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SHORT COMMUNICATIONS

Yarrowia lipolytica, a Producer of L-Lactate Oxidase

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L-lactate oxidase (EC 1.1.3.X) is a flavin enzyme catalyzing lactate oxidation to pyruvate with reduction of oxygen to hydrogen peroxide. The enzyme is extensively used in medicine for detection of the lactate content in human blood under various pathological states accompanied by inadequate oxygen supply to tissues (acute hemorrhages, severe cardiac failure, heart diseases with cyanosis, acute hypoxia, vascular collapse, etc.). Lactate oxidase is also used as a base for biosensors applied in medicine, wine making, and food industry [1]. Among microorganisms, the enzyme has been found in the bacteria *Aerococcus viridans* [2], *Streptococcus faecalis* [3], *Pediococcus* sp. [4, 5], *Lactococcus lactis* [6], and in the fungus *Geotrichum candidum* [1, 7]. The literature provides no data on lactate oxidase synthesis in yeasts. Previously [8, 9], we studied different aspects of adaptation of the yeast *Yarrowia lipolytica* to stress impacts (oxidants, high temperatures, etc.). It was shown that in *Y. lipolytica* stress conditions induce a decrease of the level of intracellular cAMP, increase of the activities of antioxidant enzymes (catalase, superoxide dismutase, glucose-6-phsophate dehydrogenase, and glutathione reductase), and appearance of an alternative, cyanide-resistant oxidase [8–10]. The goal of this study was to demonstrate the ability of the yeast *Y. lipolytica* to synthesize L-lactate oxidase.

The yeast *Yarrowia lipolytica* VKM Y-2378 (syn. *Yarrowia lipolytica* Y-155) studied in the work was obtained from the All-Russian Collection of Microorganisms, Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences. Cultivation was carried out at 29°C in 750-ml flasks containing 100 ml of Rider medium [8] with glucose (1%) or L-lactate (1%) on a shaker (200 rpm). Yeast growth was assayed by the optical density of the cell suspension (λ = 540 nm).

The cells from the exponential (8 h) or stationary (14 h) growth phase, grown on the medium with glucose, were twice washed with sterile distilled water and resuspended in 50 mM Tris–phosphate buffer, pH 7.2 ("untreated" cells). For adaptation, the cells were exposed to soft stresses, i.e., incubated in the presence of 0.5 mM H_2O_2 (60 min) or at 37°C (60 min) [8, 9].

Distribution of the enzyme activity in the cell fractions was studied as follows: the cells grown on the medium with lactate were twice washed with distilled water and suspended in 50 mM Tris–phosphate buffer (pH 7.0) containing 0.5 mM phenylmethylsulfonyl fluoride (protease inhibitor) [8]. Cell suspension (1 mg of dry cells/ml) was disintegrated in a French press, the homogenate was centrifuged at 5000 *g* for 10 min, the precipitate was discarded, and the supernatant was centrifuged at 105000 *g* for 60 min.

Lactate oxidase activity of soluble and membrane fractions, homogenate, and whole cells was determined by the rate of oxygen uptake on a polarograph using a Clark-type platinum electrode covered with Teflon film [10] in the presence of 5 mM L-lactate and was expressed in nmol O_2 min^{-1} mg^{-1} dry cells or nmol O_2 min^{-1} mg^{-1} protein.

The enzyme activity in the soluble fraction was also registered on a Shimadzu spectrophotometer by the rate of hydrogen peroxide formation in 20 mM Tris–phosphate buffer (pH 8.0) in the presence of *o*-dianisidine (0.2 mM), peroxidase (5 μg/ml), and L-lactate (2 mM) (U_{436} = 8.3 mM^{-1} cm^{-1}) [7]. The amount of the enzyme catalyzing oxidation of 1 μmol of L-lactate per 1 min was taken as a unit of activity (U).

Catalase activity was assayed by the change in H_2O_2 absorption, U_{240} = 0.32 mM^{-1} cm^{-1} [8].

The capacity for L-lactate oxidation appeared in the cells of *Y. lipolytica* from the exponential growth phase after adaptation to different stresses (cell incubation in the presence of low doses of an oxidant or at 37°C) (Fig. 1, curves *2* and *3*). Unadapted ("untreated") cells did not oxidize L-lactate (curve *1*). Transition of the yeast to the stationary growth phase caused by glucose depletion also resulted in the ability of the cells to oxidize this substrate (curve *4*). Thus, stresses induce not only an increase of activities of antioxidant enzymes (catalase, glucose-6-phosphate dehydrogenase, superoxide dismutase, and glutathione reductase), as has been shown previously [8, 9], but also lactate oxidase activity.

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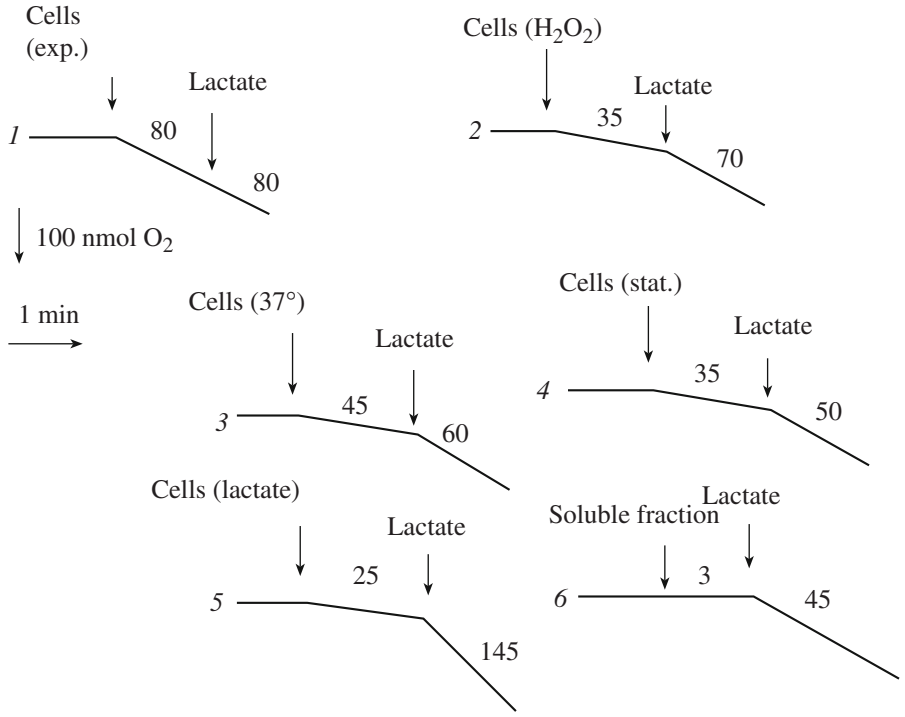

Fig. 1. L-lactate oxidation by the cells of *Y. lipolytica* from the exponential (*1, 2, 3, 5, 6*) and stationary (*4*) growth phases. *1–4*, the cells grown on glucose: untreated (*1, 4*); 0.5 mM H_2O_2, 60 min (*2*); 37°C, 60 min (*3*); *5, 6*, the cells grown on L-lactate: cells (*5*); soluble fraction (*6*). Lactate oxidase activity is shown by numbers on the curves: *1–5*, nmol O_2 min^{-1} mg^{-1} of dry cells; *6*, nmol O_2 min^{-1} mg^{-1} of protein.

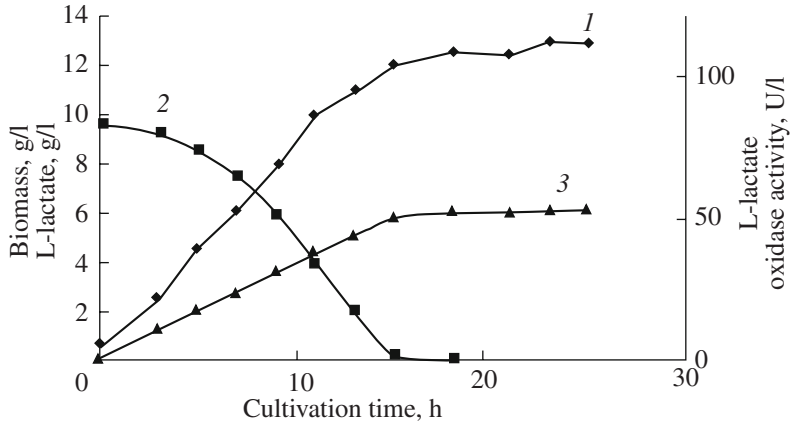

Fig. 2. Synthesis of L-lactate oxidase during the growth of *Y. lipolytica* on L-lactate: biomass accumulation (*1*); L-lactate concentration (*2*); L-lactate oxidase activity (*3*).

Lactate oxidase activity was expected to be higher in the cells grown on the medium with lactate. Indeed, cells grown in the presence of lactate showed higher rates of its oxidation (Fig. 1, curve *5*).

Figure 2 presents the data on biomass accumulation and lactate oxidase activity during *Y. lipolytica* growth on the medium with lactate. It can be seen that the lactate oxidase activity (curve *3*) correlates with the increase of the yeast biomass (curve *1*). After transition of the cells to the stationary growth phase (12–14 h) as a result of lactate depletion (curve *2*), the enzyme synthesis stopped.

Determination of the localization of lactate oxidase in the cell showed that the enzyme was present mainly in the soluble cell's fraction (Table). The presence of a certain activity in the membrane fraction was probably due to partial sorption of the enzyme on the membranes. It should be noted that the cells grown on lac-

Distribution of L-lactate oxidase activity by fractions

Fraction	Total activity, U/ml	Lactate oxidase content, %
Homogenate (1 mg of dry cells/ml)	40.0	100
Membrane fraction	1.5	4
Soluble fraction	38.5	95

tate-containing medium had a higher catalase activity (148 ± 2.5 μmol min^{-1} mg^{-1} protein) than the cells grown on glucose (28.0 ± 1.3 μmol min^{-1} mg^{-1} protein) [8, 9].

Thus, it was shown for the first time that yeasts are able to synthesize lactate oxidase. It was established that this enzyme was synthesized in *Y. lipolytica* in response to stresses and growth on lactate as a sole carbon and energy source.

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