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Evaluation of yeast capability to assimilate various carbohydrates by menadione-mediated amperometry

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Menadione-mediated amperometry at carbon paste electrodes containing yeast *Kluyveromyces lactis* grown on different carbon sources (glucose, galactose or maltose) was exploited to monitor intracellular redox activity induced after yeast subjection to several monosaccharides (glucose, fructose, galactose) and disaccharides (sucrose, maltose, lactose). Higher electrode responses to galactose and lactose or maltose for yeasts grown on galactose or maltose, respectively, compared to those obtained with yeasts grown on glucose, indicated the influence of a carbon source on yeast capability to utilize carbohydrates.

Key words: yeast, carbon paste, mediated amperometry, menadione, carbohydrates

INTRODUCTION

All presently known yeasts (nearly 1,500 species belonging to 149 genera [1]) are capable to assimilate one or more carbohydrates. The basic metabolic pathways of carbohydrate assimilation for various yeasts are considered similar with differences in transport systems, equipment of phosphorylation or in regulation of the pathways [2–6]. These differences result in different rates of carbohydrate utilization. Yeast capability to consume carbohydrates is usually evaluated by 1 or 2 day-lasting measurements of their residual concentrations [7, 8] or by measuring the uptake rates by zero *trans*-influx assays employing ^{14}C- or ^{3}H-labeled sugars [9–12]. Therefore, a rapid, simple and not expensive method to compare the yeast capabilities to assimilate various sugars is greatly desirable.

Electrochemistry provides the possibility of monitoring redox processes inside the yeast without cell disruption. To achieve electron transfer between the electrode and the redox centers of the enzymes in the cell, an electroactive mediator is added to the solution. Mediator shuttles the electrons between the electrode and the redox centers in the enzymes. Our previous research employing yeast-modified carbon paste electrodes showed the possibility to monitor intracellular redox activities related to sugar metabolism in yeast cells by using a single lipophilic mediator menadione (2-methyl-1,4-naphtalenedione) [13, 14]. Menadione can cross the cell membrane and enter the cytoplasm where it is reduced to menadiol (2-methyl-1,4-naphtalenediol) by the cytosolic and mitochondrial enzymes catalyzing electron transfer from NAD(P)H to quinone substrates. Menadiol diffuses outside the cell and is oxidized to menadione at the carbon paste electrode. After subjection of yeast-modified electrodes to various sugars, the increases in amperometric responses were obtained due to additional formation of NAD(P)H in the glycolysis and pentose phosphate pathway and, therefore, additional production of menadiol. The magnitudes of the mediated sugar currents were considered to reflect sugar assimilation rates. Continuous measurements of menadione-mediated glucose-, fructose- or sucrose-induced currents at electrodes with yeasts belonging to different genera revealed two distinct modes of current development during the first 2 to 3 min after yeast subjection to these sugars [14]. Initial transient currents and delay in current development were characteristic of electrodes with tested *Saccharomyces cerevisiae*. The currents at electrodes with tested non-*Saccharomyces cerevisiae* strains were gradually increasing with no delay in current development. The reason of this diversity is obscure. Another variation was detected in yeast capability to utilize galactose indicating possibly different mechanisms

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of galactose assimilation by *S. cerevisiae* or *Pichia guilliermondii* and yeast *Candida pulcherrima* or *Debaryomyces hansenii* [14].

Kluyveromyces lactis used in this research is one of sparse yeasts that are capable to utilize milk sugar lactose (consisting of glucose and galactose). Thus, one more sugar could be included into the research. This work presents the investigation of menadione-mediated current responses to three monosaccharides (glucose, fructose and galactose) and three disaccharides (sucrose, maltose and lactose) at *K. lactis*-modified electrodes. The influence of different carbon sources (glucose, galactose and maltose) in the yeast growth medium on current magnitudes was tested.

EXPERIMENTAL

Wild type (wt.) *K. lactis* was isolated from spontaneous fermentation of juniper berries that terminated within 7 days and possessed only one dominating yeast strain. The identification of yeast was performed using the automatised API 20C AUX (bioMerieux, France) system for clinical yeast identification and by classical methods such as assimilation of sugars and other substances [15]. Yeasts were grown on the YEPD medium containing 1% yeast extract, 2% peptone, 2% glucose (or galactose or maltose) and 2.5% agar as continuous lawn for 3 days at 30 °C until biomass stopped growing and thereafter were stored in a fridge for electrode preparation.

All chemicals were of analytical grade and used without further purification. Glucose and fructose were purchased from Fluka. Galactose was from Applichem. Sucrose and maltose were from Reakhim. Lactose was from AnalaR. Phosphate buffer was prepared from 0.1 M KH_2PO_4 (Fluka) and contained 0.1 M KCl (Fluka). The pH value was adjusted with KOH. Menadione (Sigma) solution was prepared in ethanol.

Plain carbon paste was prepared by mixing 100 mg of graphite powder (Merck) with 50 μL of paraffin oil (Fluka). The paste was packed into an electrode body consisting of a plastic tube (diameter 2.9 mm) and a copper wire serving as an electrode contact. The layers of the yeast cells on the surfaces of plain carbon paste electrodes were formed by dipping the electrode into the suspensions of yeasts prepared from 80 mg yeast in 1 mL of phosphate buffer at pH 6.5. This concentration of yeasts was considered as optimal since further increase of the yeast amount resulted in lower currents due to slower diffusion of a mediator through a thick layer of cells. The electrodes were allowed to dry at room temperature for 25–30 min and then covered with a dialysis membrane (Aldrich-Sigma). The yeast suspension was used only for 1 day. To repeat the experiments, a new yeast suspension for electrode modification was prepared.

All experiments were repeated at least 3 times.

Electrochemical experiments were carried out with a BAS-Epsilon Bioanalytical system (West Lafayette, USA) and a three-electrode cell arranged with a magnetic stirrer. Platinum wire and Ag/AgCl, 3 M NaCl served as counter- and reference electrodes, respectively. The yeast-modified carbon paste electrode served as a working electrode. The electrochemical measurements were performed immediately after the electrode preparation. Amperometry was carried out in a stirred solution at an operating potential 0.1 V (vs Ag/AgCl, 3 M NaCl) in phosphate buffer at pH 6.5. The electrode was poised at an operating potential until the steady state of the background current was obtained. Thereafter, menadione was added to a final concentration 67 μM. After the new steady state of the current was established (45 to 60 min), a sugar-containing solution was added. For repetitive measurements with the same electrode, the electrode was taken out from the solution, rinsed with water and again placed in the phosphate buffer. The electrode was again poised at an operating potential until the steady state of the background was obtained. Menadione and sugar solutions were again successively added.

All measurements were carried out at room temperature.

RESULTS AND DISCUSSION

Previously published amperometric investigations of glucose metabolism employed yeast *S. cerevisiae*, platinum electrodes and the double mediator system consisting of lypophilic menadione/menadiol and hydrophilic ferricyanide/ferrocyanide [16–19]. Direct electrochemical oxidation of menadiol at a Pt electrode was slow. Therefore, ferricyanide was used for signal amplification. When yeasts were immobilized on a carbon paste electrode, ferricyanide as the second mediator was not necessary due to the quasi-reversible menadiol/menadione redox process at the carbon paste electrode [13]. Menadione is known to produce reactive oxygen species inside the yeast cells that can cause oxidative cell damage [20, 21], therefore, its concentration for experiments with yeast-modified electrodes was chosen with respect to the stability of the currents. Reproducible steady states of the currents at menadione concentration 67 μM at *K. lactis*-containing carbon paste electrodes indicated that yeast cells were not damaged. Upon injection of a definite amount of mono- or disaccharide into the solution, the increases of the currents were observed (Fig. 1, shown for yeasts grown on glucose as a carbon source) due to increased menadiol concentrations caused by additional formation of NAD(P)H during the glycolysis and pentose phosphate pathway. The responses developed gradually and after some time reached their nearly constant values. The highest menadione mediated-currents were obtained for glucose and fructose (Fig. 1a) that are preferable sugars for all yeasts and sucrose (Fig. 1b) that consists of glucose and fructose. The response to lactose was more than twofold lower. Maltose- and galactose-induced currents were relatively very low indicating slow assimilation of these carbohydrates. The mean values and the standard errors of the currents at three separate electrodes (obtained during 10 min after subjection to sugars) are summarized in the Table.

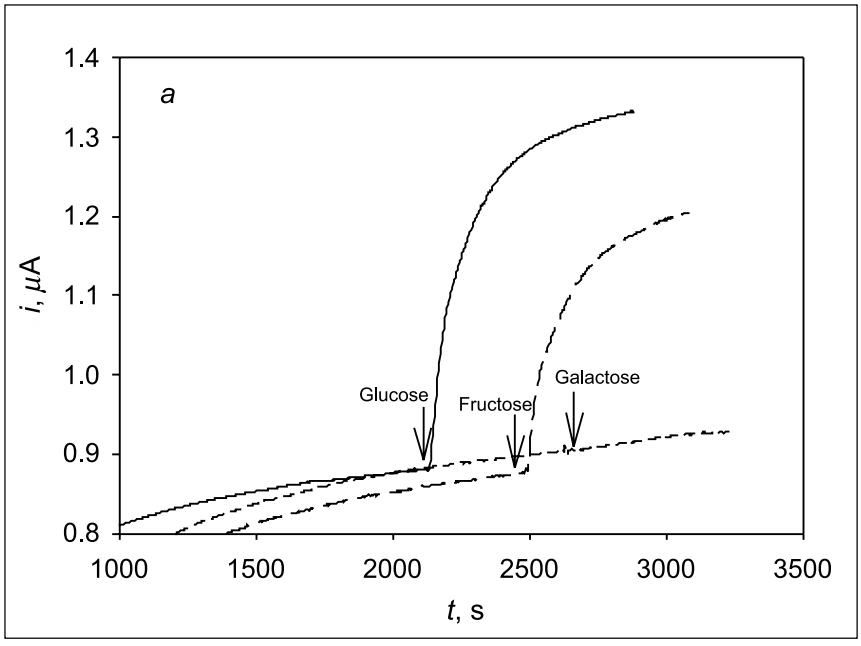

Fig. 1a. Menadione-mediated current responses of electrodes containing yeast *K. lactis* to additions of monosaccharides (as indicated) in phosphate buffer at pH 6.5, menadione concentration 67 μM, glucose, fructose and galactose concentrations 10 mM, operating potential 0.1 V

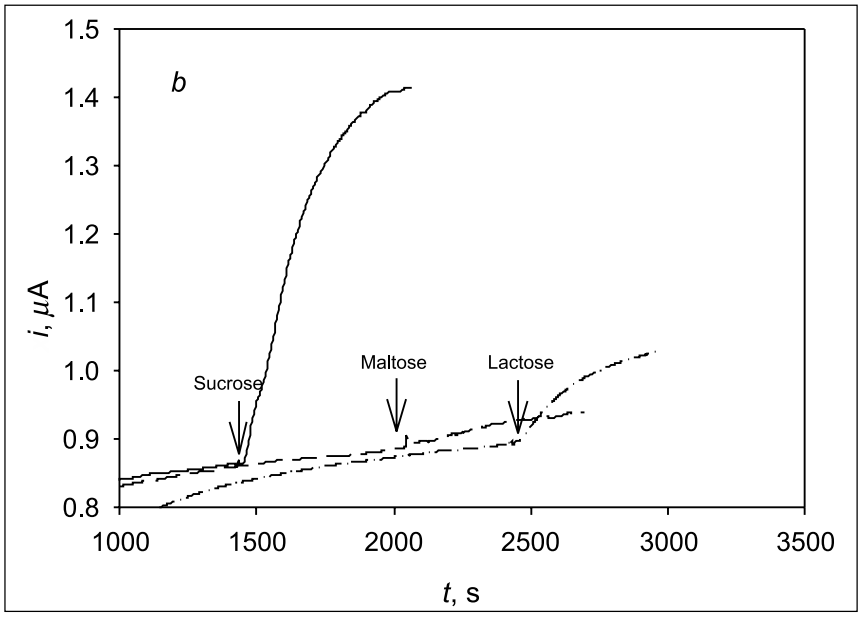

Fig. 1b. Menadione-mediated current responses of electrodes containing yeast *K. lactis* to additions of disaccharides (as indicated) in phosphate buffer at pH 6.5, menadione concentration 67 μM, sucrose, lactose and maltose concentrations 5 mM, operating potential 0.1 V

Table. **Menadione-mediated current responses to various sugars at carbon paste electrodes with immobilized *K. lactis*; menadione concentration 67 μM, glucose, fructose and galactose concentrations 10 mM, sucrose, lactose and maltose concentrations 5 mM, solution pH 6.5, operating potential 0.1V vs Ag/AgCl, 3M NaCl**

Sugar in medium of yeast growth	Current responses to					
	Glucose, nA	Fructose, nA	Galactose, nA	Sucrose, nA	Lactose, nA	Maltose, nA
Glucose	450 ± 20	390 ± 30	20 ± 5	420 ± 30	150 ± 20	50 ± 5
Galactose	430 ± 30	410 ± 30	130 ± 10		270 ± 10	40 ± 5
Maltose	460 ± 20		30 ± 5		140 ± 15	360 ± 20

Variation in the magnitudes of the currents is possibly related to some variation in cell loading caused by the dip-coating mode of cell immobilization.

Yeast cell membranes are not freely permeable for sugars [3, 5]. An obligatory step in their utilization is their transport through cell membranes either in an intact form (glucose

and fructose) or after hydrolysis to the component monosaccharides, mediated by substrate-specific transporters. Most of the investigation of sugar transport and pathways of utilization has been carried out with *S. cerevisiae*. It has been assumed that other yeasts use sugars in the same way. Hexose transport in yeast is mediated by large families of sugar transporters, sometimes called permeases [3, 5]. Hydrolysis of disaccharides may occur outside the cell membrane, in the periplasmic space, or inside the cell after the transport of disaccharide, depending on the yeast species and the nature of sugar. Sucrose is hydrolyzed to an equimolar mixture of glucose and fructose in most cases by an external invertase. Kinetic analysis of the glucose and fructose uptake in *S. cerevisiae* [10] revealed that when glucose and fructose were in the media separately, the uptake profiles indicated that both sugars were utilized at similar rates. When media contained equal concentrations of glucose and fructose, glucose was utilized at approximately twice the rate of fructose. The preferential uptake of glucose was also determined when sucrose was used as a substrate suggesting that glucose and fructose compete for the same membrane carriers in *S. cerevisiae* [10]. Some yeast strains are also able to transport sucrose via sucrose-proton symport followed by intracellular hydrolysis and metabolism [22]. Our previous research of menadione-mediated responses at yeast-modified electrodes revealed that the ratio of the current responses to glucose, fructose and sucrose were strain-dependent [14]. Similar responses to these sugars or somewhat higher glucose currents were obtained only at electrodes with commercial baker's yeast, two samples of the tested wild type *S. cerevisiae* and one sample of tested *C. pulcherrima*, whereas glucose currents at the electrodes containing the tested wild type *P. guilliermondii* and *D. hansenii* were two- to threefold higher [14]. In this research, *K. lactis*-containing electrodes gave only somewhat higher responses to glucose compared to those of fructose and sucrose (Table). Different ratios of the magnitudes of current responses to glucose, fructose and sucrose possibly reflect different sugar transport systems in yeasts belonging to different genera.

Maltose metabolism depends on two key enzymes: maltose permease that transports maltose into the yeast cell and maltase that cleaves maltose into two molecules of glucose [23, 24]. For the induction and synthesis of these enzymes, the presence of maltose in the cell environment is required [24]. In most organisms, the conversion of galactose to glucose-6-phosphate which enters glycolysis takes place by the action of five enzymes (the Leloir pathway) [25–27]. It is generally accepted that in *S. cerevisiae* the genes encoding the Leloir pathway of galactose assimilation are repressed in the presence of glucose and can be activated in the presence of galactose [27]. Therefore, low galactose and maltose currents (Table) recorded at the electrodes with *K. lactis* grown on glucose could possibly be explained by inactivity of enzymes necessary for effective assimilation of these sugars. Lactose is one of many carbon compounds that can be metabolized by *K. lactis* but not by *S. cerevisiae* [28]. The ability of this yeast to metabolize lactose results from the presence of lactose permease and β-galactosidase that hydrolyses internalized lactose into glucose and galactose [29]. Intracellular glucose can enter glycolysis while galactose follows the Leloir pathway.

To reveal whether the substitution of glucose in the yeast growth medium has influenced the yeast modified electrode responses, experiments were carried out with cells grown on galactose or maltose instead of glucose. The responses to glucose remained similar in all cases (Table). The changes were observed for responses to galactose, lactose and maltose. When yeasts were grown on galactose, the magnitudes of galactose currents were about sixfold higher (Table) indicating that the necessary enzymes were active. The responses to lactose were also higher (Table) possibly due to higher impact of galactose. Maltose-induced currents remained low. When yeasts were grown on maltose, the magnitudes of the maltose currents were about sevenfold higher (Table) also indicating that necessary enzymes were activated. Galactose- and lactose-induced currents were similar to those obtained when yeasts were grown on glucose.

CONCLUSIONS

Menadione-mediated amperometry at carbon paste electrodes modified with yeasts provides the possibility to monitor intracellular redox activity related to sugar metabolism. Different magnitudes of the currents induced by glucose, fructose, galactose, sucrose, lactose or maltose reflect potential differences in sugar transport systems and different activities of the enzymes necessary for assimilation of these sugars in yeast grown on distinct carbon sources.

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References

1. C. P. Kurtzman, J. W. Fell, T. Boekhout, *The Yeasts: a Taxonomic Study*, 5th edn., Elsevier, Amsterdam (2010).
2. S. Zaman, S. Lippman, X. Zhao, J. R. Broach, *Annu. Rev. Genet.*, **42**, 27 (2008).
3. C. L. Flores, C. Rodriguez, T. Petit, C. Gancedo, *FEMS Microbiol. Rev.*, **24**, 507 (2000).
4. M. J. Leandro, C. Fonseca, P. Goncalvez, *FEMS Yeast Res.*, **9**, 511 (2009).
5. J. Horak, *Curr. Genet.*, **59**, 1 (2013).
6. M. Conrad, J. Schothorst, H. N. Kangipati, G. van Zeebroek, M. Rubio-Texeira, J. M. Thevelein, *FEMS Microbiol. Rev.*, **38**, 254 (2014).
7. S. I. Mussatto, E. M. S. Mechado, L. M. Carneiro, J. A. Teixieira, *Appl. Energ.*, **92**, 763 (2012).
8. S. Ohara, T. Kato, Y. Fukushima, A. Sakoda, *J. Biosci. Bioeng.*, **115(5),** 540 (2013).
9. H. van Urk, E. Potsma, W. A. Scheffers, J. P. Van Dijken, *J. Gen. Microbiol.*, **135**, 2399 (1989).

10. T. D'Amore, I. Russel, G. G. Stewart, *J. Ind. Microbiol.*, **4**, 315 (1989).
11. D. M. Coons, R. B. Boulton, L. F. Bisson, *J. Bacteriol.*, **177(11)**, 3251 (1995).
12. W.-J. Lee, M.-D. Kim, Y.-Ryu, L. F. Bisson, J.-H. Seo, *Appl. Microbiol. Biotechnol.*, **60**, 186 (2002).
13. R. Garjonyte, V. Melvydas, A. Paskevicius, V. Rasomavicius, A. Malinauskas, *Cent. Eur. J. Biol.*, **9(2)**, 173 (2014).
14. R. Garjonyte, V. Melvydas, A. Malinauskas (submitted).
15. I. P. Bab'eva, V. I. Golubev, *Methods of Yeast Isolation and Identification*, Pischevaya Promyslennost, Moscow (1979) (in Russian).
16. A. Heiskanen, J. Yakovleva, C. Spegel, et al., *Electrochem. Commun.*, **6**, 219 (2004).
17. C. F. Spegel, A. R. Heiskanen, N. V. Kostesha, et al., *Anal. Chem.*, **79(23)**, 8919 (2007).
18. A. Heiskanen, C. Spegel, N. Kostesha, S. Lindahl, T. Ruzgas, J. Emneus, *Anal. Biochem.*, **384(1)**, 11 (2009).
19. N. V. Kostesha, J. R. M. Almeida, A. R. Heiskanen, M. F. Gorwa-Grauslund, B. Hahn-Hagerdal, J. Emneus, *Anal. Chem.*, **81(24)**, 9896 (2009).
20. F. A. V. Castro, R. S. Herdero, A. D. Panek, E. C. A. Eleutherio, M. D. Pereira, *Biochim. Biophys. Acta*, **1770(2)**, 213 (2008).
21. I. S. Kim, H. Y. Sohn, I. Jin, *J. Microbiol.*, **49**, 816 (2011).
22. T. O. Basso, S. Kok, M. Dario, et al., *J. Metabolic Eng.*, **13**, 694 (2011).
23. T. Jiang, D. Xiao, Q. Gao, *Ann. Microbiol.*, **58**, 655 (2008).
24. S. Novak, V. Zechner-Krpan, V. Maric, *Food Technol. Biotechnol.*, **42(3)**, 213 (2004).
25. K. Lai, M. I. Klapa, *Metabolic Eng.*, **6**, 239 (2004).
26. K. Lai, L. J. Elsas, K. J. Wierenga, *IUBMB Life*, **61(11)**, 1063 (2009).
27. C. A. Sellick, R. N. Campbell, R. J. Reece, *Int. Rev. Cell Mol. Biol.*, **269**, 111 (2008).
28. K. D. Breunig, M. Bolotin-Fukuhara, M. M. Bianchi, et al., *Enz. Microb. Technol.*, **26**, 771 (2000).
29. P. M. R. Guimarães, J. A. Teixeira, L. Domingues, *Biotechnol. Adv.*, **28**, 375 (2010)

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AMPEROMETRINIS MIELIŲ GEBĖJIMO ĮSISAVINTI ĮVAIRIUS ANGLIAVANDENIUS VERTINIMAS

S a n t r a u k a

Mielių *Kluyveromyces lactis* gebėjimas įsisavinti įvairius angliavandenius (gliukozę, fruktozę, galaktozę, sacharozę, laktozę ir maltozę) buvo tirtas amperometriškai naudojant mielių sluoksniu modifikuotus anglies pastos elektrodus ir mediatorių menadioną. Registruotų srovių dydžiai priklausė nuo to, kokiu angliavandeniu buvo veikiamos mielės ir kuris cukrus (gliukozė, galaktozė ar maltozė) buvo auginimo terpėje. Kelis kartus padidėjusios galaktozės ar maltozės srovės, kai auginimo terpėje vietoj gliukozės buvo galaktozė ar maltozė, yra susijusios su fermentų, reikalingų pastariesiems cukrams įsisavinti, aktyvavimu mielių augimo metu.