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Comamonas testosteroni Strain TI as a Potential Base for a Microbial Sensor Detecting Surfactants

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Abstract—Strain *Comamonas testosteroni* TI, capable of degrading the nonionic surfactant (NIS) nonylphenolethoxylate (OP-10), was used for constructing a pilot cellular biosensor. The lower NIS detection limit for the biosensor was 0.25 mg/l. We studied the substrate specificity of the biosensor with respect to a wide range of organic compounds: surfactants, polyaromatic compounds (PAC), carbohydrates, alcohols, etc. It was shown that the biosensor based on *Comamonas testosteroni* TI did not respond to glucose, which was an advantage over the formerly described biosensor based on *Pseudomonas rathonis* T. The amplitude of the sensor response remained stable for 10 days.

The construction of biosensor systems for detecting toxic chemicals is a topical problem. Multisensor systems based on degrading bacteria provide a promising approach to detecting xenobiotics. Surfactants rank high in the scale of commercial production. Although of low toxicity, they are hazardous because of their ability to solubilize pollutants and concentrate them in the water phase. Surfactants with the highest environmental stability are those having aromatic rings in their molecules, such as nonionic surfactants (NIS) [1]. Precise monitoring of their concentration in the environment is important, but there is still no efficient proximate method for their detection. Sensitive, inexpensive, and simple-to-operate analyzers can be developed with the use of the biosensor approach, which has been rapidly advancing in the last two decades. Microbial biosensors based on cells harboring plasmids for degrading corresponding chemicals are considered promising for tasks of environment protection. They allow simple and inexpensive assays. Numerous models of microbial biosensors, described in the literature, allow the detection of naphthalene [2], dichloromethane, catechol, phenol, chlorophenols [3], ammonia, nitrogen, nitrates, sulfides, and surfactants [4, 5]. They also allow an estimation of the biological oxygen demand (BOD) and overall toxicity [6, 7]. The sensor model described in [4] was successfully used for analysis of linear alkyl sulfonates in the Ayaza River (Japan). Another microbial sensor allows detection of surfactants in various media [3]. In addition, we described features of some biosensors for detecting NIS in [8, 9–11].

Some surfactant-degrading microorganisms have narrow ranges of substrates [11]. This makes them promising for use in microbial biosensors for surfactant analysis. Construction of multisensor systems is of special interest, because this would extend the range of detectable compounds. Such construction requires that a set of strains degrading different substrates be available.

The purpose of the study is to characterize a microbial biosensor based on NIS-degrading strain *Comamonas testosteroni* TI.

MATERIALS AND METHODS

Cultivation of microorganisms. Experiments were carried out with a strain degrading nonylphenolethoxylate, *C. testosteroni* TI, formerly isolated from surfactant-polluted soil. It can degrade OP-10 at concentrations from 100 to 300 mg/l. The strain was grown at 28°C by shaking in Erlenmeyer flasks 140 rpm with the following synthetic medium (g/l): Na_2HPO_4 (1.0), NH_4NO_3 (1.0), KCl (0.5), $MgCl_2$ (0.01), OP-10 (0.2). The biomass was harvested by centrifugation at 5000 *g* for 20 min and washed with three portions of 30 mM phosphate buffer pH 7.8.

Substrates. Various xenobiotics were used as substrates

1. Surfactants (percentage of base material). Nonionic: OP-10 (99%), Tween 80 (98%), Triton X-100 (98%), Triton X-350 (98%), polyethylene glycol dodecyl ether $n = 10$ and $n = 14$ (90%), polyethylene glycol cetyl ether $n = 6$ (90%), diethanolamide (90%), and

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sulfoethoxylate (90%). Anionic surfactants: sodium dodecyl sulfate (SDS, 100%), alkyl sulfonate (Volgonat, 60%), Metaupon (37%), alkylnaphthalene sulfonate (ANS, 50%), disodium monoalkylsulfosuccinate (DMAS, 39%), and alkylbenzenesulfonate preparations (ABS): linear ABS (90%), branched ABS (80%), branched ABS produced in Sumgait, Russia, 40%), and chlorinated sulfanole (50%). Cationic surfactants: alkoxymethyldiethylammonium methylsulfate (Alkamon, 100%), ethonium (100%), cetylpyridinium chloride (100%), tetradecyltrimethylammonium bromide (100%), alkyldimethylbenzeneammonium chloride (Katamin, 100%), and Imidostat (100%). Ampholytic surfactants: alkylamino-bis-propionate (85%) and amidobetaine (50%).

2. Aromatic and polycyclic compounds: phenanthrene, anthracene, naphthalene, fluorene, pyrene, acenaphthene, acenaphthylene, salicylate, 3-metasalicylate, and 5-metasalicylate.

3. Carbohydrates: glucose, arabitol, arabinose, xylitol, xylose, galactose, sorbitol, sorbose, sucrose, fructose, maltose, and raffinose.

4. Alcohols: methanol, ethanol, propanol, butanol, and glycerol.

5. Other classes of compounds: urea, chloroform, dimethyl formamide, bisacrylamide, acrylamide, and nonylphenol.

Biosensor analysis. A receptor element was prepared by entrapping *C. testosteroni* TI cells into 2% agar gel as described in [9, 10]. The receptor (membrane thickness, 0.3–0.5 mm) was placed onto the working surface of a Clark electrode and fixed with a Nylon net.

An Ingold 5313/010 amperometric system (USA) was used as a transducer for the biosensor. Measurements were carried out in a 5 ml cell at 20°C under constant agitation within the range of complete saturation (oxygen). The parameter to be measured (sensor signal) was the maximum rate of change of the output biosensor signal after addition of the substrate (nA/s). The operation stability (lifetime of one receptor element) was determined by repeated assay of OP-10 at 100 mg/l during 20 days. The receptor element was kept in a buffer solution at room temperature and constant agitation.

RESULTS AND DISCUSSION

Biosensor sensitivity. The biosensor based on *C. testosteroni* TI cells was highly sensitive to various NISs (Fig. 1). The lower limit of OP-10 detection was 0.25 mg/l; Triton X-100, 0.5 mg/l; and Tween-80, 0.25 mg/l. The upper limit matched the critical concentration of micelle formation and varied from 60 to 150 mg/l for various NIS.

Detection specificity. The data obtained during determination of substrate specificity are related to the OP-10 response, taken to be 100%. This facilitates the comparison of the response of the sensor to various substrates. Measurements were carried out at xenobiotic concentrations of 100, 5, and 1 mg/l. Sensor records at 5 and 1 mg/l matched in most cases. Therefore, we present data only for the concentration of 1 mg/l.

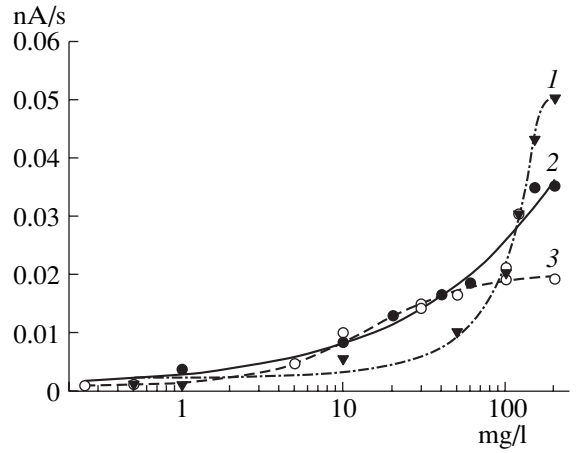

Fig. 1. Sensitivity of the biosensor based on *C. testosteroni* TI (nA/s) to nonionic surfactants (mg/l). For each concentration, values are averaged over 10 measurements. (*1*) Triton X-100, (*2*) OP-10, (*3*) Tween 80.

The amplitudes of response to various compounds at 100 and 1 mg/l are shown in Tables 1 and 2, respectively. It is worth noting that responses to various anionic surfactants at 100 mg/l varied within 80–100% of the response to OP-10, and at 1 mg/l the relative sensitivity decreased to 20–40% of the corresponding response to OP-10 (Table 2). For example, experiments on the substrate specificity to anionic surfactants at 100 mg/l demonstrated high amplitudes of the response to SDS (105% of the response to OP-10) and DMAS (127% of the response to OP-10), whereas the sensor record after the input of ANS was as low as 5% of the response to OP-10 (Table 1). However, the responses to SDS and DMAS at 1 mg/l varied within 10–50% of the response to 1 mg/l OP-10, whereas the response to ANS was completely absent (Table 2). Note that the sensor based on *P. rathonis* T did not detect Tween 80 or OP-10 at 1 mg/l [9].

Experiments on detection of cationic and ampholyte surfactants revealed stable responses only at 100 mg/l, whereas the sensor element based on *P. rathonis* T lost its activity with cationic surfactants [9]. Apparently, nonylphenolethoxylate degraders are more resistant to cationic surfactants than bacteria degrading anionic surfactants.

Investigation of the response of the sensor to carbohydrates revealed the absence of response to most substrates, including glucose. This was of particular interest, because the formerly described sensor based on *P. rathonis* T responded to glucose. Responses to sucrose and fructose amounted 22 and 13% of the response to OP-10, respectively, at 100 mg/l.

Table 1. Substrate specificity of the microbial biosensor based on strain *C. testosteroni* TI

Substrate, 100 mg/l	Sensor response*, % of the response to OP-10	Substrate, 100 mg/l	Sensor response,* % of the response to OP-10
SDS-Na	105	Sulfoethoxylate	45
Volgonat	88	Ethonium	47
Metaupon	57	Amidobetaine	40
ANS	5	Katamin	0
DMAS	127	Alkamon	0
ABS np-3	88	Imidostat	0
ABS linear	94	Glucose	0
ABS branched.	35	Arabitol	0
Sumgait ABS branched.	0	Arabinose	0
Tween 80	90	Xylitol	0
Tween 20	132	Xylose	0
Triton X-100	95	Galactose	0
Triton X-350	52	Sorbitol	0
Polyethylene glycol dodecyl ether $n = 10$	90	Cetylpyridinium chloride	35
Polyethylene glycol dodecyl ether $n = 6$	81	Tetradecyltrimethylammonium bromide	0
Polyethylene glycol cetyl ether $n = 6$	52	Alkylamino-bis-propionate	13
Diethanolamide	83	Sorbose	0
Sucrose	22	Raffinose	0
Fructose	13	Methanol	0
Maltose	0	Ethanol	84
Propanol	54	Bisacrylamide	0
Butanol	0	Acrylamide	0
Glycerol	0	Chloroform	0
Urea	0	Nonylphenol	0
Dimethylformamide	0	NP-40	48

* Sensor response to OP-10 at 100 mg/l was 0.023 nA/s.

Table 2. Substrate specificity of the microbial biosensor based on strain *C. testosteroni* TI

Substrate, 1 mg/l	Sensor response,* % of the response to OP-10	Substrate, 1 mg/l	Sensor response,* % of the response to OP-10
SDS	55	Sulfoethoxylate	0
Volgonat	48	Ethonium	75
Metaupon	52	Amidobetaine	36
ANS	0	Katamin	30
DMAS	23	Alkamon	99
ABS np-3	35	Imidostat	0
ABS linear	10	Glucose	0
ABS branched.	13	Arabitol	0
Sumgait ABS branched.	18	Arabinose	0
Tween 80	40	Xylitol	0
Tween 20	46	Xylose	0
Triton X-100	98	Galactose	13
Triton X-350	73	Sorbitol	0
Polyethylene glycol dodecyl ether $n = 10$	133	Cetylpyridinium chloride	0
Polyethylene glycol dodecyl ether $n = 6$	23	Tetradecyltrimethylammonium bromide	40
Polyethylene glycol cetyl ether $n = 6$	25	Alkylamino-bis-propionate	0
Diethanolamide	28	Sorbose	0
Sucrose	23	Raffinose	0
Fructose	0	Methanol	0
Maltose	0	Ethanol	92
Propanol	85	Bisacrylamide	0
Butanol	0	Acrylamide	0
Glycerol	0	Chloroform	0
Urea	0	Nonylphenol	56
Dimethylformamide	0	NP-40	64

* Sensor response to OP-10 at 1 mg/l was 0.004 nA/s.

Determination of the ability of the receptor element based on *C. testosteroni* TI to generate signals with the presence of polyaromatic hydrocarbons showed that the signal amplitude depended on the nature of the substrate, which was likely to be related to their different water solubilities. For example, the sensor produced intense responses (~35–45% of the response to OP-10) to naphthalene, anthracene, and pyrene, whereas fluorene, phenanthrene, and acenaphthylene caused lower responses (about 5% of the response to OP-10), and acenaphthene produced no response at all. Note that the sensor based on *P. rathonis* T could detect only naphthalene of all the polyaromatic compounds tested.

Other parameters of the sensor. The operation stability of the sensor (the duration of operation of a single receptor element) was estimated by repeated measurements of OP-10 at 100 mg/l over 20 days. It was found that the amplitude of the response varied insignificantly during the first 10 days but decreased by 30% during the following 10 days (Fig. 2). In this experiment, the receptor element was kept in the buffer solution at room temperature and constant agitation.

The reproducibility of the response was estimated by repeated measurements of OP-10 at 100 mg/l. The coefficient of variation of sensor readings in these experiments was 4.5%.

Study of the dependence of sensor response on the pH of the medium showed that the optimum response level was reached at pH 7.0–7.8. The response decreased by 17–20% at pH 5.5–6.0 (Fig. 3). Measurements were carried out at 20°C. Stabilization of the biosensor signal to OP-10 at 100 mg/l took 12–15 min.

Thus, the biosensor model based on *C. testosteroni* TI bacteria has a wide-range substrate specificity with

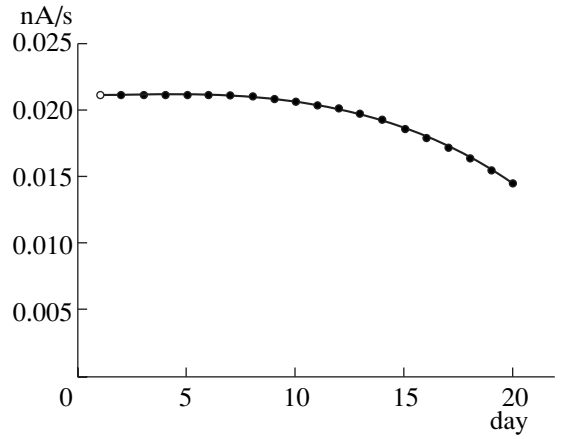

Fig. 2. Time stability of the biosensor. Change of responses (nA/s) at constant measurement for 20 days.

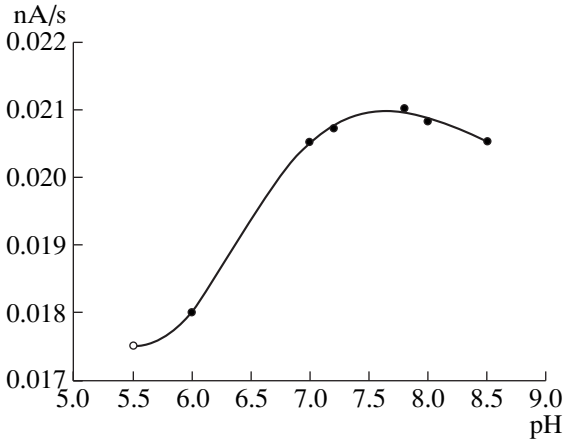

Fig. 3. Dependence of the response of the biosensor on pH.

respect to surfactants, which is an advantage. Its sensitivity to other chemicals is low. The average lower limit of detection for NIS is 0.25–0.5 mg/l. Therefore, the strain is promising for use in multisensor systems in combination with biosensors based on other strains, capable of detecting various types of surfactants.

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