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SODIUM BENZOATE ASSIMILATION BY *Rhodococcus aetherivorans* UCM AC-602

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Sodium benzoate (SB) is a widely used preservative (E211) and also serves as an active ingredient in several pharmaceuticals. Prolonged exposure to SB can disrupt aquatic ecosystems, adversely affecting aquatic organisms and potentially human health.

Aim. To investigate the features of sodium benzoate biodegradation by the *Rhodococcus aetherivorans* UCM Ac-602 strain and assess the ecological safety of the toxicant and its degradation products for higher plants.

Methods. The concentration of SB was determined using high-performance liquid chromatography. Fatty acids composition was analyzed by gas chromatography–mass spectrometry. Catalase activity was measured spectrophotometrically, and cell membrane permeability was assessed using crystal violet. Phytotoxicity was evaluated via a rapid assay using wheat (Triticum aestivum L.) as a test plant.

Results. The *R. aetherivorans* UCM Ac-602 fully utilized 0.5 g/L of SB within 7 days. A twofold decrease in the C18:1 cis-9 fatty acid and a 1.7-fold increase in 10Me-C18:0 were observed during growth on SB. Changes in catalase activity and membrane permeability during SB assimilation contributed to cellular protection against the toxic effects of the substrate. Neither SB nor its metabolites exhibited phytotoxic properties.

Conclusions. The main mechanisms of adaptation of *R. aetherivorans* UCM Ac-602 to SB assimilation are modifications in fatty acid profiles, changes in catalase activity, and alterations in membrane permeability. SB and its degradation products were shown to be non-phytotoxic and safe for plant development.

Key words: *Rhodococcus aetherivorans*, sodium benzoate, biodegradation, fatty acids, catalase, membrane permeability, phytotoxicity.

Sodium benzoate (SB) is widely used in the food industry as a food additive (E211) and preservative, as well as a pharmaceutical substance in medicinal products. SB is considered safe for humans at specific doses; however, there is evidence that when released into the environment, it can have a negative impact on aquatic flora and fauna. The prolonged presence of high SB levels in drinking water may pose a risk to human health [1]. Actinobacteria of the genus *Rhodococcus* are widely recognized as active biodegraders of a broad range of organic compounds, including toxic and persistent substances. These microorganisms demonstrate exceptional environmental resilience, retaining viability and maintaining high metabolic activity under various stress conditions [2].

This study aimed to determine the features of sodium benzoate biodegradation by *Rhodococcus aetherivorans* strain UCM Ac-602 and to assess the environmental safety of the toxicant and its metabolites for higher plants.

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Materials and Methods

The strain *R. aetherivorans* UCM Ac-602 was previously identified by our group as an efficient phenol-degrading microorganism. [3]. The strain was cultivated in liquid RS mineral medium for 7 days with shaking (n = 220 rpm) at 28 °C. SB was added to the medium at 0.5 g/L as the sole source of carbon and energy. SB concentration was determined by high-performance liquid chromatography [4]. The fatty acid composition was analyzed using a gas chromatography-mass spectrometry system as described previously [3]. Catalase activity was assessed spectrophotometrically [5]. Membrane permeability was determined using crystal violet [6]. The phytotoxicity of SB and its biodegradation products in the culture fluid after strain growth on SB was evaluated using a rapid assay on Petri dishes [7]. A wheat (*Triticum aestivum* L.) cv. "Pecheryanka" seeds were used as the test plant. On day 7 of the experiment, the following plant test functions (TF) were analyzed: number of germinated seeds, root and shoot length, and biomass. The degree of phytotoxicity was assessed using mean toxicity factor indices (TFI), and the results were evaluated according to Kabirov's toxicity scale [7].

All numerical data were processed using Microsoft Office Excel 2010. Data are presented as mean (M) and standard error (±m). Differences between groups were considered statistically significant at $P < 0.05$.

Results and Discussion

It was found that by day 7 of growth, strain *R. aetherivorans* UCM Ac-602 had completely utilized SB. Under conditions of growth on SB and glucose (control substrate), the fatty acid (FA) composition of cells was dominated by straight-chain saturated C16:0 (32.1–33.9%), straight-chain unsaturated C16:1 *cis*-9 (8.9–11.8%) and C18:1 *cis*-9 (9.6–25.3%) acids, as well as methyl-branched 10Me-C18:0 (19.8–34.8%) acid. The main difference in FA composition under SB assimilation compared to glucose was the quantitative content of individual acids. Cells grown on SB contained half the amount of C18:1 cis-9 and 1.7 times more 10Me-C18:0. Moreover, the ratio of saturated to unsaturated FAs increased 1.3–2.3-fold during SB assimilation compared to glucose. The decrease in the amount of unsaturated FA and increase of methyl-branched FAs amount, along with a higher saturated/unsaturated FA ratio during SB assimilation, may indicate on involvement of these FA in cellular adaptation to SB. These results are consistent with our previous findings on the FA composition of this strain when grown on phenol and n-hexadecane [8].

During SB assimilation by *R. aetherivorans* UCM Ac-602, significant changes in the activity of the antioxidant enzyme catalase were also observed. The initial activity of the enzyme before exposure to SB was 0.61 μmol/min × OD_{492}, taken as 100% (Fig. 1). Within the first day of cultivation, catalase activity decreased by 67.2% to 0.20 μmol/min × OD_{492}. Activity gradually recovered, reaching 78.6% on day 2 and 73.7% on days 3–4 relative to the initial level.

These findings suggest that the presence of SB in the medium induces oxidative stress in cells of *R. aetherivorans* UCM Ac-602. This leads to changes in catalase activity during SB assimilation, which protects against toxic oxygen radicals. The results align with the literature indicating that due to high catalase levels, *Rhodococcus* spp. can effectively neutralize reactive oxygen species, supporting high cellular metabolic activity [9].

A study of membrane permeability in *R. aetherivorans* UCM Ac-602 revealed that in the absence of SB, the permeability was 48.8% (Fig. 2). During the first day of growth, membrane permeability decreased to 26.0%. Over the next two to three days, membrane permeability gradually increased, reaching values close to the initial level by day 4. The observed recovery of membrane function over time is critical for strain survival. It indicates that changes in membrane permeability are one of the mechanisms by which cells adapt to toxicants. The exact mechanisms of membrane disruption and restoration during growth on SB require further in-depth study.

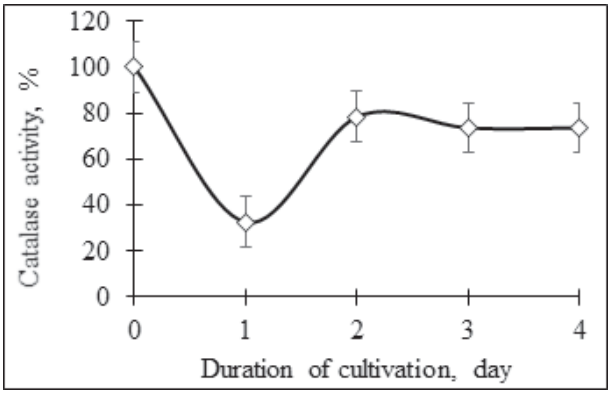

Fig. 1. **Dynamics of catalase activity in strain *R. aetherivorans* UCM Ac-602 during growth in medium containing 0.5 g/L sodium benzoate**

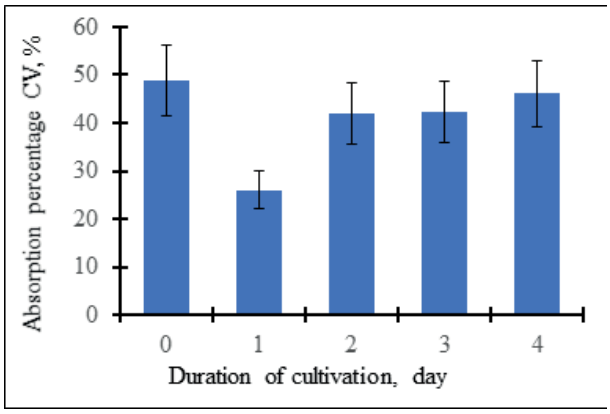

Fig. 2. **Changes in membrane permeability based on crystal violet (CV) absorption in *R. aetherivorans* UCM Ac-602 cells in the presence of SB**

Phytotoxicity assessment of SB showed a minor effect on the test plant at the studied concentration: compared to the control, the number of germinated seeds decreased by 6.8 %, root length increased by 17.1 %, root mass remained at the control level, shoot length increased by 6.2 %, and shoot mass increased by 15.2 %. Metabolites in the culture fluid (CF) of *R. aetherivorans* UCM Ac-602 after growth on SB most strongly (by 18.0 %) inhibited root development but significantly stimulated shoot development, with shoot mass exceeding the control by 26.6 %.

Dilution of the CF reduced the adverse effects of SB metabolites to control levels in distilled water. Phytotoxicity assessment showed that SB (TFI = 1.06), CF (TFI = 0.94), and its 10-fold dilution (TFI = 1.04) were not phytotoxic and belonged to toxicity class V (TFI = 0.91–1.1) — "normal". Thus, it was established that at the studied concentrations, SB and its biodegradation products are safe for higher plants.

Conclusions

R. aetherivorans UCM Ac-602 is an active degrader of SB. The main adaptive responses of this strain during SB assimilation include changes in cellular fatty acid composition, catalase activity, and membrane permeability. According to phytotoxicity levels for higher plants, the studied concentrations of SB and its metabolites are classified as non-toxic. They are safe for the functioning of higher plants in the agroecosystem.

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Not Applicable.

Conflicts of Interest
The authors declare no conflicts of interest.

Author Contributions
Conceptualization, writing — review and editing, supervision — V.S. Pidgorskyi, methodology, data collection, writing — original draft preparation — T.M. Nogina, O.G. Kisten. All authors have read and approved the final version of the manuscript.

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ЗАСВОЄННЯ БЕНЗОАТУ НАТРІЮ *Rhodococcus aetherivorans* UCM AC-602

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Бензоат натрію (БН) широко використовують як консервант (Е211), а також, як діючий компонент деяких фармацевтичних препаратів. Проте тривалий вплив БН може порушувати водні екосистеми, негативно впливаючи на водні організми та потенційно на здоров'я людини.

Мета — визначити особливості біодедеструкції бензоату натрію *Rhodococcus aetherivorans* UCM Ac-602 та екологічну безпечність токсиканту і його метаболітів для вищих рослин.

Методи. Кількість БН визначали за допомогою високоефективної рідинної хроматографії. Жирнокислотний склад клітин досліджували методом газової хромато-мас-спектрометрії. Активність каталази оцінювали спектрофотометрично, а проникність мембрани визначали за допомогою кристалічного фіолетового. Фітотоксичність досліджували експрес-методом з використанням пшениці (*Triticum aestivum* L.) як тест-рослини.

Результати. *R. aetherivorans* UCM Ac-602 повністю засвоював 0,5 г/л БН за 7 діб. За умов росту на БН спостерігалося зменшення в 2 рази кількості в клітинах жирної кислоти С18:1 *цис*-9 і збільшення в 1,7 рази кількості кислоти 10Ме-С18:0. Зміни активності каталази та проникності мембран під час асиміляції БН сприяли захисту клітинних структур від токсичної дії субстрату. БН та продукти його метаболіти не виявляли фітотоксичних властивостей.

Висновки. Основними механізми адаптації *R. aetherivorans* UCM AC-602 до засвоєння бензоату натрію є модифікація профілів жирних кислот, зміни каталазної активності та проникності мембран. Бензоат натрію та продукти його деструкції не проявляють фітотоксичності та безпечні для розвитку рослин.

Ключові слова: *Rhodococcus aetherivorans*, бензоат натрію, біодеструкція, жирні кислоти, каталаза, проникність мембран, фітотоксичність.