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NONYLPHENOL BIODEGRADATION BY THE BACTERIUM *RAOULTELLA PLANTICOLA* STRAIN F8 ISOLATED FROM THE SEDIMENT OF THE GULF OF FINLAND, THE BALTIC SEA

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Nonylphenol (NP) is a ubiquitous environmental pollutant of major concern due to its toxicity to hydrobionts, animals, and humans. Moreover, NP is known as an endocrine disruptor. The aim of this study is to isolate from bottom sediments sampled in the southern Gulf of Finland (the Baltic Sea) and identify a highly-efficient NP-degrading bacterial strain and to analyze its NP-degrading capacity at different levels of temperature, initial pH, dissolved oxygen concentrations, and initial NP content. The isolated strain F8 was identified by phenotypic traits using standard methods and by Sanger sequencing of a fragment of the 16S rRNA gene sequence (*rrs*). NP content was determined by high-performance liquid chromatography. The novel NP-degrading bacterium *Raoultella planticola* F8 was isolated from the bottom sediments sampled in the Gulf of Finland. *R. planticola* F8 isolate was deposited in the Russian Collection of Agricultural Microorganisms (RCAM), All-Russia Research Institute for Agricultural Microbiology, as the strain RCAM 05450. The *rrs* sequence of the F8 isolate was deposited in the GenBank database (No. OL831016). This strain is highly efficient for NP degradation in aerobic conditions at different NP concentrations (up to 900 mg·L^{-1}), in the temperature range of +5…+35 °C, the initial pH range of 5–9, and the dissolved oxygen concentration range of 0.8–2.46 mg·L^{-1}. This is the first study to demonstrate the ability of *R. planticola* to degrade NP. Results of this investigation provide useful information for *R. planticola* F8 application in bioremediation processes.

Keywords: *Raoultella planticola* F8, sediments, identification, nonylphenol, biodegradation

Nonylphenol (hereinafter NP), an endocrine disrupting xenobiotic of anthropogenic origin, is a widespread environmental pollutant worldwide. NP is actively used in manufacture of modified phenolic and epoxy resins and non-ionic surfactants, more specifically NP ethoxylates [Bhandari et al., 2021]. NP pollution in aquatic and terrestrial ecosystems occurs mainly due to a massive discharge into the environment of domestic and industrial wastewater, insufficiently treated at wastewater treatment plants [Barber et al., 2015].

NP pollution in the environment is of great concern due to its toxicity to hydrobionts, animals, and humans. Besides, NP is known as an endocrine disruptor [Bhandari et al., 2021; Khalid, Abdollahi, 2021; Uğuz et al., 2009]. For these reasons, NP is referred to in the list of priority hazardous substances under the Environmental Quality Standards Directive 2013/39/EU and in the list of hazardous substances in the Baltic Sea.

Because of its widespread use, NP is frequently detected in all natural environments, *inter alia* rivers, lakes, coastal waters, and bottom sediments. NP concentration in water can reach hundreds of micrograms *per* L [Bhandari et al., 2021; Solé et al., 2000]. Due to its high hydrophobic nature and low solubility in water, NP can be adsorbed on sediment particles, and this leads to its accumulation in bottom sediments of freshwater and marine ecosystems [Soares et al., 2008]. NP concentration in sediments is several orders of magnitude higher than in water, up to several thousand milligrams *per* kg. Considering NP persistence, its half-life in bottom sediments may exceed 60 years [Bhandari et al., 2021; Soares et al., 2008]. Sediments can serve as a secondary NP contamination source for aquatic ecosystems due to the desorption of part of sediment-bound NP fraction followed by its dissolution in the water phase [De Weert et al., 2008].

In the natural environment (soil, water, and bottom sediments), NP may be transformed into less toxic compounds due to abiotic (like hydrolysis and photolysis) and biological processes [Bhandari et al., 2021]. As known, microbial degradation is one of the main strategies to reduce NP pollution in the environment. The rate and extent of the degradation of pollutants, including NP, are largely determined by the physiological activity of microorganisms and conditions of their incubation (temperature, pH, pollutant content, *etc.*) [Abatenh et al., 2017; Khan et al., 2009; Xie et al., 2015]. It is also known that microbial degradation of NP can occur under both aerobic and anaerobic conditions: methanogenic and nitrate- and sulfate-reducing [Mao et al., 2012; Soares et al., 2008; Wang et al., 2015a].

Various microorganisms of different taxonomic groups, such as bacteria [Corvini et al., 2006; Ma et al., 2018; Reddy et al., 2017], blue-green algae [Baptista et al., 2009; Zaytseva, Medvedeva, 2019], microalgae [Feng et al., 2022], yeast [Rajendran et al., 2017; Vallini et al., 2001], and filamentous fungi [Kuzikova et al., 2020; Yang et al., 2018], were reported to be able to degrade alkylphenols, *inter alia* NP.

This finding prompted the search for more bacterial species that may serve as efficient NP biodegraders in bioremediation processes.

The aim of this study is to isolate from the bottom sediments sampled in the southern Gulf of Finland (the Baltic Sea) and identify the highly-efficient nonylphenol-degrading bacterial strain and to analyze its NP-degrading capacity at different levels of temperature, initial pH, dissolved oxygen concentration, and initial NP content.

MATERIAL AND METHODS

The sediments used in this research were sampled in the southern Gulf of Finland, the Baltic Sea (N59.99007°, E28.96475°) in June 2018. The sample (0–10-cm depth) was taken with a Box Corer, placed into a glass jar, and stored at +4 °C.

Technical NP (CAS 84852-15-3) was purchased from Sigma-Aldrich (the USA). Since NP has low solubility in water and mineral salt medium, NP stock solutions in ethanol were used in the tests.

The sediment sample was contaminated with NP (300 $mg \cdot kg^{-1}$) and incubated in the dark at +25 °C for 240 days.

The sediment sample (5 g, wet weight) was added to 50 mL of minimal mineral medium (hereinafter MMM) containing: $(NH_4)_2SO_4$, 4.0 $g \cdot L^{-1}$; KH_2PO_4, 1.5 $g \cdot L^{-1}$; K_2HPO_4, 1.5 $g \cdot L^{-1}$; and $MgSO_4 \cdot 7H_2O$, 0.2 $g \cdot L^{-1}$ supplemented with NP (50 $mg \cdot L^{-1}$) as a selective agent, pH (7.2 ± 0.2). The mixture was incubated on a rotary shaker Certomat BS-1 (230 rpm) at +28 °C in the dark for 7 days and then transferred to a fresh medium with NP and incubated under the same conditions. After that, the cultures were transferred regularly, every 3–4 days.

After 3 times of repeated subculturing, 0.1 mL of culture broth was pipetted and spread on solid MMM containing glucose (5.0 $g \cdot L^{-1}$), yeast extract (2.0 $g \cdot L^{-1}$), agar (20 $g \cdot L^{-1}$), and NP (50 $mg \cdot L^{-1}$). Single colonies were selected and streaked on nutrient agar supplemented with NP (50 $mg \cdot L^{-1}$). The cultures were incubated at +28 °C for 3 days. Morphologically different colonies of bacteria were selected for further study of their NP degrading ability.

Selected bacterial isolates were incubated on MMM supplemented with NP (100 $mg \cdot L^{-1}$) on a rotary shaker at 230 rpm, at +28 °C, in the dark for 7 days. After that, samples were taken to measure NP concentrations.

Phenotypic traits of the strain F8 were determined using standard methods and culture media [Krige, Padgtt, 2011].

The isolated strain F8 was identified according to the Bergey's Manual [1994] and the Sanger sequencing method for a 1450-bp fragment of the 16S rRNA gene (*rrs*) using primers fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rD1 (5'-CTTAAGGAGGTGATCCAGCC-3') [Weisburg et al., 1991]. Direct sequencing of PCR products was conducted on an ABI PRISM 3500xl genetic analyzer (Applied Biosystems, the USA).

The NCBI GenBank database (https://www.ncbi.nlm.nih.gov) and the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) were used to search for homologous sequences. To construct a phylogenetic tree, we applied the MEGA software v. 6 and used the neighbor joining method [Tamura et al., 2011]. Evolutionary distances were calculated by the Maximum Composite Likelihood method. The statistical reliability of the clusters was assessed with bootstrap analysis (1,000 replicas).

The inoculum was obtained by harvesting the strain F8 grown on solid MMM containing glucose (5.0 $g \cdot L^{-1}$), yeast extract (2.0 $g \cdot L^{-1}$), agar (20.0 $g \cdot L^{-1}$), and NP (50 $mg \cdot L^{-1}$) for 3 days. Cells were washed three times in 20 mM phosphate buffered saline (pH 7.0) and inoculated into 50 mL of MMM supplemented with NP. The initial cell density was $(3 \pm 1) \times 10^8$ $cells \cdot mL^{-1}$. The strain F8 was cultivated on MMM with NP in the dark for 7 days. NP was added to the medium in the form of ethanol solutions. Equal amounts of ethanol were added to abiotic controls.

The following cultivation conditions were manipulated in order to investigate their effects on NP biodegradation: temperature (+5, +10, +16, +22, +28, and +35 °C), initial pH (5.0, 6.0, 7.0, 8.0, and 9.0), dissolved oxygen (hereinafter DO) concentration (0.8, 1.08, 1.31, 1.53, and 2.46 $mg \cdot L^{-1}$), and initial NP content (100, 300, 500, 700, and 900 $mg \cdot L^{-1}$).

The effects of initial pH, DO concentration, and temperature on NP biodegradation were estimated at 100 $mg \cdot L^{-1}$ of NP in MMM.

To study the effects of NP content, initial pH, and DO concentration on the biodegradation capacity of the strain F8, cells were cultivated on NP-containing MMM on a rotary shaker Certomat BS-1 in the dark at +28 °C.

Various levels of DO concentration were created during the strain F8 cultivation in the Erlenmeyer flasks with different volumes of MMM (25, 50, 75, 100, and 125 mL). Winkler iodometric method was used to measure DO amount in the medium [Water Quality, 1983].

The effect of temperature on NP biodegradation rate was estimated during the bacteria cultivation under static conditions in the dark.

Non-inoculated variants were kept as blank controls to determine the abiotic loss of NP and incubated throughout the cultivation period. Each treatment in different tests was replicated three times for accuracy.

NP concentrations in the entire content of bacterial culture (cells with medium) and in abiotic controls were measured by high-performance liquid chromatography on an HP1090 chromatograph (Hewlett-Packard, the USA), according to the technique presented earlier [Kuzikova et al., 2020].

The kinetics of NP degradation during its fast phase under different bacterial cultivation conditions was analysed in accordance with the first-order model described by the following equation:

$$\ln (C_t/C_0) = -k \times t \,,$$

where C_0 is initial NP concentration ($mg \cdot L^{-1}$);

C_t is NP concentration at the time t ($mg \cdot L^{-1}$);

k is the degradation rate constant, $days^{-1}$ [Baptista et al., 2009].

All statistical analysis was carried out applying PAST 4.0 software. Statistical significance was determined using one-way ANOVA and Tukey's post-hoc test for normally distributed data; differences were considered significant at $p < 0.05$. Shapiro–Wilk and Levene's tests were performed to assess data normality and variance equality. The obtained data are given in tables and graphs as mean values with a standard deviation ($M \pm SD$) of three independent replicates ($n = 3$). Spearman's correlation coefficients (r_s) were used to identify relationships between NP degradation parameters and NP cultivation variables; $p < 0.05$ was considered statistically significant.

RESULTS

Ten bacterial strains isolated from the sample of NP-contaminated bottom sediments had the capacity to degrade NP. Extent of NP (100 $mg \cdot L^{-1}$) degradation after 7 days of cultivation was found to be between 43.1 and 91.5% depending on a bacterial strain (no data provided).

The highest biodegradation capacity (91.5%) was recorded for the strain F8. It should be pointed out that in the abiotic controls (without bacterial cells), NP degradation did not occur in the medium.

Cells of the strain F8 are gram-negative, non-spore-forming, and non-motile rods with capsules. The strain F8 forms circular beige colonies on nutrient agar, with a diameter of 2–3 mm, a smooth edge, smooth and shiny surface, fine-grained structure, and liquid consistency. The strain F8 is catalase-positive and oxidase-negative. It is a facultative anaerobic bacterium. Voges–Proskauer reactions and acid formation are positive; indole is not formed. This strain is capable of using urea, assimilating nitrogen from the atmosphere, performing denitrification, consuming nitrogen from mineral salts, and catabolizing lactose, sucrose, rhamnose, fructose, galactose, mannose, xylose, mannitol, sorbitol, glucose, arabinose, and starch with acid and gas formation; it does not use inositol. The strain shows amylolytic and lipolytic activity. Its cells are capable of growing in a wide range of temperature (+5…+36 °C) and pH (5–10, but not at pH of 3).

Phenotypically, the isolate F8 is close to the genus *Klebsiella* (Enterobacteriaceae family) [Bergey's Manual of Determinative Bacteriology, 1994].

As revealed by sequencing, the *rrs* gene fragment of the isolate F8 has the highest similarity (99.72%) with a similar fragment of the type strain *Raoultella planticola* NBRC 14939, belonging to Enterobacteriaceae family (Table 1).

Raoultella genus was separated from the closely related *Klebsiella* genus on the basis of the *rrs* and *rpoβ* gene sequences analysis, DNA-DNA hybridization, and biochemical studies [Drancourt et al., 2001]. Initially, in addition to *R. planticola*, this genus included two species: *R. ornithinolytica* and *R. terrigena* [Drancourt et al., 2001]. Later, the species *R. electrica* was described as well [Kimura, 2014].

Table 1. Similarity between the isolate F8 and the closest type strains belonging to Enterobacteriaceae family based on the 16S rRNA gene sequencing

Type strain	NCBI accession number	Similarity with the isolate F8 (%)
Raoultella planticola NBRC 14939	NR113701	99.72
Raoultella ornithinolytica JCM 6096	NR114736	99.45
Klebsiella aerogenes KCTC 2190	NR102493	99.24
Raoultella electrica 1GB	AB762091	99.16
Raoultella terrigena NBRC 14941	AB680714	98.69
Klebsiella grimontii SB73	NR159317	98.54
Klebsiella oxytoca ATCC 13182	NR041749	98.01
Klebsiella pneumoniae DSM 30104	NR036794	97.80
Enterobacter asburiae JM-458	NR145647	96.46
Erwinia amylovora DSM 30165	NR041970	95.73

A phylogenetic tree based on the *rrs* gene sequences, representing the taxonomic status of the isolate F8 within Enterobacteriaceae family, is shown in Fig. 1. As can be seen, the studied isolate formed a single cluster with the type strain *R. planticola* NBRC 14939T at a high level of statistical support (91%).

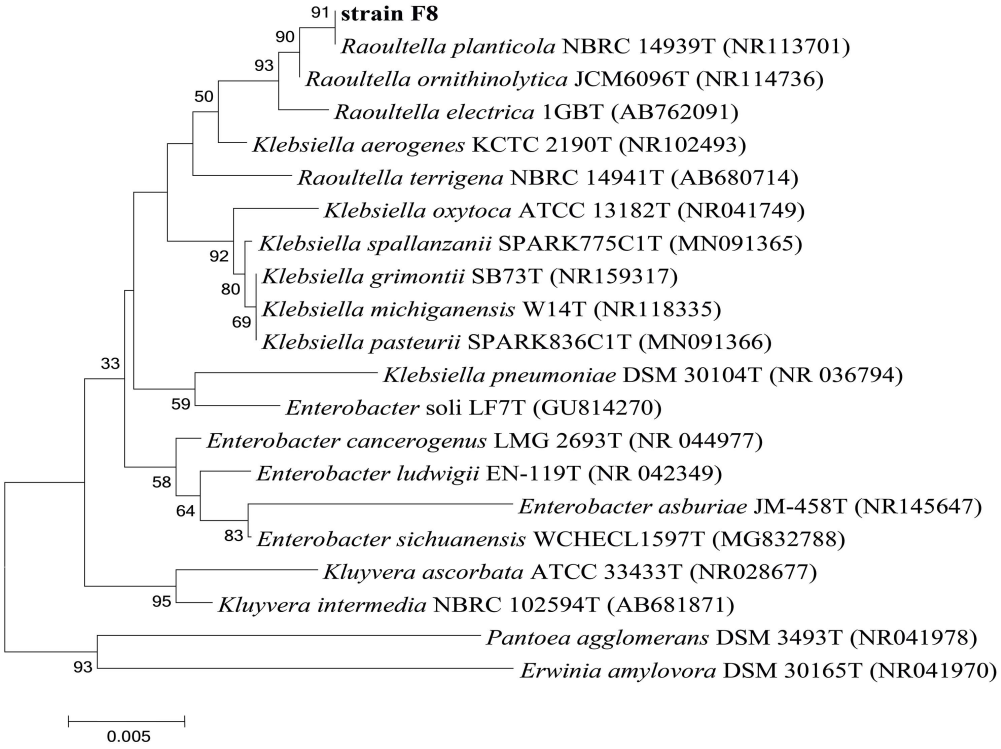

Fig. 1. Phylogenetic tree generated by the neighbor joining method using partial 16S rRNA gene sequences reflecting the taxonomic position of the strain F8 isolate within Enterobacteriaceae family. The isolated strain is highlighted in bold. Type strains are indicated by the letter T. Bootstrap values of more than 30% are given

Summing all the phenotypic traits with the reported sequence of the 16S rRNA gene fragment, the strain F8 was identified as *R. planticola* F8. The isolate *R. planticola* F8 was deposited in the Russian Collection of Agricultural Microorganisms (RCAM) as the strain RCAM 05450 and stored at −80 °C in the automated Tube Store (Liconic Instruments, Liechtenstein). The *rrs* sequence of the isolate F8 was deposited in the GenBank database (No. OL831016).

The results of studying the effect of temperature on NP degradation revealed the capacity of *R. planticola* F8 to degrade NP in a wide range, +5…+35 °C (Fig. 2). A high level of correlation was found between temperature and NP biodegradation rate constant k (r_s = 0.818; p = 0.0038).

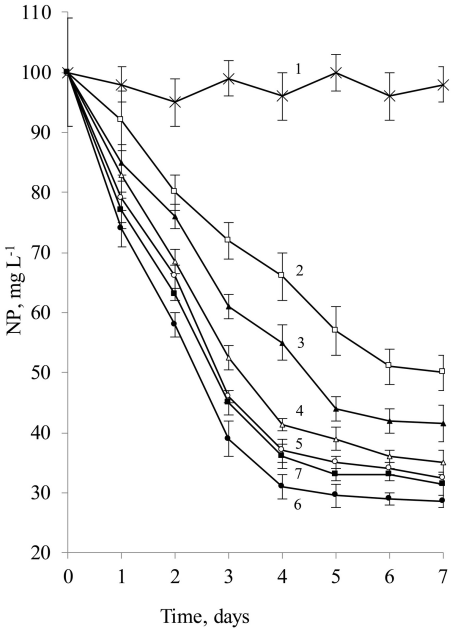

Fig. 2. Dependence of nonylphenol content in the culture liquid of the bacterium *Raoultella planticola* F8 on temperature: 1, abiotic control; 2, +5 °C; 3, +10 °C; 4, +16 °C; 5, +22 °C; 6, +28 °C; 7, +35 °C

At +5 °C, the degradation rate constant k during the fast phase was 0.111 days^{-1}, while half-life t_{50} was 6.2 days. NP degradation by the isolated strain was accelerated with a rise in the incubation temperature up to +28 °C, which resulted in a statistically significant ($p < 0.5$) increase in k and decrease in NP half-life by 2.7 times, as well as in an increase in NP degradation degree from 51 to 71.5%. A further rise in temperature, up to +35 °C, led to a decrease in k and increase in t_{50} by 1.2 times (Table 2).

Table 2. Effect of cultivation conditions on destruction of nonylphenol (100 mg·L^{-1}) by *Raoultella planticola* F8

Cultivation condition	T, °C	Dissolved oxygen, mg·L^{-1}	Initial pH	k, days^{-1}	R^2	t_{50}, days	Nonylphenol degradation degree after 7 days, %
Temperature	+5	0.8	7	0.111 ± 0.004	0.95	6.2 ± 0.2	51 ± 2
	+10	0.8	7	0.161 ± 0.005	0.991	4.3 ± 0.3	58.5 ± 1.9
	+16	0.8	7	0.212 ± 0.011	0.992	3.3 ± 0.1	65 ± 3
	+22	0.8	7	0.251 ± 0.004	0.979	2.8 ± 0.1	67.5 ± 1.2
	+28	0.8	7	0.307 ± 0.005	0.99	2.3 ± 0.2	71.5 ± 1.8
	+35	0.8	7	0.26 ± 0.01	0.995	2.7 ± 0.2	70 ± 2
Dissolved oxygen concentration	+28	0.8	7	0.307 ± 0.006	0.99	2.26 ± 0.01	71.5 ± 1.2
	+28	1.08	7	0.525 ± 0.005	0.999	1.32 ± 0.04	81 ± 2
	+28	1.31	7	0.66 ± 0.01	0.998	1.05 ± 0.03	85 ± 2
	+28	1.53	7	1.15 ± 0.11	0.986	0.6 ± 0.1	91.5 ± 1.9
	+28	2.46	7	0.916 ± 0.005	0.944	0.76 ± 0.03	89 ± 2
Initial pH	+28	1.53	5	0.569 ± 0.003	0.985	1.22 ± 0.14	80 ± 2
	+28	1.53	6	0.655 ± 0.011	0.999	1.06 ± 0.09	82 ± 2
	+28	1.53	7	1.15 ± 0.11	0.986	0.6 ± 0.1	91.5 ± 1.9
	+28	1.53	8	0.886 ± 0.009	0.997	0.78 ± 0.04	88 ± 1
	+28	1.53	9	0.458 ± 0.006	0.998	1.51 ± 0.08	76 ± 2

Taking all data into account, it can be concluded that the maximum biodegradation rate was observed at +28 °C.

As shown, NP degradation by the bacterium *R. planticola* depends on DO concentration (Fig. 3).

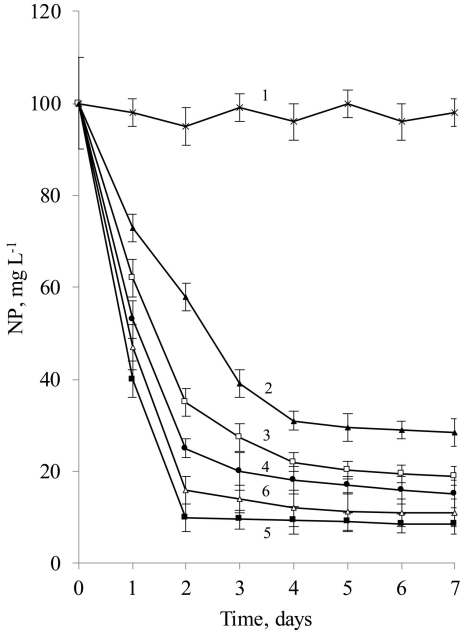

Fig. 3. Dependence of nonylphenol content in the culture liquid of the bacterium *Raoultella planticola* F8 on dissolved oxygen concentration: 1, abiotic control; 2, 0.8 mg·L^{-1}; 3, 1.08 mg·L^{-1}; 4, 1.31 mg·L^{-1}; 5, 1.53 mg·L^{-1}; 6, 2.46 mg·L^{-1}

A rise in DO concentration from 0.8 to 1.53 mg·L^{-1} led to a statistically significant increase in k and decrease in t_{50} by 3.8 times. The degree of NP degradation after 7 days of cultivation dropped by 1.3 times (Table 2). A further rise in DO concentration, up to 2.46 mg·L^{-1}, resulted in a decrease in NP biodegradation rate constant and an increase in half-life by 1.3 times.

Based on the results, DO concentration for effective NP degradation by *R. planticola* should be within 1.53–2.46 mg·L^{-1}.

The results of studying the effect of initial pH on NP degradation by *R. planticola* revealed that the highest NP degradation degree, 88–91.5%, was reached after 7 days of cultivation in the pH range of 7.0 to 8.0 (Fig. 4, Table 2).

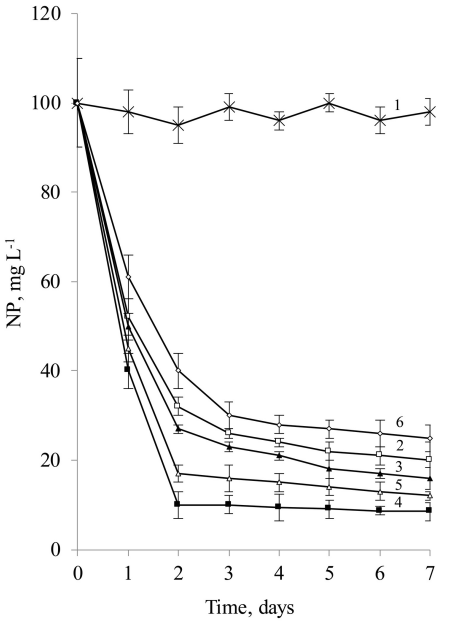

Fig. 4. Dependence of nonylphenol content in the culture liquid of the bacterium *Raoultella planticola* F8 on initial pH: 1, abiotic control; 2, pH 5; 3, pH 6; 4, pH 7; 5, pH 8; 6, pH 9

The highest degradation rate constant k (1.15 $days^{-1}$) and the lowest half-life t_{50} (0.6 days) were revealed at pH of 7.0. A decrease in pH from 7.0 to 5.0 and an increase to 9.0 resulted in a statistically significant ($p < 0.5$) drop in k and a rise in half-life t_{50} by 2 and 2.5 times, respectively.

Finally, optimal initial pH value for NP biodegradation by *R. planticola* was determined as 7.0–8.0.

As found, the bacterium *R. planticola* F8 degrades NP in a wide range of NP concentrations, from 100 to 900 $mg \cdot L^{-1}$ (Table 3).

Table 3. Effect of initial nonylphenol concentration on its destruction by *Raoultella planticola* F8

Nonylphenol, $mg \cdot L^{-1}$	k, $days^{-1}$	R^2	t_{50}, days	Nonylphenol degradation degree after 7 days, %
100	1.15 ± 0.11	0.986	0.6 ± 0.1	91.5 ± 1.9
300	0.866 ± 0.005	0.914	0.8 ± 0.1	84.8 ± 0.6
500	0.292 ± 0.002	0.976	2.4 ± 0.1	84 ± 2
700	0.22 ± 0.01	0.88	3.2 ± 0.4	78.6 ± 1.4
900	0.12 ± 0.01	0.986	5.8 ± 0.5	55.6 ± 1.3

A high level of correlation ($p < 0.001$) was registered between NP biodegradation rate constant and initial NP content ($r_s = -0.983$) and between t_{50} and initial NP concentration ($r_s = 0.999$). A rise in NP content in the medium from 100 to 900 $mg \cdot L^{-1}$ led to a statistically significant ($p < 0.05$) decrease in the degradation rate constant k and an increase in half-life t_{50} by 9.6 times. The degree of NP degradation dropped by 1.6 times (Table 3).

DISCUSSION

Recently, one of the main ecological problems was environmental contamination by endocrine disrupting chemicals, in particular NP which affects the endocrine system of living organisms.

NP degradation in natural environments is caused by its abiotic destruction and biodegradation. A wide range of bacteria belonging to different genera are known to have NP-degrading capacity: *Acinetobacter*, *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Citrobacter*, *Corynebacterium*, *Desulfobacterium*, *Klebsiella*, *Pseudomonas*, *Serratia*, *Sphingomonas*, *etc.* [Corvini et al., 2006; Gabriel et al., 2005; Ma et al., 2018; Reddy et al., 2017; Xie et al., 2015].

The pathways of NP biodegradation by bacteria are widely presented in scientific literature. Previously, it was revealed that aerobic NP degradation by bacteria can be initiated either by oxidative cleavage of the alkyl chain or by oxidative action on an aromatic ring. A putative mechanism for degradation of the alkyl chain includes hydroxylation at the terminal carbon atom (as the first step), oxidation of the resulting alcohol into the corresponding carboxylic acid, and further degradation *via* β-oxidation. NP degradation *via* oxidation of the alkyl chain is characteristic of NP isomers, in which the side chain is linear or at least not highly branched. NP isomers with highly branched side chains can initially be destroyed by hydroxylation phenolic ring. Type II *ipso*-substitution mechanism (hydroxylation at the carbon atom-4) was described as the first step of degradation pathway, which occurs by oxidation and replacement of aromatic carbon atom of NP by an alkyl side chain [Bhandari et al., 2021].

As mentioned earlier, the bacterium *R. planticola* F8, an active NP biodestructor, was isolated from bottom sediments sampled in the southern Gulf of Finland. This strain belongs to Proteobacteria phylum, Gammaproteobacteria class. Gammaproteobacteria, along with Alphaproteobacteria, are known as the most abundant bacterial groups in the microbiome of NP-contaminated bottom sediments [Wang et al., 2015b].

Raoultella representatives have been associated with degradation of various organic contaminants, such as drugs [Palyzová et al., 2019], pesticides [Bhatt et al., 2019], polycyclic aromatic hydrocarbons [Ping et al., 2017], and so on. The ability of *R. planticola* to degrade NP was revealed for the first time.

Two phases of NP biodegradation by *R. planticola* were identified analyzing the degradation curves under test conditions: the fast and the slow one. It should be noted that a similar two-phase nature of a decrease in alkylphenols content was previously found during their destruction by cyanobacteria [Baptista et al., 2009; Zaytseva, Medvedeva, 2019] and micromycetes [Kuzikova et al., 2020]. The limitation of NP degradation process at the end of the fast phase can be caused by a decline in the medium quality which results from formation of metabolites toxic to bacteria [Bai et al., 2017].

Due to their metabolism and capacity to adapt to adverse environmental conditions, microorganisms can degrade a wide range of organic pollutants, including alkylphenols. However, their efficiency depends on many factors, *inter alia* pollutant concentration and physicochemical characteristics of the environment, such as temperature, pH, DO concentration, *etc.* [Abatenh et al., 2017; Watanabe et al., 2012].

This study allowed revealing that the rate of NP degradation by *R. planticola* F8 depends on temperature, initial pH, DO concentration, and initial NP content to a large extent. It is well known that temperature is one of the most relevant abiotic factors affecting the degradation of xenobiotics. Temperature variations can accelerate or decelerate biodegradation by affecting the physiological properties of microbial degraders, in particular *via* direct effect on the biological enzymes involved in the degradation pathway [Abatenh et al., 2017; Khan et al., 2009].

The temperature dependence of NP biodegradation, as well as the optimum temperature (+30 °C), were revealed earlier during NP degradation by bacterial strains *Pseudomonas* sp., *Acidovorax* sp., *Pseudomonas putida*, *Citrobacter freundii*, and complex microorganisms ZJF composed by three strains combined: *Serratia* sp., *Klebsiella* sp., and *Ps. putida* [Ma et al., 2018; Watanabe et al., 2012; Xie et al., 2015].

It is worth noting that previous studies were focused on the ability of bacteria to degrade NP at temperatures above +14 °C [Ma et al., 2018; Watanabe et al., 2012; Xie et al., 2015]. To date, information on NP degradation at lower temperatures is still lacking.

As shown in our tests, NP destruction by *R. planticola* F8 also significantly depends on temperature. This bacterium was found to be highly efficient for NP degradation in a wide temperature range, +5…+35 °C. The fact that *R. planticola* F8 is capable of degrading NP even at such a low temperature, as +5 °C, is of certain interest. Biodegradation rate increases as temperature rises from +5 to +28 °C, reaching its maximum at +28 °C. A rise in temperature from +28 to +35 °C led to a drop in biodegradation rate. It is assumed that contaminant biodegradation is slowed down at relatively high and low temperatures due to a decrease in the activity of bacterial and extracellular enzymes [Xie et al., 2015].

Aeration and pH levels significantly affect the biodegradation of organic pollutants.

Oxygen is the most common electron acceptor in the bacterial respiration. During aerobic biodegradation of aromatic compounds, oxygen acts as an electron acceptor for aromatic pollutants, besides participating in substrate activation *via* oxygenation reactions [Cao et al., 2009]. As known, in aerobic conditions, the bacterial biodegradation of alkylphenols, *inter alia* NP, involves mono- and dioxidases and multicomponent phenol hydroxylases. These enzymes catalyze chemical reactions cleaving chemical bonds and assisting the transfer of electrons from reduced organic substrate (donor) to another chemical compound (acceptor). Oxidases play a key role in metabolism of organic compounds,

increasing their reactivity or water solubility or causing the aromatic ring cleavage. Generally, introduction of O_2 atoms into the organic molecule by oxygenase results in the aromatic ring cleavage [Cao et al., 2009; Karigar, Rao, 2011; Tuan et al., 2011].

We established that *R. planticola* F8 is capable of degrading NP in a wide range of DO concentrations in the medium (0.8–2.46 mg·L^{-1}). The rate of NP degradation by this bacterium was minimal at the lowest DO content used in the tests, 0.8 mg·L^{-1}. An increase in DO concentration up to 1.53 mg·L^{-1} resulted in a rise in degradation rate. Optimal DO content for NP degradation by *R. planticola* F8 was determined as 1.53–2.46 mg·L^{-1}.

The level of pH is known to affect the physiological properties of microorganisms, thus playing a noticeable role in biodegradation of organic pollutants. Like other proteins, microbial enzymes, *inter alia* those catalysing biodegradation processes, are extremely sensitive to a medium pH. The changes in pH level cause alterations in the electric charge of various chemical groups which are present in enzyme molecules. An imbalance in electrical charges in very acidic and alkaline pH ranges leads to destruction of chemical bonds that support the structure of the enzyme, decrease in enzymatic activity, and denaturation of the enzyme. Consequently, there is a significant deterioration in pollutant biodegradation [Alneyadi et al., 2017].

As previously reported, pH level affects the bacterial degradation of various organic pollutants: polyaromatic hydrocarbons, phenol and its derivatives, antibiotics, *etc.* [Ibrahim et al., 2018; Khan et al., 2009; Lakshmi, Sridevi, 2009; Liu et al., 2017]. As shown earlier, pH value significantly affects NP degradation by the bacteria *Ps. putida* and *C. freundii* with optimal pH levels of 5–7 and 6–7, respectively, and by complex microorganisms ZJF with optimal pH of 6.0 [Ma et al., 2018; Xie et al., 2015].

This work investigated effects of initial pH in the range of 5.0–9.0 on NP degradation by the bacterium *R. planticola* F8, and optimal pH level for NP biodegradation was revealed, 7.0–8.0. Both increasing pH to 9.0 and decreasing it to 5.0 decelerate pollutant biodegradation.

Initial NP concentrations affect the bacterial degradation as well. Earlier studies showed the effect of initial NP content on efficiency of NP removal by different bacrteria: *Acidovorax* sp., *C. freundii*, *Serratia* sp., *Klebsiella* sp., and *Ps. putida* [Ma et al., 2018; Xie et al., 2015]. For example, an increase in efficiency of NP degradation by *Ps. putida* and *C. freundii* was recorded when initial pollutant concentration was raised from 1 to 5 μg·L^{-1}. However, a further rise in NP content, up to 9 μg·L^{-1}, caused no statistically significant changes in extent of degradation [Xie et al., 2015]. A rise in initial NP concentration from 5 to 10–15 mg·L^{-1} also resulted in an increase in efficiency of pollutant degradation by the bacteria *Serratia* sp., *Klebsiella* sp., and *Ps. putida* up to 60%. But a further rise in initial NP concentration, up to 100 mg·L^{-1}, led to a drop in degradation efficiency to 30% [Ma et al., 2018].

Our data show as follows: an increase in NP concentration from 100 to 900 mg·L^{-1} led to a suppression of degradation efficiency by *R. planticola* F8, a decrease in degradation rate, and an increase in NP half-life. The slowdown in biodegradation of pollutants at high concentrations is explained by their toxic effect on pollutant-degrading microorganisms [Abatenh et al., 2017]. However, it should be noted that the isolated strain *R. planticola* F8, despite a statistically significant drop in the degradation rate constant k, a rise in half-life t_{50} by 9.6 times, and a decrease in the degree of NP degradation by 1.6 times, was capable of degrading NP at such a high content, as 900 mg·L^{-1}. The same degradative activity at such a high toxicant concentration was previously registered only in the microbial consortium NP-M2 isolated from bottom sediments, mainly consisting of bacteria of the genera *Sphingomonas*, *Pseudomonas*, *Alicycliphilus*, and *Acidovorax* [Bai et al., 2017].

Conclusion. We isolated the bacterial strain F8 from the nonylphenol-contaminated bottom sediments sampled in the southern Gulf of Finland (the Baltic Sea). *Raoultella planticola* F8 is capable of degrading nonylphenol in aerobic conditions at its different concentrations (up to 900 mg·L^{-1}) and in a wide range of temperature, initial pH, and dissolved oxygen content.

The results of this study provide useful information for the potential application of the bacterium *R. planticola* F8 in bioremediation processes.

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БИОДЕГРАДАЦИЯ НОНИФЕНОЛА БАКТЕРИЕЙ *RAOULTELLA PLANTICOLA* F8, ВЫДЕЛЕННОЙ ИЗ ДОННЫХ ОСАДКОВ ФИНСКОГО ЗАЛИВА БАЛТИЙСКОГО МОРЯ

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Нонилфенол (НФ) — ксенобиотик антропогенного происхождения — является широко распространённым во всём мире загрязнителем окружающей среды. Попадание НФ в объекты окружающей среды вызывает серьёзную озабоченность вследствие его токсичности для водных организмов, животных и человека. Кроме того, НФ известен как эндокринный деструктор. Цель данной статьи — выделение из донных отложений, отобранных в южной части Финского залива (Балтийское море), и идентификация высокоэффективного штамма бактерий, способного деструктировать НФ, а также изучение его способности к деградации НФ при различных уровнях температуры, pH, концентраций растворённого кислорода и исходных концентраций НФ. Идентификацию выделенного штамма F8 проводили по фенотипическим признакам с использованием стандартных методов, а также методом секвенирования по Сэнгеру фрагмента последовательности гена 16S рРНК (*rrs*). Содержание НФ определяли методом высокоэффективной жидкостной хроматографии. Новая НФ-деструктирующая бактерия *Raoultella planticola* F8 выделена из донных отложений, отобранных в Финском заливе. Изолят *R. planticola* F8 депонирован в Ведомственной коллекции микроорганизмов сельскохозяйственного назначения ВНИИСХМ под регистрационным номером RCAM 05450. Последовательность гена *rrs* изолята *R. planticola* F8 депонирована в базе данных GenBank под номером OL831016. Этот штамм высокоэффективен для деградации НФ в аэробных условиях при различных концентрациях НФ (до 900 мг·л$^{-1}$), в диапазоне температур от +5 до +35 °C, начальных значений pH от 5 до 9 и концентраций растворённого кислорода от 0,8 до 2,46 мг·л$^{-1}$. Данное исследование — первое, демонстрирующее способность *R. planticola* транформировать НФ. Результаты этой работы предоставляют полезную информацию для применения *R. planticola* F8 в процессах биоремедиации.

Ключевые слова: *Raoultella planticola* F8, донные осадки, идентификация, нонилфенол, биодеградация