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Biosensor approach for electrochemical quantitative assessment and qualitative characterization of the effect of fusaric acid on a culture-receptor

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ABSTRACT

Fusaric acid (FA) is a secondary fungal metabolite, which is widespread on corn and corn-based feed and food; FA has non-specific toxicity. Biosensor method is an express and easy-to-use method for quantitative and qualitative assessment of FA effect. Search for cultures has been performed for the formation of laboratory models of FA biosensor with the Clark-type oxygen electrode as transducer: respiration intensity of chosen cultures changed in the presence of FA. Resting cells of *Fusarium oxysporum* f. sp. *vasinfectum* and *Bacillus subtilis* were used as receptors of the amperometric biosensor for FA determination in aqueous solution. To enhance the sensitivity of detection, induction by substrate was performed for *Bacillus subtilis*. Response-concentration linear dependencies were obtained in a range of 0.5–500 FA mg/L. Biosensor models were applied to characterize influence of FA on microbial cells and investigate some features of FA transport. The dependences of the cells' response to FA on FA concentration were obtained; the kinetic parameters $S_{0.5}$ and V_{max} were determined for each culture. Inhibition-threshold FA (S^{it}) concentrations were similar for both studied cultures. At concentrations lower than S^{it}, the process of simple diffusion governed FA transport into cells and caused the cells' response to FA for non-induced culture.

1. Introduction

Mycotoxins are toxic secondary metabolites, which are produced by fungi of the genera *Aspergillus*, *Penicillium* and *Fusarium* (Ismaiel and Papenbrock, 2015). Fusaric acid (FA) is one of the oldest known secondary metabolites. This acid is produced by some fungi of the genus *Fusarium*.

Grains of cereals are contaminated with mycotoxins during cereal growth, grain storage and processing. Cereals are the important constituent of feed for farm animals and birds. As a result, mycotoxins from contaminated livestock product may appear in human organism. Fusaric acid is frequently found in grains. FA is widespread on corn as well as not only in corn-based feed but also food (Bacon et al., 1996; Porter et al., 1995).

FA (5-*n*-butylpyridine-2-carboxylic acid, $C_{10}H_{13}O_2N$) is a mild, weak organic acid with high phytotoxic activity and nonspecific toxicity to animals and humans. Furthermore, FA may augment toxicity of other mycotoxins. It synergistically acts with other mycotoxins eliciting a organism response to toxin (Bacon et al., 1995; Niehaus et al., 2014). FA promotes virulence of *Fusarium oxysporum* for a plant (López-Díaz et al., 2018). That is a reason why FA is used as an herbicide. "The toxic effects of FA on plants include alteration of membrane permeability (modification of cell membrane potential), a decrease in mitochondrial activity and oxygen uptake, inhibition of ATP synthesis and inhibition of root growth" (D'Alton and Etherton, 1984; Dong et al., 2012; Ismaiel and Papenbrock, 2015; Marré et al., 1993; Wang et al., 2014). The toxic concentration of FA was 10^{-5} M (1.8 FA mg/L) for *Arabidopsis thaliana* cells (Bouizgarne et al., 2006). For tomato, potato, basal root of lily, *Ricinus* roots and culture of potato cell suspension, the toxic concentration of FA was about 10^{-3} M (179 FA mg/L) (Curir et al., 2000; Ismaiel

Abbreviations: FA, fusaric acid.

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and Papenbrock, 2015; Pavlovkin et al., 2004; Sapko et al., 2011; Singh and Upadhyay, 2014; Singh et al., 2017). The nontoxic concentration of FA was 10^{-6} M and lower. FA induced synthesis of the phytoalexins (Bouizgarne et al., 2006) at nontoxic concentrations ($<10^{-6}$ M).

An important ecological role of mycotoxins is that they are essential chemical language mediating communication between microorganisms (Venkatesh and Keller, 2019), but Tung et al. (2017) showed that FA was an inhibitor of bacterial quorum sensing. Depending on the concentration used, FA had bactericidal or bacteriostatic effect (Bacon et al., 2006; Landa et al., 2002; May et al., 2000), and also inhibited production of secondary metabolites (Ruiz et al., 2015; Van Rij et al., 2005). At the same time, despite FA toxicity, some microorganisms consumed this acid as a sole carbon source for growth or transformed it (Venkatesh and Keller, 2019).

FA also exhibits pharmacological activity (Parma et al., 2010; Wang and Ng, 1999). FA disrupts the functioning of the systems involved into adrenaline metabolism. It was confirmed that FA was an antihypertensive agent. The pharmacokinetics of fusaric acid was determined in rats to clarify the mechanism of FA activity against head and neck squamous cell carcinoma (Gross, 1977; Hidaka et al., 1969; Hidaka and Takeya, 1972; Jucker, 1971; Porter et al., 1995; Stack et al., 2014; Terasawa and Kameyama, 1971). Ogata et al. (2001) found that FA induced apoptosis in human leukemia cells. To obtain this toxin, microbial production of FA was optimized (Bacon et al., 1996; Davis, 1970; Han et al., 2014; Parma et al., 2010).

It is obvious that easy and rapid methods for monitoring of FA and assessing toxicity of the acid are needed. Until recently, conventional methods were used. Different kinds of chromatography techniques (thin-layer chromatography, liquid chromatography and gas-liquid chromatography) were employed to monitor the process of FA production and determine the FA content (Amalfitano, 2002; Chen et al., 2017; Fung et al., 1976; Paterson and Rutherford, 1991). Microbiological methods were applied for assessment of the phytotoxicity of fusaric acid (Stipanovic et al., 2011) and determination of the FA content (Chandramohan and Mahadevan, 1968; Li et al., 2012; Šrobárová et al., 2009; Venter et al., 1996). A biosensor approach has not yet been used to determine FA concentration and to study FA effect on microorganisms.

A biosensor technique is an express method of analysis without expensive equipment; a microbial membrane sensor would be a suitable tool for the study of processes with FA. A microbial receptor of a biosensor is formed on the basis of a culture-receptor, which recognizes the substance (the substrate of the biosensor). It is noteworthy that the magnitude of a response to a substrate for a culture-receptor is stipulated by concentration of substrate and features of substrate metabolism. Therefore, a biosensor approach is an approach composed of quantitative assessment and qualitative characterization of FA influence.

It is known that the rate of a response to a substrate for the biosensor' culture-receptor is caused by two processes: substrate transport into the cells of the culture and substrate metabolism by cell enzymes (Turner et al., 1987). Bacterial cultures-indicators of the genus *Bacillus* were used in microbiological methods described by Šrobárová et al. (2009); the quantity of FA was determined by the size of transparent zones on the plates with *Bacillus subtilis*. Surely, FA transport into bacterial cells preceded the inhibition of *Bacillus subtilis* growth by this toxin.

Some fungi of the genus *Fusarium* produce fusaric acid. Transport of the own secondary metabolite into cells of a culture-producer and its metabolism are distinctive for fungi-producers (Kozlovskii et al., 2013). Thus, in the presence of FA, processes of FA transport and metabolism are supposed to be activated in cells of some fungi of the genus *Fusarium* and strains of the bacterium *Bacillus subtilis*. Hence, a search for culture-receptors for microbial membrane FA sensors (with the Clark-type oxygen electrode as the transducer) has been performed among fungi of the genus *Fusarium* and strains of the bacterium *Bacillus subtilis*. Cultures that consumed oxygen more intensively in the presence of FA have been chosen and used in the present biosensor study.

This is the first research that shows the possibility of the use of bacteria of the genus *Bacillus* and fungi of the genus *Fusarium* for formation of a bioreceptor of a microbial amperometric FA sensor and for biosensor-based characterization of FA influence on microbial cells.

2. Materials and methods

2.1. The microorganisms and culture conditions

Fusarium oxysporum strains and *Bacillus subtilis* strains, stored in a culture collection created by the author of this paper, have been used in the present study. The bacterial cultures and fungal strains were maintained on agarized Luria-Bertani (LB) medium and malt agar, respectively, at + 4 °C. Cultures were transferred every 6 months.

2.2. Formation of bioreceptors based on the cells of cultures-receptors

Bioreceptors were formed on the basis of the cultures under study (cultures-receptors).

Bacterial *B. subtilis* cultures were grown on agarized LB medium at + 28 °C for 24 h. Bacterial cells were harvested and then suspended in a 50 mM potassium-sodium phosphate buffer (pH 7.4) at 100 mg of wet cells per milliliter. Suspensions were stored at + 4 °C for 12 h and then used for bioreceptor formation. For the formation of a *B. subtilis*-based bioreceptor, resting bacterial cells were immobilized on Whatman paper by the method of physical adsorption (This method for cell immobilization has been used for 20 years and has shown good scientific output.). Bacterial suspension (10 μL) was spotted (3–4 mm in diameter) onto a piece of paper (4 ×4 mm^2). The prepared bioreceptor was air-dried and used for biosensor formation.

Fusarium strains were grown on malt agar at + 25 °C. A bite of grown mycelium (3 × 3 mm^2) was used as a bioreceptor.

2.3. Formation of laboratory models of a microbial membrane sensor

Laboratory models were formed on the basis of obtained bioreceptors. The bioreceptor, formed on the basis of the culture under study, was fixed on the measuring surface of the Clark-type oxygen electrode by means of nylon net. The electrode with the bioreceptor was then placed in an open measuring cell (working volume of the cell was 5 mL) equipped with a magnetic stirrer. The design of the recognizing part of the microbial membrane sensor was shown on Fig. 1. The oxygen electrode was used with an amplifier (Ingold 531–04 O_2 Amplifier, Switzerland-USA). A two-coordinate XY Recorder-4103 (Czech Republic) was applied as a response recorder. The construction of laboratory models of the biosensor was described in the previous paper of the author (Emelyanova and Solyanikova, 2021).

2.4. Detection of the cultures-receptors' responses to FA

The measurements of cultures' responses to FA were carried out in the air-saturated 50 mM K-Na-phosphate buffer (pH 7.0) at + 20–22 °C. When basal (endogenous) respiration of culture-receptor cells stabilized, solution of FA was injected into buffer solution. Then, the change in oxygen concentration was measured with the Clark-type oxygen electrode. The culture-receptor response to FA was determined as the rate of culture respiration change in response to injection of FA solution into the measuring cell. The rate of culture respiration change was detected by the rate of change in oxygen concentration at the measuring area of the oxygen electrode, where the bioreceptor element was fixed. The Clark-type oxygen electrode transduced the rate of change in oxygen concentration in solution to the rate of change in the electrode current. The recorded signal reflected the rate of change in oxygen consumption by culture in response to injection of FA. The response to FA was calculated as the first derivative of the electrode current change (pA/s) in response to addition of FA. Then, after signal registration, the system was washed with buffer solution. The next measurement was made

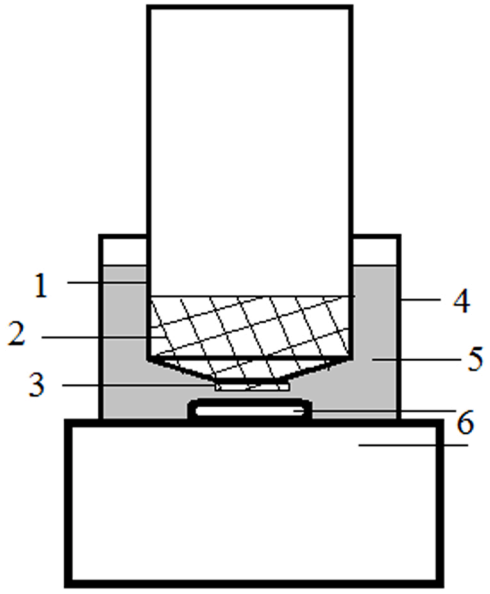

Fig. 1. The design of the recognizing part of the laboratory model of the microbial membrane sensor:1 – the Clark-type oxygen electrode; 2 – a nylon net for bioreceptor fixation; 3 – the bioreceptor; 4 – an open measuring cell; 5 – buffer solution; 6 – a magnetic stirrer.

when the line of basal respiration was stabilized (a horizontal line of recorded signal).

2.5. Induction of immobilized B. subtilis cells 128

For induction, the electrode with *B. subtilis* 128-based bioreceptor fixed on the electrode surface was placed in a 50 mM solution of K-Na-phosphate buffer (pH 7.0) containing FA. After 15–18-h induction, the system was washed with buffer solution and then the measurement of the culture's response (the response of induced culture) to FA was performed.

2.6. Statistics

The measurements were taken in two independent series of experiments. Results of one of the series of experiments are presented in this paper. These results are averaged values of triplicate measurements. Statistical data analysis was carried out using a Student's *t*-test ($p < 0.05$).

3. Results and discussion

3.1. Response-FA concentration curves

Fungi of the genus *Fusarium* and strains of the bacterium *Bacillus subtilis* were used as cultures-receptors for the formation of laboratory models of a biosensor for FA. Changes in the respiration intensity of cultures-receptors in the presence of FA were investigated. The best results were obtained for two cultures, *Fusarium oxysporum* f. sp. *vasinfectum* Coll and *Bacillus subtilis* 128 (Emelyanova et al., 2016). Cells of these cultures were chosen for the formation of bioreceptors and laboratory models of the microbial membrane sensor. *F. oxysporum* f. sp. *vasinfectum*- and *B. subtilis*-based sensor models were employed in this research.

Dependencies of the rate of response to FA (*V*, pA/s) on the initial FA concentration (*S*, mg/L) were investigated for cells of cultures chosen. The lowest FA concentration, which triggered the registered response to the acid, was 12.5 FA mg/L. 1250 FA mg/L was the highest FA concentration used in the experiment to study FA influence on the cultures' respiration. Response-concentration curves (*V* vs. *S*) for the fungus and bacterium are presented in Fig. 2a and c, respectively. The shapes of curves were typical of toxic substrates (FA is a toxic substrate). These curves were characterized by the presence of inhibition-threshold substrate concentration (S^{it}). S^{it} was FA concentration above which the substrate inhibition of processes was observed.

In our study, the rate of the response to FA rose with an increase of FA concentration and the rate reached a characteristic point $V = 0.5\ V_{max}$. It was observed at $S_{0.5}$ substrate concentration. The values of $S_{0.5}$ have been calculated. $S_{0.5}$ was 247 and 169 FA mg/L for *F. oxysporum* f. sp. *vasinfectum* Coll and *B. subtilis* 128, respectively. Then, the rate got up to its highest point (V_{max} is the maximum rate of the response to FA) at FA concentration about 550 FA mg/L (the inhibition-threshold substrate concentration). The value of V_{max} was 12.2 and 14.8 pA/s for the fungus and bacterium studied, respectively. When FA concentration was higher than the inhibition-threshold substrate concentration, the inhibition by substrate was observed: the rate of the response to FA began to slowdown.

Comparing the concentration-response curves at concentration lower than the inhibition-threshold substrate concentration (Fig. 3), similar slopes of curves of *V* vs. *S* dependencies are obvious for cultures studied. Magnitudes of V_{max} and $S_{0.5}$ obtained for cultures, *F. oxysporum* f. sp. *vasinfectum* Coll and *B. subtilis* 128, were close. Probably, similar patterns of the formation of the response to substrate for our cultures occurred in the presence of FA.

For both *F. oxysporum* f. sp. *vasinfectum* Coll and *B. subtilis* 128, almost linear *V* vs. *S* dependences at FA concentrations lower than the inhibition-threshold substrate concentration are demonstrated in Fig. 2a and c (Linear intervals of *V* vs. *S* dependences – calibration plots – are shown for the fungus and bacterium in Fig. 2b and d, respectively.). These findings could indicate that similar processes caused the response to FA for cultures-receptors. Furthermore, constitutive systems were involved in the formation of the cultures' responses to FA, since the responses to the substrate – FA – were recorded for the culture non-induced by the substrate (culture were grown in FA-free media).

3.2. Inhibition by substrate (FA)

Detecting the inhibition-threshold substrate concentration, S^{it}, by means of the conventional microbiological method is a rather laborious process. The biosensor method is a suitable tool for the assessment of inhibition-threshold substrate concentration. In this biosensor study, cultures' responses to FA decreased when the concentration of the acid was higher than S^{it}. S^{it} was almost the same (about 550 mg/L of FA) for both cultures under study (Fig. 3). This might be due to the similar influence of the chemical compound, FA, on the cells of both cultures.

While studying the FA influence on the growth of cultures, Bacon et al. (2006) and Landa et al. (2002) showed that FA concentration higher than 200 mg/L exhibits a bactericidal effect on the most of *Bacillus* species, and an inhibition-threshold FA concentration ranged from 200 to 500 mg/L for eight fluorescent pseudomonades. These concentrations were of the same order as concentrations obtained in our study. Similar to our study with *F. oxysporum* f. sp. *vasinfectum* Coll, Crutcher et al. (2015) found that FA differentially inhibited the growth of *Fusarium oxysporum* f. *vasinfectum* (Fov). However, for our fungus, we observed only retardation in a rise of the magnitude of the rate in response to FA, while Crutcher et al. (2015) reported no growth for their fungus culture at 500 FA mg/L. Such behavior can be explained by greater resistance to toxic effect of FA for cultures used in this study. It should be noted, however, that in our research we used immobilized bacterial cells, which were more resistant to stress factors than intact cells.

In addition, in the present study, the constant of inhibition by substrate ($S^{si}_{0.5}$) for culture response to FA has been determined for *F. oxysporum* f. sp. *vasinfectum* Coll. $S^{si}_{0.5}$ was the FA concentration higher than the inhibition-threshold FA concentration, S^{it}. At concentration

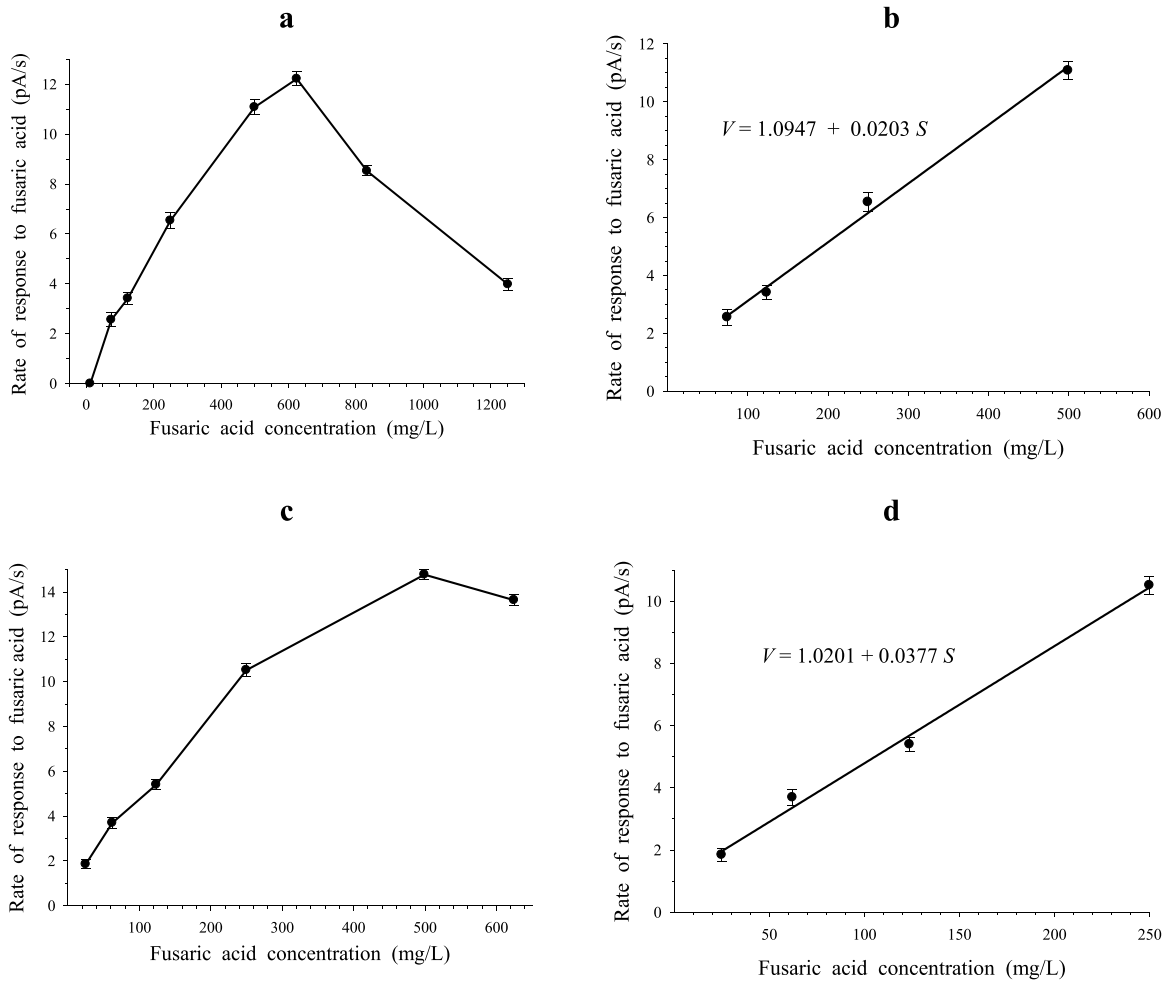

Fig. 2. Dependences of the rate of the response (*V*, pA/s) to FA on the initial FA concentration (*S*, mg/L) for *F. oxysporum* f. sp. *vasinfectum* Coll (**a, b**) and *B. subtilis* 128 (**c, d**). **a, c**: Curves of *V* vs. *S* dependencies. **b, d**: Calibrated plots.

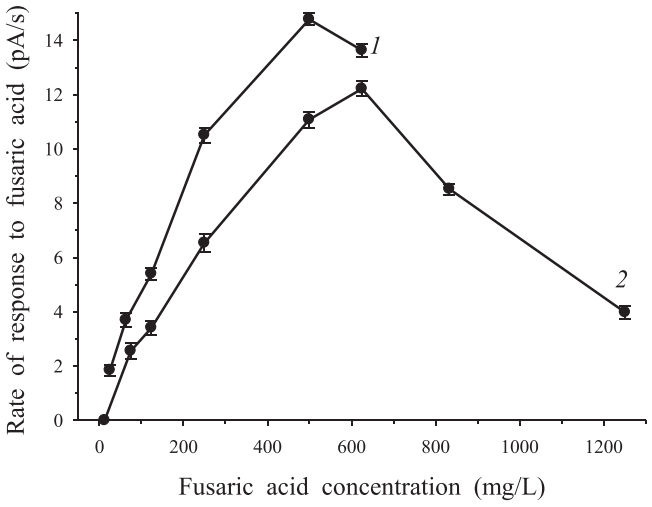

Fig. 3. Comparison of responses (*V*, pA/s) to FA for *B. subtilis* 128 (*1*) and *F. oxysporum* f. sp. *vasinfectum* (*2*).

higher than the S^{it}, the rate of the response to FA began to decrease and reached the value of $0.5V_{max}$ at $S^{si}_{0.5}$ FA concentration. $S^{si}_{0.5}$ was about 1100 mg/L. We detected only 50% inhibition of the rate of the fungus's response at FA concentration of about 1000 mg/L, but Landa et al. (2002) observed no growth of fungi at the said concentration.

In this study, instead of the analysis of growth of cultures, we used a biosensor approach to evaluate the inhibition effect of FA on microbial cells. Results of investigations do not contradict a general notion provided in the literature so far. Furthermore, the use of the biosensor technique led to significantly faster evaluation (without cell cultivation), and this biosensor approach was less labor intensive than a microbiological method.

3.3. Induction of cell activity by FA

It is known that cell activity determines the sensitivity of a kinetically controlled biosensor with low microbe loading (Riedel et al., 1989), the laboratory model of which on the basis of resting cells of *B. subtilis* 128 was formed in this study. Additionally, the detection limit can be also improved by enhancing the sensitivity of a biosensor system. It is a matter of knowledge that activity of microbial cells depends both on the activity of substrate catabolism in cells and on the activity of substrate transport into cells. Both processes usually are induced by substrate of the catabolism pathway (Rojo et al., 1988). Therefore, to induce the cell's system associated with FA transport and metabolism and to enhance the activity of a culture-receptor of the biosensor, we used an incubation approach: *B. subtilis* 128-based receptor was incubated in the presence of FA.

Induction by FA resulted in the enhancement of response to the acid. Dependences of the rate of the response to FA (*V*, pA/s) on the initial concentration of the acid (*S*, FA mg/L) for *B* subtilis 128 cells before and

after induction are shown in Fig. 4. Also, improvement of the detection limit by an order of magnitude occurred due to induction by FA. If before induction the minimum concentration, at which the response to FA was registered, was 12.5 FA mg/L, then after induction this concentration equaled to 0.25 FA mg/L. At the same time, the magnitude of the culture's response to FA increased by 3–5-times. The results of induction indicated that FA-induced *B* subtilis 128 exhibited the increased resistance to FA.

Induction by FA should activate the inducible systems of FA transport into *B* subtilis 128 cells and/or of FA metabolism in cells of the bacterium. Activation of any of the mentioned systems caused an alteration in the magnitudes of the response to and detection limit of FA.

Activation of the inducible systems of FA transport into *B* subtilis 128 caused primarily an alteration in the limit of FA detection. Hu et al. (2012) reported that Gram-negative bacterium *Stenotrophomonas maltophilia* K279 exerted FA resistance owing to the presence of ABC (the ATP-binding cassette) proteins-transporters in cells; and ABC proteins were also constituents of influx and efflux systems of Gram-positive bacteria (Lorca et al., 2007). In the present study, a Gram-positive bacterium, *B. subtilis* 128, was used as a culture-receptor. Probably, after incubation in the presence of FA, induction of proteins-transporters could occur, resulting in the FA transport into bacterial cells at FA concentration lower than that before induction. The induction of proteins-transporters could be supported by the fact that the saturation plot (Fig. 5) was obtained after the induction instead of linear *V* vs. *S* dependence that passes through the origin of coordinates (Fig. 2c). The *V* vs. *S* saturation curve is attributed to the involvement of proteins-transporters in the movement of the substrate. Nevertheless, active transport requires chemical energy (due to ATP-binding proteins) but it is hardly possible in resting cells such as immobilized *B. subtilis* 128 cells. Facilitated diffusion also involves specific transporters, while they can be ATP-independent. Because of the breach of the cell-medium proton gradient in resting cells, the process of facilitated diffusion can also be disturbed in immobilized *B. subtilis* 128 cells. Thus, further study is needed to confirm the presence of proteins-transporters in resting *B. subtilis* 128 cells after induction by FA.

The changes, which were recorded after induction of *B. subtilis* 128, could be associated with the activation of inducible enzymes involved in FA catabolism. As a result, significant increase of *B. subtilis* 128 response to FA after induction (Fig. 4) might occur. Furthermore, after incubation in the presence of FA, the shape of the plot of *V* vs. *S* dependency changed from linear dependence (Fig. 2c) to the saturation plot (Fig. 5), typical of the classical Michaelis-Menten kinetics of enzyme-substrate

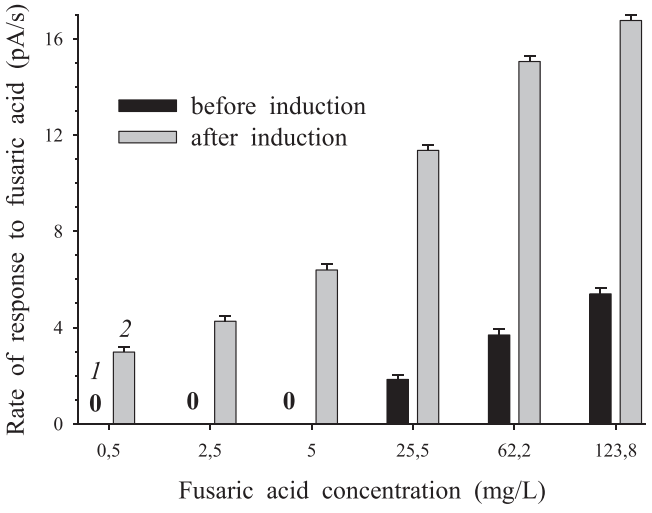

Fig. 4. Comparison of responses (*V*, pA/s) to FA for immobilized *B. subtilis* 128 cells before (1) and after (2) induction by FA. 0 implies that a response to FA was 0 pA/s.

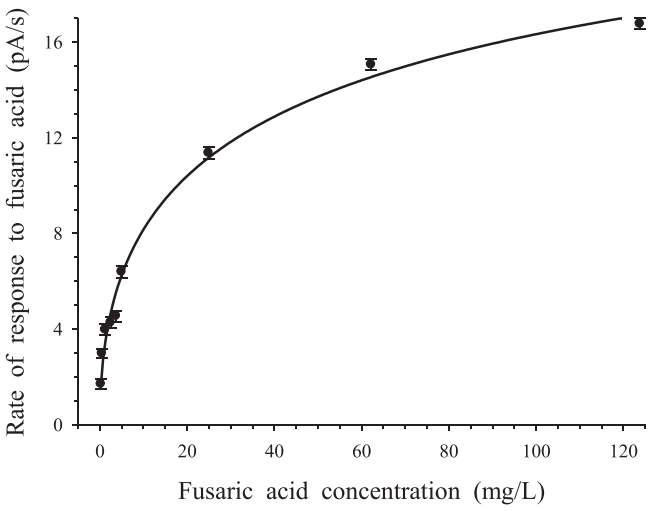

Fig. 5. *V* vs. *S* dependence for induced immobilized *B. subtilis* cells 128.

interaction. Most likely, induction of enzymes of FA metabolism took place after incubation of bacterial cells in the presence of FA taking into account that the value of $S_{0.5}$ changed to 8 FA mg/L (as opposed to 169 FA mg/L before induction), thereby indicating a high enzyme-substrate affinity.

More research will provide an in-depth understanding of what processes are activated in immobilized *B. subtilis* 128 cells as a result of induction by FA.

3.4. FA transport into microbial cells

A similar slope of *V* vs. *S* curves was detected for *F. oxysporum* f. sp. *vasinfectum* Coll and *B. subtilis* 128. The plots are depicted in Fig. 3. $S_{0.5}$ values calculated for the bacterium and fungus were the same order of the magnitude. Probably, it is the evidence for the similarity of processes that occurred in the cells of cultures-receptors in the presence of FA. It is known that the response to a substrate for the culture-receptor of a microbial sensor is caused by processes of substrate transport into microbial cells and of substrate metabolism by the cells' enzymes (Turner et al., 1987). When the enzymes initiated the metabolism of substrate are absent, the response to a substrate is governed by the processes of substrate transport into the cell and vice versa. In the present study, for both cultures of interest, the plots of *V* on *S* dependences in the double-reciprocal coordinates (*1/V* vs. *1/S*) were the straight lines (data not shown). Hence, in accordance with the principle of "bottle neck", only one factor was rate-determining, only one process (substrate transport or substrate metabolism) stipulated the form of the plot of *V* vs. *S* for the cultures' responses to FA.

The shape of curves of *V* vs. *S* was inherent for toxic substrates (Fig. 2a and c). For enzymatic reactions inhibited by substrate, *V* vs. *pS* dependency is usually depicted by a symmetrical bell-shaped curve (Webb, 1963). The curve of *V* vs. *pS* dependency for *F. oxysporum* f. sp. *vasinfectum* Coll is shown in Fig. 6. It is evident that the bell-shaped curve in Fig. 6 was not perfectly symmetrical. Imperfect symmetry indicates that *F. oxysporum* f. sp. *vasinfectum* Coll response to FA was caused by processes, which were not only the processes of enzyme-substrate interaction. It can be assumed that without induction by FA, transport processes took part in the formation of a response to FA for *F. oxysporum* f. sp. *vasinfectum* Coll and *B. subtilis* 128.

Bacon et al. (2006) showed that the toxicity of FA is due to its activity as a lipophilic agent. Transport of lipophilic compounds is carried out via simple diffusion (passive transport): the lipophilic compound diffuses through the lipid layer of the membrane without energy consumption (Kotyk and Janaček, 1977). Crutcher et al. (2015) found that FA might enter cells of *Fusarium oxysporum* f. *vasinfectum* (Fov) by a

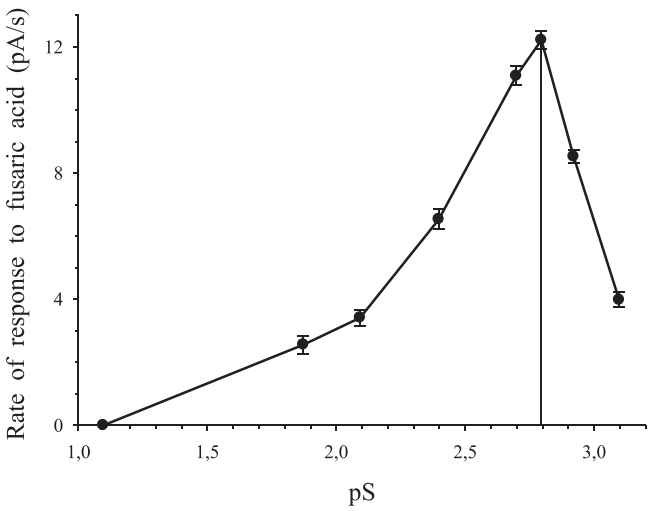

Fig. 6. Dependence of *V* vs. *pS* for *F. oxysporum* f. sp. *vasinfectum* Coll.

mechanism, which was independent of the transporter: via passive transport without saturation. As a result, in the present study, the transport of FA into cells of cultures-receptors might be carried out via passive transport without saturation (without a transporter via simple diffusion). Furthermore, passive diffusion is of great importance when vital functions of cells are disturbed, for example, in resting cells (such as resting cells of the cultures-receptors, which were used in the present study). In our study, plots of *V* vs. *S* dependencies were linear at concentrations lower than S^{it} and lines passed through the origin of coordinates; it is characteristic of the process of substrate entry via simple diffusion. Consequently, for non-induced cells of *F. oxysporum* f. sp. *vasinfectum* Coll and *B. subtilis* 128 at FA concentration lower than S^{it}, plots of the cultures' responses to FA vs. FA concentration (Fig. 2a and c) depicted the process of simple diffusion of FA into cells of cultures-receptors.

3.5. Calibrated plots for FA determination

Respiration of the cultures-receptors (*F. oxysporum* f. sp. *vasinfectum* Coll and *B. subtilis* 128), registered in the absence of FA, was endogenous in nature. Injection of FA into the measuring cell led to a contact of microbial cells with the acid and an increase in the respiration rate of the culture-receptor. In this study, the rate of respiration of *F. oxysporum* f. sp. *vasinfectum* Coll and *B. subtilis* 128 depended on FA concentration. Signal registered after FA injection was proportional to FA concentration. Hence, using a linear dependence of the rate of electrode current change on FA concentration, the acid concentration in injected solution could be determined. Calibrated plots of *V* vs. *S* dependencies are depicted in Fig. 2b and d for *F. oxysporum* f. sp. *vasinfectum* Coll and *B. subtilis* 128, respectively. Registered rates of the response to FA (*V*, pA/s) were expressed by formulas: $V = 1.0947 + 0.0203\,S$ (for the fungus) or $V = 1.0201 + 0.0377\,S$ (for the bacterium), where *S* (mg/L) was FA concentration in the measuring cell. The calibrated plot for *F. oxysporum* f. sp. *vasinfectum* Coll was obtained, when FA concentration ranged between 70 and 500 FA mg/L. The calibrated plot for *B. subtilis* 128 could be used for determination of FA concentration in a range of *S* from 20 FA mg/L to 250 FA mg/L. After *B. subtilis* 128 induction by FA, the calibrated plot ($V = 2.7710 + 0.6396\,S$) was obtained for FA concentration ranged between 0.5 and 5 FA mg/L.

Li et al. (2012) observed linearity of *V* vs. *S* dependence only at FA concentration higher than 100 mg/L (100–2000 mg/L) using HPLC analysis of FA concentration. For luminescent bacterium assay, linearity was obtained within a range of 5–40 FA mg/L (Li et al., 2012). The limit of microbiological detection of FA reported by Li et al. (2012) was by an order of magnitude worse than that recorded with the use of the bacterium in the present study. Moreover, in our research, total duration of the assay including the system washing was no longer than 20 min. This offers the possibility to carry out up to four analyses per hour without the necessity to cultivate the culture-receptor. Furthermore, it is possible to obtain quickly the information about changes in FA concentration using the receptor elements prepared in advance; they can be immediately replaced if necessary.

4. Conclusion

This study is the first research which demonstrates the possibility of using *Fusarium oxysporum* f. *vasinfectum* and *Bacillus subtilis* to form a bioreceptor of a microbial amperometric sensor to FA. The biosensor model on the basis of chosen cultures could be used for rapid assay of FA concentration in aqueous solution.

The microorganism-based receptor was the recognition element of the laboratory model of the biosensor system described. It was not necessary to grow a new culture for each analysis due to the availability of interchangeable receptor elements. As a result, total duration of single assay including the system washing was no longer than 20 min (up to four assays per hour).

The laboratory model of the microbial sensor based on FA-recognizing microbial culture is attractive as a tool for investigation of special features of FA effect on a culture-receptor and its metabolism. The promising potential of the biosensor approach is not yet fully recognized to use widely the opportunities, which this approach offers to study the substrate effect on microbial metabolism.

We have demonstrated that the biosensor approach is easy-to-use, efficient and economic method to estimate some characteristic features of the effect of toxic substance on microorganisms and to get rapidly the information about changes in a state of the culture in response to the presence of a toxic substrate.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Competing interests

The authors declare that they have no conflict of interest.

Ethics approval

This study was performed without the participation of humans and/or animals and no ethical approval is required.

Authors' contributions

Conception and design were from E.V. Emelyanova. Material preparation, data collection and analysis were performed by E.V. Emelyanova. Investigations were performed by E.V. Emelyanova and T.V. Antipova. Resources were from E.V. Emelyanova and T.V. Antipova. The first draft of the manuscript was written by E.V. Emelyanova. All authors read and approved the final manuscript.

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