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Microbial Biosensors for Selective Detection of Disaccharides

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Abstract

Seven microbial strains were screened for their ability to detect disaccharides as components of Clark-type oxygen biosensors. Sensors responded to varying degrees to maltose, cellobiose, sucrose, and melibiose, but none responded strongly to lactose. Although microbial sensors are relatively nonspecific, it is possible to obtain differential measurements of specific substrates using multiple sensors with different relative specificities. For example, Escherichia coli strain K-802 oxidized maltose but had low activity against sucrose, while Bacillus subtilis strain VKM B-434 responded more strongly to sucrose than maltose. Furthermore, signals from these two sensors were additive for selected samples. Results suggest that a two-component biosensor utilizing these strains could be used for differential detection of sucrose and maltose.

INTRODUCTION

Whole cell microbial biosensors offer advantages for the real-time quantitative measurement of analytes (D'Souza, 2001). They are simple and inexpensive to construct, offer sensitivity and stability, and are rugged and durable under field conditions. However, microbial biosensors often suffer from a lack of specificity towards related substrates. This limitation may be overcome by the use of multiple sensors with complementary specificities. For example, we previously demonstrated that a nonspecific microbial sensor could be used for the specific detection of ethanol in a two-component sensor system (Reshetilov et al., 1998). Sophisticated techniques of chemometrics and artificial neural networks greatly enhance the processing of data from a microbial sensor array (Lobanov et al., 2001).

Relatively few works have focused on microbial sensors for the detection of disaccharides. Riedel et al. (1990) describe the use of Bacillus subtilis- or Trichosporon cutaneum-based microbial sensors for the detection of maltose, lactose, and sucrose. Svitel et al. (1998) describe the use of coimmobilized Gluconobacter oxydans and Saccharomyces cerevisiae for the detection of sucrose, while modified cells of Kluyveromyces marxianus were used for lactose detection. Held et al. (2002) constructed a microbial sensor array for simultaneous detection of mono- and disaccharides using various transport mutants of Escherichia coli.

Direct quantitation of disaccharides would be useful in numerous commercial food processes. Mixtures of sucrose and maltose are used for the enzymatic production of glucan oligosaccharides, useful as prebiotics and low glycemic index sweeteners (Monsan and Paul, 1995; Carlson and Woo, 2004; Cote and Holt, 2007; Grysman et al., 2008). Since the concentration of sucrose and maltose affects the type of oligosaccharides produced (Reh et al., 1990), it would be useful to have a convenient method to monitor and control these disaccharides in real time.

In this work we survey microorganisms for their ability to detect disaccharides as components of microbial biosensors, and identify two strains that show promise for the development of a two-component biosensor for sucrose and maltose.

METHODS

I. MICROBIAL STRAINS AND CULTURE MEDIA

Pichia angusta strain Y-1397, Arxula adeninovorans strain Y-78(6), Gluconobacter oxydans strain B-1280, Gluconobacter oxydans strain B-1227, Bacillus subtilis strain VKM B-434 and Rhodococcus sp. strain R-20 were obtained from the All-Russian Collection of Microorganisms, G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms (IBPM), Russian Academy of Science (RAS). Escherichia coli strain K-802 was kindly provided by the Laboratory of the Structure-functional Analysis of Genetic Systems of Microorganisms,

IBPM, RAS.

Strains of G. oxydans were grown on medium containing (g/l): sorbitol, 20; yeast extract, 2. Cells were grown for 18 h in 750-ml Erlenmeyer flasks containing 100 ml of growth medium on a shaker (200 rpm, 28°C). P. angusta strain VKM Y-1397 was grown for 24 h in 750-ml Erlenmeyer flasks (medium volume 100 ml) at 28°C on a shaker (220 rpm) in a liquid medium of the following composition (g/l): $(NH_4)_2SO_4$, 2.5; $MgSO_4$, 0.2; K_2HPO_4, 0.7; NaH_2PO_4, 3.0; yeast extract, 0.5; 100 μl of vitamin solution per 100 ml of the medium (thiamin and biotin – 5 mg in 10 ml of methanol); 1 ml of solution of trace elements per 100 ml of the medium ($CaCl_2 \times 2H_2O$, 0.11; $FeSO_4 \times 7H_2O$, 0.017; $ZnSO_4 \times 6H_2O$, 0.009; $MnSO_4 \times 5H_2O$, 0.00023; $CuSO_4 \times 5H_2O$, 0.0045); glycerol (1%, v/v). A. adeninovorans strain Y-78(6) was grown at 37°C for 24 h on medium containing (g/l): glucose, 10; peptone, 5; yeast extract, 0.5. Rhodococcus sp. R-20, E. coli K-802, and B. subtilis VKM B-434 were grown on the following nutrient medium of IBPM (g/l): aminopeptide from animal blood hydrolysate, 60.0; tryptone, 50.0; fodder yeast extract, 10.0; soybean extract, 30.0 (medium developed in G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms). Cells were grown at 28°C for 18 h. Strain E. coli K-802 was grown at 37°C.

II. BIOSENSOR FABRICATION AND OPERATION

Cells were separated by centrifugation at 10,000 x g for 5 min and twice washed with potassium-phosphate buffer (30 mM, pH 7.5). For G. oxydans strains, potassium-phosphate buffer (30 mM, pH 6.6) was used. Cells were immobilized by physical sorption on glass fiber filters (GF/A, Whatman). For this purpose, 5 μl of cell suspension containing biomass in the concentration of 100 mg of wet weight/ml was applied to the filter and dried at room temperature for 20 min.

For the fabrication of biosensors, a bioreceptor of 3×3 mm^2 was fixed to the measuring surface of a Clark oxygen-type electrode by a capron net and a fitting ring. Measurements were performed in an open cuvette, and the sensor signal was recorded by an IPC2L amperometric potentiostat connected to a desktop computer. An analyte sample (5-100 μl) was introduced into a 2-ml cuvette, and measurements were performed under constant stirring. Biosensor responses were recorded as the maximal rate of change in signal (nA/s).

RESULTS AND DISCUSSION

RESPONSES OF MICROBIAL SENSORS TO SACCHARIDES

Table 1 shows the responses of microbial sensors to 1 mM glucose and 1 mM disaccharides. Glucose serves as a positive control, and as expected, all strains responded most strongly to this sugar. Most of the strains responded to maltose and cellobiose. Both G. oxydans strains responded to melibiose. None of the strains was highly sensitive to lactose. Only B. subtilis strain VKM B-434 responded strongly to sucrose. Its response to maltose was about half that of sucrose. In contrast, E. coli strain K-802 responded strongly only to maltose. The complementary nature of these responses suggests that these strains might be useful for selective biosensor detection of disaccharides. Based on these results, the latter two strains were chosen for further testing as a model microbial sensor for differential detection of sucrose and maltose.

Figure 1

Table 1. Microbial sensor responses to 1 mM glucose and 1 mM disaccharides.

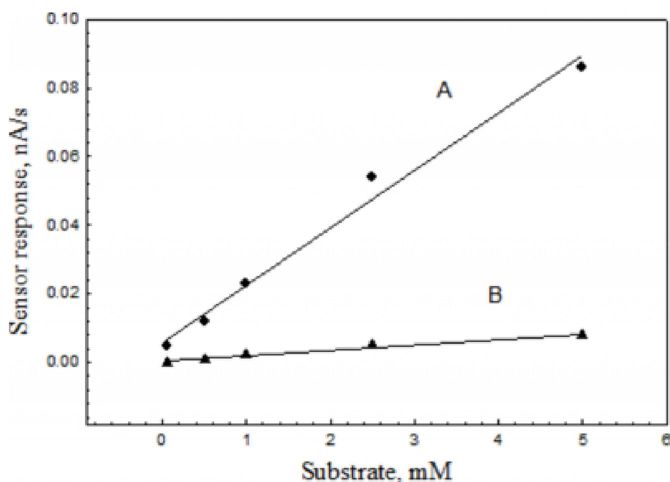
Microorganism, Strain Number	Sensor response, nA/sec					
	Glucose	Maltose	Cellobiose	Sucrose	Lactose	Melibiose
Arxula adeninovorans VKM Y-78(6)	0.040	0.012	0.020	0.010	0.002	0
Bacillus subtilis VKM B-434	0.130	0.050	0.013	0.100	0.005	0.002
Escherichia coli K-802	0.440	0.070	0.009	0	0	0.005
Gluconobacter oxydans B-1280	0.360	0.022	0.050	0.002	0	0.040
Gluconobacter oxydans B-1227	0.520	0.024	0.038	0.006	0	0.015
Pichia angusta VKM Y-1397	0.115	0.007	0.018	0.003	0	0.003
Rhodococcus sp. R-20	0.020	0.010	0.030	0	0	0

CALIBRATION DEPENDENCES OF MICROBIAL SENSORS

Fig. 1 shows the calibration dependence of a microbial sensor based on E. coli strain K-802 for sucrose and maltose. The range of linear dependence of the signal is 0.05-5.0 mM for both sucrose and maltose, although clearly the response is much stronger for maltose.

Figure 2

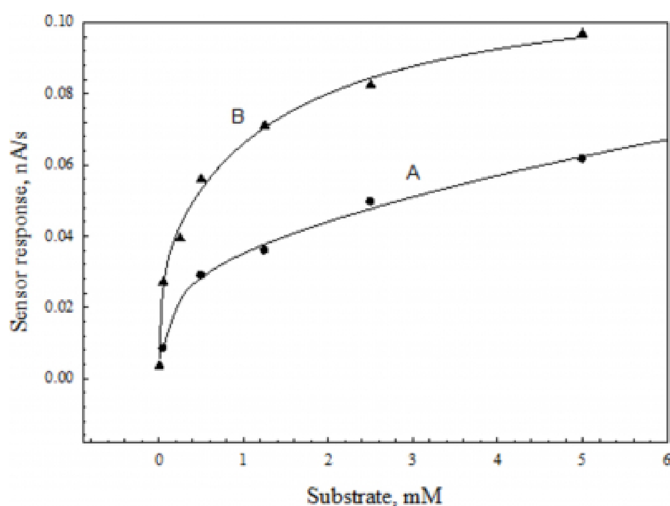
Fig. 1. Calibration dependence of the strain K-802-based sensor for detection of maltose (A) and sucrose (B).



By contrast, Fig. 2 shows the calibration dependence of a microbial sensor based on B. subtilis strain VKM B-434 for sucrose and maltose. Although the kinetics are more complex in this case, practical response ranges were determined to be 0.05-5.0 mM for maltose and 0.005-0.5 mM for sucrose. Clearly, this sensor responds more strongly to sucrose.

Figure 3

Fig. 2. Calibration dependences of sensor based on strain VKM B-434 for detection of maltose (A) and sucrose (B).



ADDITIVITY OF MICROBIAL SENSOR SIGNALS

The additivity of microbial sensor signals was assessed within the initial part of the determined response ranges. Multi-component analysis requires that the sensor signal corresponding to one substance changes upon the addition of the second substance to the sample. The simplest case is complete (linear) additivity, in which the sensor response to a sample containing two substances is equal to the sum of sensor responses to each component separately. Nonlinearity usually requires a thorough study of weighted coefficients or the introduction of approximating dependences. For example, in our previous study on the selective detection of ethanol, we used the classical model of multi-component analysis (cluster analysis) suggesting the linear additivity of sensor responses: , where S_K is the total response of sensor K, being a linear combination of individual responses $S_K(i)$ to m of the substrates present in the mixture with coefficients a_i (Lobanov et al., 2001). The range of linear additivity makes it possible to consider coefficients a_i before individual responses $S_k(i)$ to be concentration-independent, which significantly simplifies calibration.

For the current study, additivity was tested by measuring the responses to 0.025 mM sucrose, 0.25 mM maltose, and a mixture of these two disaccharides at the same final concentration. As shown in Table 2, the sensor signals were linearly additive for selected samples.

Figure 4

Table 2. Additivity of microbial sensor signals.

Bioreceptor	Sensor signals, nA/sec		
	Sucrose, 0.025 mM	Maltose, 0.25 mM	sucrose (0.025 mM) + maltose (0.25 mM)
Bacillus subtilis VKM B-434	0.025	0.025	0.048
Escherichia coli K-802	–	0.006	0.006

These findings suggest that a system based on the bacteria B. subtilis strain VKM B-434 and E. coli strain K-802 can be considered as a model for microbial sensors for the selective detection of a mixture containing the disaccharides maltose and sucrose. Such sensors could find practical application in the food processing industry, particularly in the enzymatic production of glucan oligosaccharides. Similarly, results suggest that microbial sensors based on strains of G. oxydans could be developed for the selective detection of cellobiose or melibiose. Cellobiose sensors could be useful in the biorefining of cellulosic biomass. The general screening method employed in this study could be extended to identify microbial strains able to oxidize additional substrates, including lactose and oligosaccharides.

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