Metabolomic study of the biodegradation pathway of sodium-benzoate in *Pseudomonas aeruginosa* san ai

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Introduction

Many aromatic compounds are considered to be environmental pollutants that can adversely affect flora and fauna, resulting in the entry of toxic compounds into the food chain causing serious health problems and genetic damage in humans.

Benzoate is often used as a model compound to investigate the possibility of microbial degradation of aromatic compounds because it is the simplest known aromatic intermediate in the biodegradation of various complex polyaromatic compounds. The obtained information on the bacterial degradation of benzoate can be further used to understand and predict the degradation pathways of complex aromatic compounds.

Analytical omics methods enable the study of early molecular changes in the organism to sources of pollution and as such can be used to identify a specific metabolic response to a toxic substance, detect new biomarkers and predict the effects of pollutants on organisms and the environment [1].

The goal of this study was to analyze the products of benzoate degradation by polyextremophilic, hydrocarbonoclastic *Pseudomonas aeruginosa* san ai [2] using targeted metabolomic analysis in order to determine the specificity of the metabolic pathway of sodium-benzoate (NaB).

Material and Methods

For determination of NaB metabolites, after 11 h, 24 h, 48 h and 7 days of incubation, ethyl acetate extracts were prepared and derivatized samples were analyzed using a GC x GC-MS system. Sample preparation method, GC x GC-MS instrumental condition and identification of metabolites were performed as previously described in our study [3].

Results

The mass spectrum and retention time of compounds clearly confirmed presence of seven benzoate metabolites such as: 3,4-dixydroxybenzoate, catechol, *cis, cis*-muconic acid, muconolactone, β-ketoadipate enol-lactone and succinic acid as the end product of the benzoate transformation.

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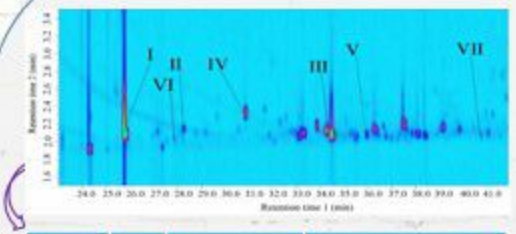
References: [1] D. Gouveia et al., *J. Proteomics*, 198 (2019) 66-77. **[2]** I. Karadžić et al., in *Unique ecosystems - amazing microbes, series extreme environments*, CRC Press (Taylor & Francis group), 2020, 343-358.; **[3]** A. Medić et al., RSC Adv. 10, 14060.

Discussion



Compound number	Retention time (min)	m/z (% of relative intensity)	Metabolite identification according to NIST library
I	25.70	179 (100), 105 (67), 135 (50), 77 (44)	Benzoic acid trimethylsilyl ester
II	28.20	73 (100), 239 (16), 72 (15), 74 (13), 254 (12)	1,2-Benzenediol bis(trimethylsilyl) ether (Catechol, 2TMS)
III	34.40	169 (100), 73 (82), 147 (62), 170 (19), 168 (17), 271 (13)	2-Furanacetic acid, α-[[(trimethylsilyl)oxy]-, trimethylsilyl ester (cis, cis- Muconate, 2TMS)
IV	30.80	75 (100), 73 (89), 157 (76), 199 (51), 81 (48), 255 (43), 111 (41), 85 (40)	Cyclohexanone-3-carboxylic acid, trimethylsilyl ester (Muconolactone, TMS)
V	35.03	73 (100), 147 (75), 77 (46), 271 (51), 75 (33)	2,4-Hexadienedioic acid, bis(trimethylsilyl) ester, (E,E) (β- ketoadipate enol-lactone, 2TMS)
VI	27.80	147 (100), 73 (87), 148 (22), 75 (21), 293 (14), 74 (14), 146 (13), 72 (13), 247 (12)	Butanedioic acid, bis(trimethylsilyl) ester (Succinic acid, 2TMS)
VII	40.60	73 (100), 193 (70) 72 (29) 75 (18), 194 (15), 370 (15), 147 (14), 74 (13), 355 (11)	Benzoic acid, 3,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester (Protocatechuate, 3TMS)

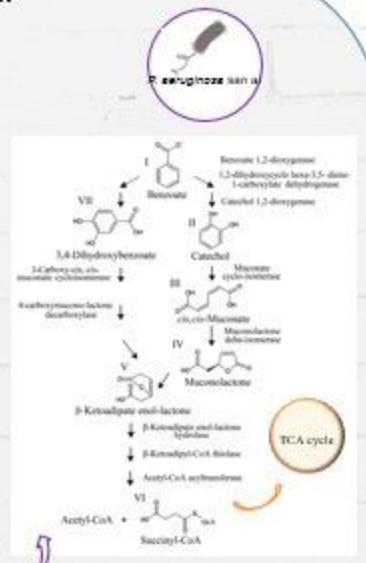


Fig. 1. identified metabolites and proposed metabolic pathways of benzene degradation by *P. aeruginosa* san ai

Our results indicate the degradation of NaB through the catechol branch of β-ketoadipate degradation pathway, followed with *ortho*- cleavage of catechol. Furthermore, the identified protocatechuate implies the existence of the second branch of β-ketoadipate pathway.

Conclusion

Metabolomic study showed that almost 99% of benzoate was removed / metabolized within 48 hours and clearly indicates that aromatic degradation occurs via β-ketoadipate. *P. aeruginosa* san ai can be considered as a good candidate for application in bioremediation of environments polluted by different aromatic compounds.