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# Mediated amperometry reveals two distinct modes of yeast responses to glucose

**Research Article**

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**Abstract:** Mediated amperometry was exploited to monitor intracellular redox activity without cell disruption. Continuous measurements of menadione-mediated glucose currents at carbon paste electrodes with various immobilized intact wild type yeasts (*Saccharomyces cerevisiae, Candida pulcherrima*, *Clavispora lusitaniae*, *Wickerhamomyces anomalus*, *Pichia guilliermondii*, *Kluyveromyces lactis* var. *lactis*, *Debaryomyces hansenii, Candida zeymolaydes* and *Candida tropicalis*) revealed two distinct and previously unreported modes of development of the currents during the first 2 to 3 min. after subjection to glucose. A correlation among the values of the currents and the capacities of wild type yeasts to secrete various substances was observed.

**Keywords:** *Yeasts • Menadione • Mediated amperometry • Carbon paste • Glucose*



## 1. Introduction

Yeasts are extensively employed in the baking of bread, the making of fermented beverages, the production of various pharmaceuticals and proteins, and the protection of food and forage against spoilage. There are 149 genera and nearly 1,500 yeast species already described in the literature [1]. The yeasts show great diversity both in nutrient assimilation and in product secretion. Microbiologists face the difficult task of selecting the most competitive strains for scientific research and biotechnological application. A rapid, simple and reliable method to compare the yeasts is greatly desirable.

Electrochemistry provides the possibility of monitoring redox processes related to the metabolic activity of living cells [2-12]. Direct electron transfer between the electrode and the redox centers of the enzymes in the cells is, as a rule, not achievable. An electroactive compound, usually called a mediator, added to the solution acts as an electron shuttle between the electrode and the redox centers in the biological compound.

The yeast *Saccharomyces cerevisiae* is the most commonly used in scientific research as a model organism to reveal the mechanisms of various physiological processes within eukaryotic cells and to understand their interactions with their surrounding environment. Glucose is a preferential nutrient and a substance that affects yeast physiology at many levels. The mechanisms involved in the response of yeast cells to glucose are very complex (as reviewed in [13-16]) with many unanswered questions.

Electrochemical investigations of glucose metabolism in *Saccharomyces cerevisiae* employed the double mediator system consisting of lypophilic menadione/menadiol (2-methyl-1,4-naphtalenedione/2-methyl-1,4-naphtalenediol) and hydrophilic ferricyanide/ferrocyanide serving for signal amplification [2-12]. Menadione can cross the cell membrane and enter the cytoplasm where it is reduced to menadiol by the cytosolic and mitochondrial enzymes catalyzing

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electron transfer from NAD(P)H to quinone substrates. The reduced menadiol can diffuse outside the cell and reduce ferricyanide to ferrocyanide. Ferrocyanide is then oxidized to ferricyanide at the electrode and an anodic current is recorded. In the presence of glucose, an increase in amperometric responses is observed due to additional formation of NAD(P)H in glycolysis and pentose phosphate pathways. The magnitudes of the mediated glucose currents were considered to reflect glucose assimilation rates.

The aim of this research was to test the feasibility of amperometry for fast and simple yeast screening for glucose assimilation by using carbon paste electrodes and only a single mediator—menadione.

## 2. Experimental Procedures

### 2.1 Yeasts

All yeast strains are listed in Table 1. Baker's yeast *Saccharomyces cerevisiae* (SEMA, Panevezys, Lithuania) was obtained from a local market (shelf life not less than 2 weeks as specified by the producer). *Saccharomyces cerevisiae* α'1 MATα leu2-2 (haploid strain), *Saccharomyces cerevisiae* M437 wt. (wild type) HM/HM (kill-K2), *Saccharomyces cerevisiae* K28 wt. HM/HM (kill-28) and *Saccharomyces cerevisiae* Rom K-100 HM/HM wt. (kill-K2) were obtained from the collection of the Institute of Botany (Nature Research Center, Vilnius, Lithuania). *Candida pulcherrima* (*Metschnikowia pulcherrima*) was obtained from the CBS-KNAN Fungal Biodiversity Center, an Institute of the Royal Netherlands Academy of Arts and Sciences, Utrecht, the Netherlands.

Wild type strains *Saccharomyces cerevisiae*, *Wickerhamomyces anomalus*, *Pichia guilliermondii*, *Kluyveromyces lactis* var. *lactis*, *Debaryomyces hansenii*, *Candida tropicalis* and *Candida zeylanoides* were isolated from spontaneous fermentations of various fruits and berries collected in different localities of Lithuania. The yeasts were isolated from those fermentations that terminated within 7 days and possessed only one dominating yeast strain. The isolated strains varied in rates of glucose assimilation and ethanol production, biocide and biostatic properties and in abilities to secrete killer toxins, pigments and other yet unidentified substances [17-19]. The identification of yeasts was performed using the automatised API 20C AUX (bioMérieux, France) system for clinical yeast identification and by classical methods such as assimilation of sugars and other substances [20]. The identification of *Saccharomyces cerevisiae* SRV1 ($K^-$) wt., Kx wt. and N1M Kn wt. was performed at the Institute of Biotechnology (Vilnius, Lithuania). The latter two strains were additionally identified by polymerase chain reaction according to protocols described in [21]. *Candida* species were additionally identified using VITEK 2 (bioMérieux, France). The yeasts were grown on the YEPD medium containing 1% yeast extract, 2% peptone, 2% glucose and 2.5% agar as continuous lawn for 3 days at 30°C until biomass stopped growing. The nutrients were exhausted during this time and the cells began to starve. The cells were then tested microscopically to be sure that non-budding cells comprised 90-95%. Yeasts were stored in the fridge and used for electrode preparation within 7 days. Several yeast strains were tested again after 12 to 15 weeks.

Levels of ethanol were determined by gas chromatography at the National Veterinary laboratory according to LST EN ISO/IEC requirements.

### 2.2 Chemicals

All chemicals were of analytical grade and used without further purification. D-glucose was purchased from Merck. Phosphate buffer was prepared from 0.1 mol $L^{-1}$ $KH_2PO_4$ (Fluka) and contained 0.1 mol $L^{-1}$ KCl (Fluka). The pH value was adjusted with KOH. Menadione (Sigma) solution was prepared in ethanol.

### 2.3 Electrode preparation

Plain carbon paste was prepared by mixing 100 mg of graphite powder (Merck) with 50 µL of paraffin oil (Fluka). The paste was placed into the cavity of a homemade electrode consisting of a plastic tube (diameter 2.9 mm) and a copper wire serving as an electrode contact. The surface was then smoothened on a weighing paper. A layer of the yeast cells was formed by dipping the electrode into the suspension prepared from 40 mg yeast in 0.5 mL of phosphate buffer at pH 6.5. This concentration of yeasts was considered optimal since further increase resulted in lower currents due to slower diffusion of mediators through a thicker layer of cells. The electrode was allowed to dry at ambient temperature for 25-30 min and then covered with a dialysis membrane (Aldrich-Sigma). Yeast suspensions were prepared daily. All experiments were repeated at least 3 times.

### 2.4 Electrochemical measurements

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 N NaCl served as counter- and reference electrodes, respectively. A modified carbon paste electrode served as a working electrode. The electrochemical measurements were performed either immediately after the electrode

| Yeast sample | Current responses to | |
|---|---|---|
| | menadione, nA | glucose, nA |
| 1. Baker's yeast *Saccharomyces cerevisiae* | 980 ± 40 | 340 ± 20 |
| 2. *Saccharomyces cerevisiae* α' 1 MATα leu2-2 | 500 ± 30 | 550 ± 50 |
| 3. *Saccharomyces cerevisiae* * Rom K-100 wt. HM/HM [kill-K2] | 950 ± 40 | 80 ± 10 |
| 4. *Saccharomyces cerevisiae* * SRV1 wt. ($K^-$) | 460 ± 50 | 30 ± 5 |
| 5. *Saccharomyces cerevisiae* * Kx wt. | 640 ± 30 | 30 ± 5 |
| 6. *Saccharomyces cerevisiae* * M437 wt. HM/HM (kill-K2) | 920 ± 50 | 70 ± 5 |
| 7. *Saccharomyces cerevisiae* * N1M Kn wt. | 1,530 ± 110 | 200 ± 50 |
| 8. *Saccharomyces cerevisiae* * K28 wt. HM/HM (kill-K28) | 760 ± 60 | 170 ± 10 |
| 9. *Wickerhamomyces anomalus* * WA 6M wt. ($K^-$) | 1,060 ± 110 | 40 ± 5 |
| 10. *Wickerhamomyces anomalus* * W008 wt. ($K^-$) | 480 ± 50 | 20 ± 5 |
| 11. *Candida pulcherrima* (*Metschnikowia pulcherrima*) biocid. (p) | 1,470 ± 140 | 270 ± 20 |
| 12. *Candida tropicalis* CKB5 wt. ($K^-$) | 900 ± 30 | 20 ± 5 |
| 13. *Candida zeylanoides* CAMIK wt. ($K^+$) | 1,360 ± 100 | 240 ± 10 |
| 14. *Kluyveromyces lactis* var. *lactis* KK2K wt. ($K^+$) | 1,370 ± 60 | 240 ± 15 |
| 15. *Kluyveromyces lactis* var. *lactis* KK1K ($K^+$) | 1,100 ± 40 | 280 ± 20 |
| 16. *Debaryomyces hansenii* DRV3 wt. biocid. (p) | 1,370 ± 350 | 830 ± 60 |
| 17. *Debaryomyces hansenii* D212 wt. biocid. (p) | 1,230 ± 90 | 1,170 ±110 |
| 18. *Debaryomyces hansenii* DKR 20 wt. ($K^+$) (p) | 770 ± 60 | 120 ± 10 |
| 19. *Clavispora lusitaniae* CAL17 wt. biocid. (p) | 1,190 ± 160 | 300 ± 25 |
| 20. *Clavispora lusitaniae* C111 wt. biocid. (p) | 1,160 ± 110 | 570 ± 40 |
| 21. *Pichia guillermondii* P041 wt. ($K^-$) | 430 ± 30 | 60 ± 5 |
| 22. *Pichia guillermondii* PRV2 wt. biostat. (p) | 1,460 ± 70 | 430 ± 30 |
| 23. *Pichia guillermondii* P071 wt. biostat. (p) | 1,450 ± 60 | 380 ± 30 |
| 24. *Pichia guilliermondii* P179 wt. ($K^+$) (p) | 1,690 ± 250 | 240 ± 30 |
| 25. *Pichia guilliermondii* P150 wt. ($K^+$) (p) | 1,450 ± 150 | 280 ± 20 |
| 26. *Pichia guilliermondii* P15B wt. biostat. (p) | 1,770 ± 80 | 320 ± 30 |

**Table 1.** Current responses to 67 μmol $L^{-1}$ menadione and menadione-mediated 10 mmol $L^{-1}$ glucose at carbon paste electrodes with immobilized yeast cells; solution pH 6.5, operating potential 0.1 V *vs.* Ag/AgCl, 3N NaCl.

** - Yeast strains with transient menadione-mediated glucose response. $K^-$ - non-killer strains; $K^+$ - killer strains secreting killer toxins of yet not identified type, kill-K2 and kill- K28 – strains secreting, respectively, Saccharomyces cerevisiae K2 and K28 type killer toxins, Kx – strain secreting a new type of killer toxin and a novel X factor [18], Kn – strain secreting a new type of killer toxin of yet not identified type (not published), biocid. – strains secreting yet not identified toxic substances with killer effect, biostat. – strains secreting yet not identified toxic substances that suppress yeast growth,*
*p – strains producing red pigments.*

preparation or after keeping the prepared electrodes in 10 mmol $L^{-1}$ glucose for 30 min. Amperometry was carried out in a stirred solution at an operating potential of 0.1 V (*vs*. Ag/AgCl, 3 N NaCl) in phosphate buffer at pH 6.5. The electrode was poised at operating potential until the steady state of the background current was obtained. Thereafter, menadione- and glucose-containing solutions were successively added. For repetitive measurements with the same electrode, the electrode was taken out from the solution, washed and again placed into the phosphate buffer at pH 6.5. The electrode was again poised at operating potential until the steady state of the background current was obtained. Menadione and glucose were again successively added.

All measurements were carried out at room temperature.

## 3. Results

Cyclic voltammetry is the most widely used technique for acquiring information about the properties of redox-active substances in the solution [22]. It consists of linearly scanning he potential of a stationary working electrode using a triangular potential waveform. Anodic and cathodic currents are registered duringa positive-going forward potential scan and a negative-going reverse potential scan, respectively (Figure 1). The resulting plot of current vs. potential (a cyclic voltammogram) gives various parameters, *i.e*., the peak current values ($i_{pa}$ and $i_{pc}$, anodic and cathodic, respectively), the peak potential values ($E_{pa}$ and $E_{pc}$, anodic and cathodic, respectively), the value of the formal potential $E^{o\prime}$ ($E^{o\prime}=(E_{pa}+E_{pc})/2$), the value of the peak potential separation $\Delta E_p$ ($E_p=E_{pa}-E_{pc}$), and the ratio of $i_{pa}$ to $i_{pc}$ ($i_{pa}/i_{pc}$) [22]. The cyclic voltammogram in the solution containing menadione (Figure 1) shows both anodic and cathodic peaks at -0.09 V and –0.31 V respectively, indicating that both oxidation and reduction of the menadiol/menadione redox pair is possible at a carbon paste electrode. The value of the $E^{o\prime}$ is -0.2 V. The peak potential separation $\Delta E_p$=0.22 V and $i_{pa}/i_{pc}<1$ values specify a quasi-reversible redox process [22].

An operating potential for the measurements of menadione and menadione-mediated glucose currents of 0.1 V was chosen for our research, *i. e*., 0.3 V more positive than the $E^{o\prime}$ value. The current recorded at the yeast-containing carbon paste electrodes after sequential addition of menadione and glucose is shown in Figures 2a and 2b.

The electrode responses to both menadione and glucose started immediately after the injections. Two distinct modes in the development of menadione-mediated glucose currents were observed. The currents at electrodes containing non-*Saccharomyces cerevisiae* yeasts (except *Wickerhamomyces anomalus*), commercial baker's yeast and *Saccharomyces cerevisiae* α'1 MATα leu2-2 (haploid strain) increased gradually and, after some time, approached their steady states (Figure 2a, electrodes containing *Candida pulcherrima*, *Pichia guilliermondii* PRV2 wt. and *Debaryomyces hansenii* DKR 20 wt., solid, long- and short-dashed lines, respectively) whereas quite a different transient mode of current development was found at electrodes with the investigated wild type yeasts *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus*. The currents initially increased for 30 to 40 s after glucose addition; some decrease during 60 to 80 s was then observed, followed again by relatively slow (Figure 2b, solid and short-dashed lines, electrodes containing, respectively, *Saccharomyces cerevisiae* Rom K-100 HM/HM wt. and *Wickerhamomyces*

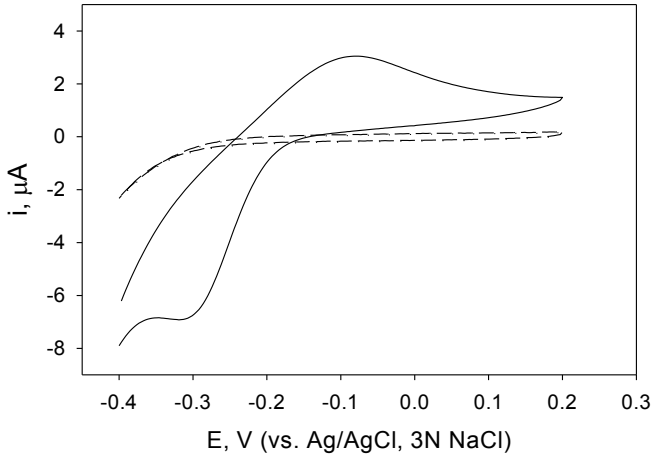


**Figure 1.** Cyclic voltammogram of carbon paste electrode in phosphate buffer pH 6.5 (dashed line) and in phosphate buffer pH 6.5 containing 0.2 mmol $L^{-1}$ menadione (solid line), potential scan rate 25 mV $s^{-1}$

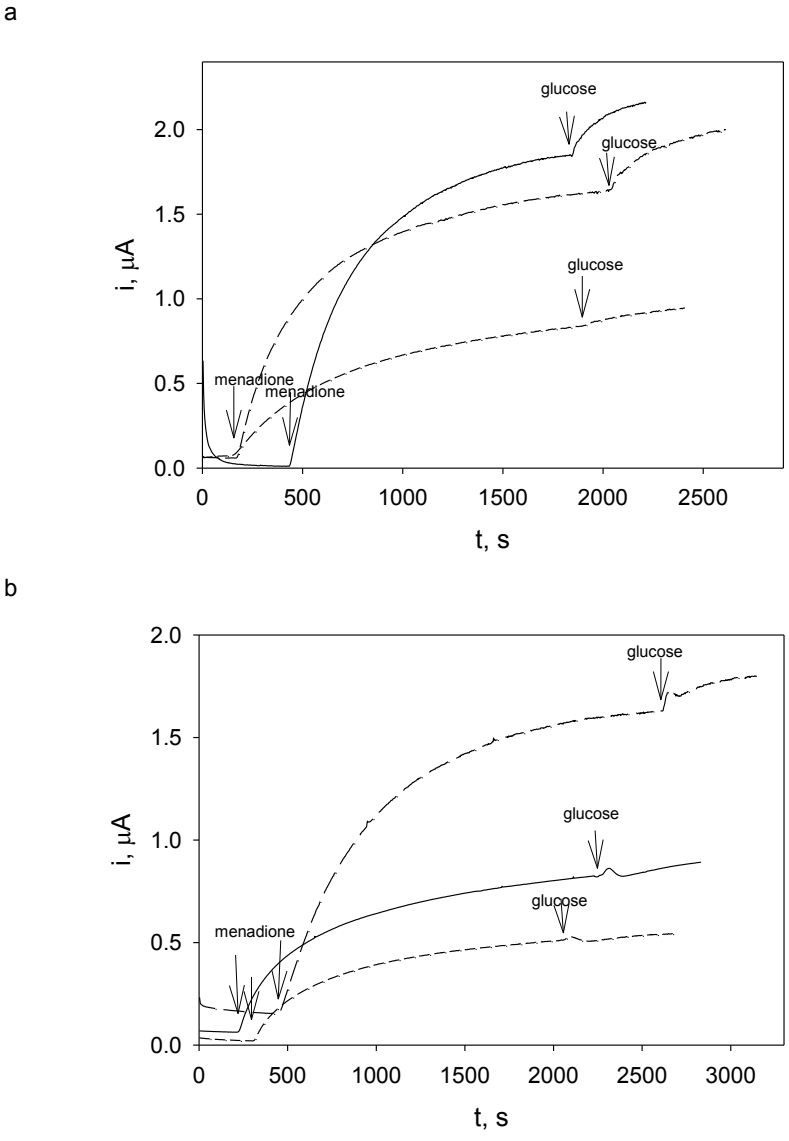


**Figure 2.** a- Current responses of electrodes containing yeast *Candida pulcherrima* (solid line), *Debaryomyces hansenii* DKR 20wt. (short-dashed line) and *Pichia guilliermondii* PRV2 biostat. (long-dashed line), to additions of 67 $\mu$mol L$^{-1}$ menadione and 10 mmol L$^{-1}$ glucose in phosphate buffer at pH 6.5, operating potential 0.1 V. b- Current responses of electrodes containing yeast *Saccharomyces cerevisiae* Rom K-100 (solid line), *Wickerhamomyces anomalus* W008 wt. (short-dashed line) and *Saccharomyces cerevisiae* N1M wt. (long-dashed line) and to additions of 67 $\mu$mol L$^{-1}$ menadione and 10 mmol L$^{-1}$ glucose in phosphate buffer at pH 6.5, operating potential 0.1 V.

*anomalus* W008 wt.) or fast (Figure 2b, long-dashed line, electrode with *Saccharomyces cerevisiae* N1M wt.) increase in currents. The results (the mean values of the responses of 3 electrodes prepared from the same yeast suspension with standard errors) obtained for electrodes prepared with all investigated yeasts are summarized in Table 1.

Two well-characterized strains, *Saccharomyces cerevisiae* Rom K-100 HM/HM wt. and *Candida pulcherrima* (*Metschnikowia pulcherrima)*, were taken for comparison. The spike recorded in glucose currents at electrodes containing *Saccharomyces cerevisiae* Rom K-100 HM/HM wt. (Figure 2b, solid line) was also characteristic of all other wild type *Saccharomyces cerevisiae* and two *Wickerhamomyces anomalus*. The development of menadione-mediated glucose currents at electrodes containing non-*Saccharomyces cerevisiae* (except *Wickerhamomyces anomalus)* strains was gradual as in the case of electrodes containing *Candida pulcherrima* (*Metschnikowia pulcherrima)* (Figure 2a, solid line). The highest menadione-mediated glucose responses were obtained at electrodes containing

*Debaryomyces hansenii* DRV3 wt., *Debaryomyces hansenii*, D212 wt. and *Clavispora lusitaniae* C111 wt. indicating the highest rates of NAD(P)H formation in glycolysis and pentose phosphate pathways, *i.e.*, the highest glucose assimilation rates.

When the electrodes were kept in 10 mmol $L^{-1}$ glucose for 30 min. before menadione and glucose addition (starving cells were fed before amperometric measurement) a gradual increase of currents was observed in all cases. The spikes in the menadione-mediated glucose responses at electrodes containing wild type *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* disappeared. The loss of spikes was also observed with repetitive menadione and glucose injections using the same electrode, *i.e.*, with prolonged subjection of cells to glucose. The magnitudes of glucose currents after the fourth injection were higher: 200 to 450% compared to the first one. Glucose-induced currents at electrodes with investigated *Candida pulcherrima, Clavispora lusitaniae, Pichia guilliermondii*, *Kluyveromyces lactis var. lactis*, *Debaryomyces hansenii*, *Candida tropicalis* and *Candida zeylanoides strains*, commercial baker's yeast and haploid strain *Saccharomyces cerevisiae* α'1 MATα leu2-2 increased only by 5 to 8% with each repetitive addition.

Electrodes were repetitively prepared after keeping the yeasts in the fridge (4°C) for 12 to 14 weeks. The results obtained with all repetitively examined strains (*Saccharomyces cerevisiae* α'1 MATα leu2-2, *Saccharomyces cerevisiae* Rom K-100 HM/HM wt, *Wickerhamomyces anomalus* W008 wt., *Candida pulcherrima* (*Metschnikowia pulcherrima*), *Kluyveromyces lactis* var. *lactis* KK2K wt., *Pichia guilliermondii* P179 wt. and *Clavispora lusitaniae* C111 wt.) were very similar to those obtained previously suggesting that this period of time of yeast storage in the fridge did not alter the mode and the values of menadione-mediated glucose currents.

## 4. Discussion

The majority of past electrochemical investigations of glucose metabolism in yeast employed *Saccharomyces cerevisiae* cells, the double mediator system consisting of lypophilic menadione/menadiol and hydrophilic ferricyanide/ferrocyanide and Pt electrodes [2-12]. Direct electrochemical oxidation of menadiol at a Pt electrode is a competing process, however, occurring at a lower rate than oxidation *via* ferricyanide and is usually not taken into consideration. Direct oxidation of menadiol at carbon electrodes is faster compared to that at Pt electrode [8]. Cyclic voltammetry showed both oxidation and reduction of menadiol/menadione redox pairs at carbon paste electrodes; therefore, ferricyanide was not necessary as a second mediator for signal amplification. Some of the investigated non-*Saccharomyces cerevisiae* yeasts also responded to glucose in the presence of only ferricyanide. The origin of these currents is not clear. By using only a single menadione instead of two mediators, additional ferricyanide-mediated glucose currents were eliminated.

Menadione is known to produce reactive oxygen species inside the yeast cells that can cause oxidative cell damage [23,24]. To avoid the detrimental effect and to obtain a stable electrochemical response at electrodes modified with *Saccharomyces cerevisiae,* menadione concentration should not exceed 100 µmol $L^{-1}$ [8]. The menadione concentration of 67 µmol $L^{-1}$ used in this research permitted stable and relatively high menadione currents for all investigated *Saccharomyces cerevisiae* and non-*Saccharomyces cerevisiae* strains. The variation in the magnitudes of responses to menadione possibly reflected the difference in activities of the cytosolic and mitochondrial enzymes catalyzing electron transfer from NAD(P)H to menadione and/or different permeability of yeast cell membranes. After the addition of glucose, continuous measurement of currents showed two distinct modes of electrode responses gradually increasing glucose currents at electrodes containing non-*Saccharomyces cerevisiae* yeasts (except *Wickerhamomyces anomalus*), commercial baker's yeast and *Saccharomyces cerevisiae* α'1 MATα leu2-2 (haploid strain) and transient glucose currents at electrodes with *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* yeasts. These two modes could not be observed by typical periodical determination of glucose concentrations employed by microbiologists to evaluate glucose assimilation rates. The difference in the development of menadione-mediated glucose currents at electrodes containing yeast strains of different genera suggests the possibility of different mechanisms of glucose assimilation by starving cells at the initial stage. As could be seen from amperometric measurements, the currents initially increased for 30 to 40 s after glucose addition. Decrease during 60 to 80 s was then observed, suggesting some delay in glucose assimilation. The time-scale of the development of transient menadione-mediated glucose currents (2 to 3 min. after the first glucose addition) observed at electrodes containing wild type *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* strains was very similar to that (also up to 3 min) of transient increases of protein kinase acitivities and intracellular

concentrations of cyclic adenosine monophosphate [25-27] or glycolytic and tricarboxylic acid cycle intermediates (such as glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-biphosphate, 6-phosphogluconate and some other) [28] determined for the wild type yeast *Saccharomyces cerevisiae* after glucose addition. To the best of our knowledge analogous detailed investigation of concentrations of these substances for non-*Saccharomyces cerevisiae* yeast strains is not presented in the literature; therefore, comparison with other electrochemical data is not possible.

An increase in amperometric responses after addition of glucose is ascribed to additional formation of NAD(P)H in glycolysis and pentose phosphate pathways. The investigation of the contribution of the cofactors NADH and NADPH to the measured currents and which of them is responsible for the transient current mode was outside the scope of this work. An attempt to determine how the cofactors are involved in menadione-mediated glucose currents has already been made for *Saccharomyces cerevisiae* by inhibiting glycolytic or pentose phosphate pathways with, respectively, iodoacetate or epiandrosterone [9] or by employing deliberately constructed mutant strains [8]. The results were contradictory. Inhibition-based research established that more than 90% of the current originated from NADH produced during glycolysis, whereas experiments with mutant strains showed higher contribution from NADPH.

The loss of the transient mode of menadione-mediated glucose responses and the increase of current magnitudes at electrodes with yeasts pre-incubated with glucose showed that something has changed in the state of the yeasts. The increase of glucose currents was more pronounced for wild type *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* compared to that obtained for other investigated non-*Saccharomyces cerevisiae* strains. This suggests some delay in "switching on" glucose assimilation by *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus*. The "spiked mode" of glucose currents was not detected during the former investigation of yeast redox activities [2-12] since the experimental conditions were different. Non-wild type *Saccharomyces cerevisiae* strains were pre-incubated with glucose before measurements; therefore, the "spiked mode" of responses to glucose was not observed.As determined previously [17-19], the investigated wild type yeast strains varied in rates of glucose assimilation and ethanol production, biocide and biostatic properties, and in abilities to secrete killer toxins, pigments and other yet not identified substances. These properties were compared with electrochemical results. The well-characterized strain *Candida pulcherrima* (*Metschnikowia pulcherrima)* taken for comparison is a strain producing the red pigment pulcherrimin [29].

The highest menadione-mediated glucose responses were obtained at electrodes containing *Debaryomyces hansenii* DRV3wt., *Debaryomyces hansenii*, D212 wt. and *Clavispora lusitaniae* C111 wt. These strains secreted a red pigment (probably also pulcherrimin; the composition is now under examination) and produced as-yet unidentified substances with killer effect. Certain yeasts have the ability to secrete low molecular weight glycoproteins (killer toxins) [30-33], which are lethal to receptive cells. Therefore, yeasts with killer effect can prevent the proliferation of wild sensitive yeasts and could be useful for developing microbiological protection of food against spoilage microorganisms. The strains that did not possess killer activity and did not produce pigments (*Saccharomyces cerevisiae* SRV1, both *Wickerhamomyces anomalus*, *Candida tropicalis* CKB5 and *Pichia guilllermondii* P041) were among the strains with low glucose currents.

Among investigated *Saccharomyces cerevisiae* strains, the highest current responses to glucose were obtained for electrodes modified with *Saccharomyces cerevisiae* N1M wt. This strain (already commercialized) also secreted a new killer toxin of as-yet unidentified type and exceeded other strains in the rates of glucose assimilation and ethanol production (up to 18%). Low glucose currents for electrodes with *Saccharomyces cerevisiae* Kx strain correlated with a relatively low level of ethanol production (up to 11%) by this strain (unpublished results). Preliminary investigation showed that other observed *Saccharomyces cerevisiae* and non-*Saccharomyces cerevisiae* strains produced up to 12% ethanol (unpublished results). Overall, certain correlation between a yeast's ability to secrete various substances and menadione-mediated glucose current was noticed. The higher the yeast "vital activity" was observed, the higher the currents determined. Therefore, amperometric measurements could help to discriminate between "intense" and "non-intense" activity of yeasts and facilitate the selection of "faster working" strains for further research.

## 5. Conclusions

Electrochemical measurements employing carbon paste electrodes and only one mediator, menadione, can be exploited to monitor intracellular redox activity without cell disruption. Continuous measurements of glucose-induced currents *in vivo* at electrodes with

various immobilized intact wild type yeasts allowed the observation of two distinct modes in the development of the currents during the first 2 to 3 min after subjection to glucose. This suggests that the mechanism of cell responses to glucose at the initial stage could potentially be different for yeasts belonging to different genera. The increase of currents after repetitive subjection of yeasts to glucose (or the effect of time that yeasts have been subjected to the nutrient) was obviously higher for *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* compared to that for non-*Saccharomyces cerevisiae* strains, suggesting some delay in "switching on" glucose assimilation.

Although the origin of the difference in the development of glucose currents is obscure, menadione-mediated glucose currents could nevertheless be useful for fast screening of a yeast ability to assimilate glucose for comparison and control of the isolated wild type yeasts.

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