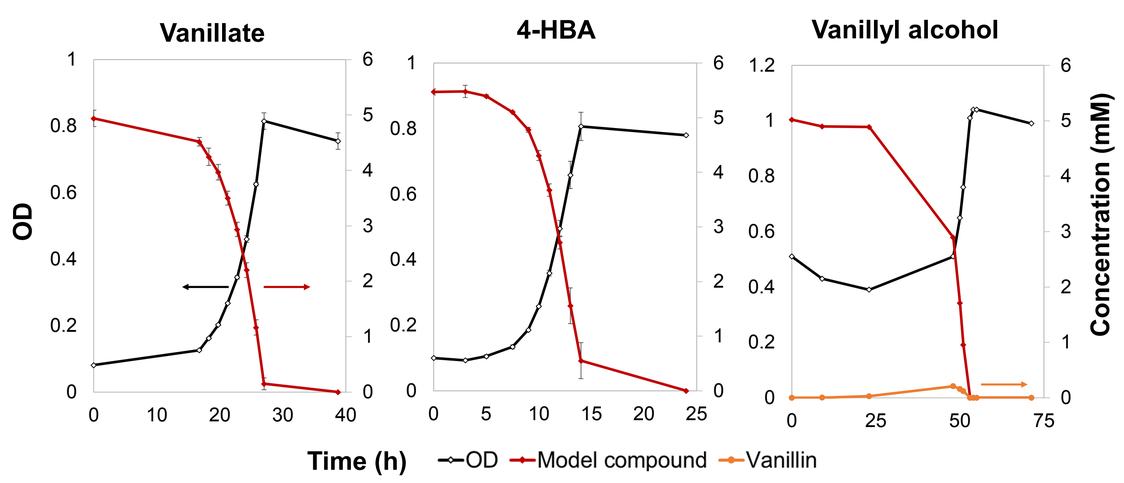

Figure 4: Growth of *P. deceptionensis* on vanillate, 4-HBA and vanillyl alcohol. No growth was observed on *cis-cis* muconate (not shown), which may be due to the absence of related transporters.

Table 1: Specific growth rates, yields and specific uptake rates of *P. deceptionensis* on lignin model compounds

Compounds	Growth rate [h^{-1}]	Yield [g/mmol]	Yield [g/g]	Uptake rate [mmol/(g*h)]
Vanillin	0.15 ± 0.001	0.09 ± 0.001	0.62 ± 0.006	3.20 ± 0.065
Benzoate	0.30 ± 0.019	0.09 ± 0.001	0.76 ± 0.007	4.20 ± 0.155
***p*-Coumarate**	0.29 ± 0.006	0.11 ± 0.005	0.68 ± 0.030	2.90 ± 0.197
Ferulate	0.18 ± 0.001	0.09 ± 0.001	0.48 ± 0.005	1.98 ± 0.025
Vanillate	0.18 ± 0.002	0.09 ± 0.004	0.55 ± 0.024	1.95 ± 0.032
4-HBA	0.30 ± 0.021	0.09 ± 0.001	0.65 ± 0.006	2.87 ± 0.360
Vanillyl alcohol	0.12 ± 0.004	0.06 ± 0.001	0.42 ± 0.006	1.67 ± 0.021

The highest growth rate and uptake rate were observed for benzoate, which indicates that the benzoate branch is faster. When vanillin was given as a carbon source, *P. deceptionensis* consumed it at a rate of 3.20 mmol/g/h and flowed through vanillate at the rate of 1.95 mmol/g/h. The excess uptake of vanillin was converted into **vanillyl alcohol.**

CONCLUSION

- No growth was observed on *B. licheniformis* and *B. safensis* on lignin model compounds
- ***P. fasciculus* and *R. erythropolis*** were able to grow on some model compounds, but they showed a **long lag phase** on most compounds.
- ***P. deceptionensis* was able to grow on most of the given model compounds and it excretes several intermediates during growth.**
- ***P. deceptionensis* excretes vanillyl alcohol during growth on vanillin**, which is quite unique from other *Pseudomonas* species which converts excess vanillin to vanillic acid.

REFERENCES

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2. Salvachúa D, Karp EM, Nimlos CT, Vardon DR, Beckham GT (2015) Towards lignin consolidated bioprocessing: simultaneous lignin depolymerization and product generation by bacteria. Green Chem 17(11):4951-4967 doi:10.1039/c5gc01165e

Acknowledgements
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