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Extracellular Phosphomannan as a Phosphate Reserve in the Yeast *Kuraishia capsulata*

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Abstract—We have found that extracellular phosphomannan is the main phosphate reserve in the yeast *Kuraishia capsulata*, in contrast to other yeast species effectively absorbing P_i. Under nitrogen starvation, *K. capsulata* absorbed essentially all P_i from the medium containing 240 mM glucose, 2.5 mM $MgSO_4$, and 11 mM KH_2PO_4. Inorganic polyphosphate level in the cells was about 14% of the P_i absorbed. Most of the P_i (~60%) was found in the fraction of extracellular phosphomannan that can be used as a carbon and phosphorus source by this yeast in deficient media.

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Phosphorus is a vital element and, therefore, microorganisms are able to reserve phosphorus to use it under phosphate depletion in the medium. Reserve phosphate compounds are highly variable in both chemical nature and localization in microorganisms. Thus, the halophilic archae form extracellular poorly soluble salts $MgPO_4OH{\cdot}4H_2O$, and bacteria of the genus *Brevibacterium* accumulate $NH_4MgPO_4{\cdot}6H_2O$ within their cells [1, 2]. In *Acetobacter xylinum*, orthophosphate or high molecular weight inorganic polyphosphates dominate as reserve phosphorus compounds depending on cultivation conditions [3]. Many bacteria and yeast accumulate inorganic polyphosphates as reserve phosphorus compounds [4]. In contrast to prokaryotes, the biodiversity of reserve phosphorus compounds in yeasts is still poorly studied.

The objective of the present work was to study the peculiarities of reserving phosphorus compounds by the yeast *Kuraishia capsulata* capable of synthesizing extracellular phosphomannan.

MATERIALS AND METHODS

Strains and culture conditions. The object of research was the yeast *Kuraishia* (*Hansenula*) *capsulata* VKM Y-2514 from the All-Russian Collection of Microorganisms. The yeast kept on malt agar slants was grown at 30°C on a circulatory shaker at 140 rpm for 24 h (stationary phase) in the YPD medium (2% glucose, 1% yeast extract, 2% peptone). Then the biomass was separated from the culture medium by 15-min centrifugation at 13,000*g*, washed with 1% KCl solution, weighed, and used in experiments for determination of P_i uptake.

The cells of *K. capsulata* (10 g of wet biomass per liter) were placed in 50 ml of the medium containing 240 mM glucose, 2.5 mM $MgSO_4$, and KH_2PO_4 (concentrations are indicated in the tables and figures) and incubated at 30°C and 140 rpm. After the incubation, the cells were harvested by 15-min centrifugation at 13,000*g*, washed with 1% KCl solution, and used for analysis. The resulting supernatant was used for the assay of phosphorus compounds, for isolation of phosphomannan, and as a carbon and phosphate source in the experiments described below.

Phosphomannan utilization was studied by cultivating *K. capsulata* cells at 30°C and 140 rpm in a medium (1 liter) supplemented with 3 g of $(NH_4)_2SO_4$, 0.7 g of $MgSO_4$, 0.4 g of $Ca(NO_3)_2$, 1.31 g of K_2SO_4, 0.5 ml of microelement solution [5], and 0.5 g of yeast extract from which P_i had been removed by the method described earlier [6]. Glucose or phosphomannan-containing supernatant was added as a carbon source.

Phosphomannan isolation. The cells of *K. capsulata* were incubated for 24 h in a shaker (140 rpm) at 30°C in the medium containing 240 mM glucose, 2.5 mM $MgSO_4$, and 10 mM KH_2PO_4. Phosphomannan was iso-

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lated from the supernatant obtained after precipitation of the cells according to the method described earlier [7]. Sodium borate (8 ml of 5% solution) and cetavlon (8 ml of 5% solution) were added to 20 ml of the supernatant. The precipitate was washed with water, dissolved in 5 ml of concentrated acetic acid, lyophilized, and weighed for phosphomannan quantification.

Analysis of phosphorus compounds. Several polyphosphate fractions were isolated from the biomass. Acid-soluble and salt-soluble fractions were obtained by a known method [5]. Acid-insoluble polyphosphates were extracted by adding 10 ml of 0.5 N $HClO_4$ to residual biomass, followed by boiling for 30 min. The suspension was centrifuged at 5000*g* for 20 min, and polyphosphate level in the supernatant was estimated by the amount of P_i. The levels of P_i and labile phosphorus were determined in the fractions of acid-soluble and salt-soluble polyphosphates [8]. The polyphosphate levels can be judged by the amount of labile phosphorus. The level of total phosphorus was determined in the biomass. Inorganic P_i and labile and total phosphorus were determined in the supernatant after incubation of the cells with glucose, $MgSO_4$, and KH_2PO_4. The samples were mineralized in 32% $HClO_4$ at 150°C to assay total phosphorus by the level of released P_i. The optical density of the culture was determined at 600 nm in a 1-cm cell. The biomass weight was determined after 15-min centrifugation of the cells at 13,000*g*.

The mean values of three independent experiments are presented in the tables and figures.

RESULTS AND DISCUSSION

The model conditions used previously to study this process in bacteria and other yeasts [2, 9] were used to analyze the P_i absorption capacity of the yeast *K. capsulata* in minimal media. Similar to other yeast species [9], *K. capsulata* needed the carbon and Mg^{2+} sources for P_i uptake (Table 1). On addition of a nitrogen source, P_i uptake decreased.

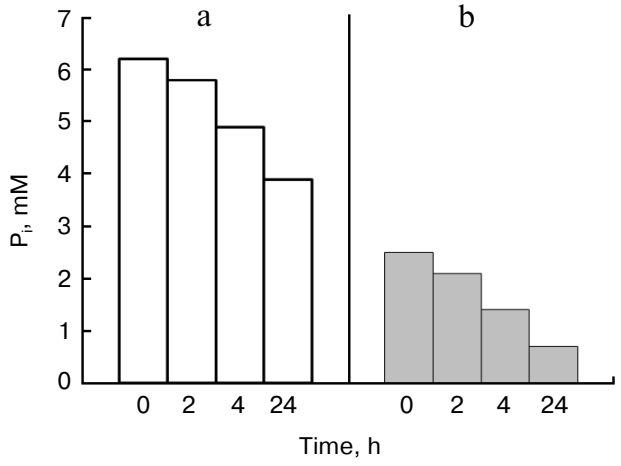

Fig. 1. Influence of incubation time on decrease in P_i concentration under incubation of *K. capsulata* cells in the presence of 30 mM glucose and 5 mM $MgSO_4$: a) the initial P_i concentration was 6.2 mM; b) the initial P_i concentration was 2.5 mM.

Table 1. Compositions of incubation media for the study of P_i accumulation by the cells of *K. capsulata*. The initial optical density at 600 nm was 10.5. Incubation time was 24 h

Incubation medium content, mM	OD_{600}	P_i in medium after 24 h, mM
Glucose, 30; KH_2PO_4, 1.9	15.7	1.3
Glucose, 30; KH_2PO_4, 1.9; $MgSO_4$, 2.5	15.5	0
Glucose, 30; KH_2PO_4, 1.9; $MgSO_4$, 5.0	13.4	0
KH_2PO_4, 1.9; $MgSO_4$, 5.0	9.5	1.9
Glucose, 30; KH_2PO_4, 1.9; $MgSO_4$, 5.0; $(NH_4)_2SO_4$, 5.0	16.0	0.95

Under the used model conditions, P_i concentration in the medium (Fig. 1) decreased more slowly than in other yeast species studied previously [9]. Substantial decrease in P_i concentration was reached after 24 h of incubation. Incubation of cells of *Saccharomyces cerevisiae* and *Cryptococcus humicola* under the same conditions was accompanied by decrease in P_i concentration (5 mM) by 80% during 5 h [9]. The 24-h incubation period was chosen for further experiments with *K. capsulata*.

The dependence of P_i concentration on glucose concentration in the medium is shown in Fig. 2. In the medium containing 240 mM glucose and 2.5 mM Mg^{2+}, P_i concentration decreased from 11 to 0.12 mM in 24 h. The cells of *K. capsulata* effectively decreased P_i in the medium, similar to the cells of other species [9].

We tried to reveal the phosphorus compounds accumulated in *K. capsulata*. Table 2 demonstrates the data on the content of different polyphosphate fractions in *K. capsulata* under nitrogen starvation. Polyphosphate level in the cells of *K. capsulata* was lower compared to those of *S. cerevisiae* and *Cr. humicola* (Table 2). The distinctive feature of this yeast was a salt-soluble fraction representing the greater part of the polyphosphates. The polyphosphate content was more than 60% of the accumulated P_i in *S. cerevisiae* and *Cr. humicola* but only 14% in *K. capsulata* under the same model conditions (Table 2). Therefore, polyphosphates cannot be considered as the main phosphorus reserve of *K. capsulata*.

Table 2. Polyphosphate content in different fractions (μmol/g wet weight) of *K. capsulata*, *S. cerevisiae*, and *Cr. humicola* under the model conditions with carbon and phosphate excess and nitrogen starvation. The total polyP percentage of the initial P_i in the medium is given in parentheses. The incubation medium contained 240 mM glucose, 11 mM P_i, and 2.5 mM Mg^{2+}; incubation time was 24 h

Polyphosphates	*K. capsulata*	*S. cerevisiae* [9]	*Cr. humicola* [9]
Acid-soluble	8	23	166
Salt-soluble	44	110	11
Acid-insoluble	36	157	59
Total	88 (14%)	290 (69%)	236 (63%)

It is known that a special feature of this yeast is formation of extracellular phosphomannan [10]. We suggested that P_i was used for the synthesis of this polymer under growth-limiting nitrogen starvation, since the medium was rich in the carbon source and P_i. Different phosphorus forms were determined in the biomass and supernatant after the incubation of *K. capsulata* to test this assumption (Table 3). The content of P_i in the biomass and supernatant was low compared to that in the starting medium. In contrast to the biomass, the incubation medium contained no labile phosphorus, indicating the absence of polyphosphates. It is notable that the medium has a high content (66%) of organic phosphorus compounds, the phosphorus of which can be determined only after mineralization. We suggested that the total phosphorus of the medium was present as phosphomannan.

Phosphomannan preparation was isolated from the incubation medium using a known method [7], and its amount was 34 mg/ml under the above conditions, i.e. much above the amount produced by *K.* (*Hansenula*) *capsulata* in the optimal media used by other authors, 1.9 mg/ml [11]. In the work cited, a slightly higher glucose concentration (360 mM) and a substantially higher P_i concentration (36 mM) were used. It seems likely that the greater amount of phosphomannan under our conditions was a result of nitrogen starvation. These conditions are of certain biotechnological interest, since extracellular polysaccharides of the yeast can be considered as promising immunomodulators [12] and dental preparations [13].

The phosphomannan preparation obtained in our experiments contained 3.35 μmol of total phosphorus per gram of the preparation, making ~57% of total phospho-

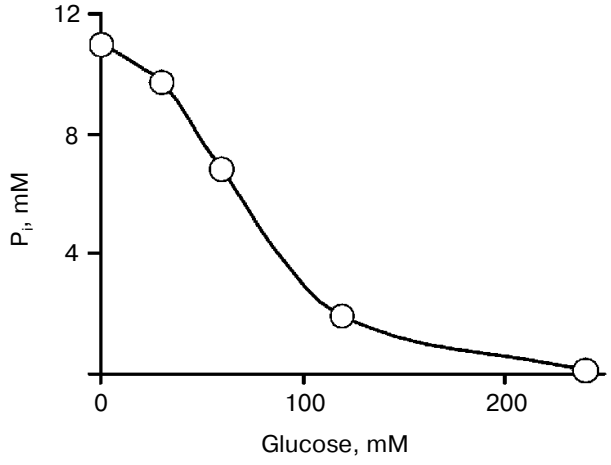

Fig. 2. Dependence of P_i uptake by *K. capsulata* cells on glucose concentration. The incubation medium contained 2.5 mM $MgSO_4$. The incubation time was 24 h.

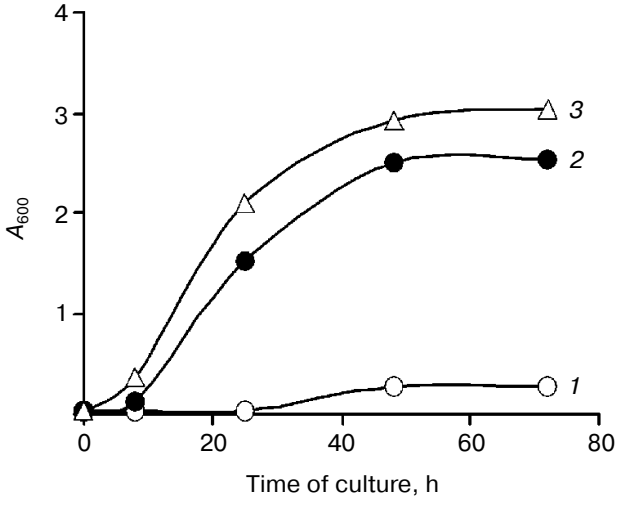

Fig. 3. Growth curves of *K. capsulata*: *1*) medium with 0.1 mM P_i and without a carbon source; *2*) 120 mM glucose was added to medium (*1*); *3*) the supernatant containing ~1 g of phosphomannan was added to medium (*1*).

Table 3. Distribution of P_i, labile and total phosphorus between the biomass and the incubation medium containing 240 mM glucose, 2.5 mM Mg^{2+}, and 10 mM P_i; incubation time was 24 h

Phosphorus form	% of the initial P_i content in the medium	
	biomass	medium
P_i	1.3	0.5
Labile phosphorus	14.0	0.5
Total phosphorus	34.0	66.0

rus in the incubation medium. The phosphomannan preparation contained neither P_i nor labile phosphate. Under hydrolysis of this preparation with 2 N HCl at 100°C, 25% of phosphorus was released as P_i in 2 h, followed by 64% release in 6 h. Such character of hydrolysis is in agreement with the literature data [10].

The question of the function of extracellular phosphomannan has not been discussed in other works [7, 10, 11]. We have tested the ability of *K. capsulata* to use phosphomannan for its growth. *Kuraishia capsulata* was cultivated in a P_i-deficient medium (0.1 mM P_i) with addition of a supernatant containing ~1 g of phosphomannan as the only carbon source (Fig. 3). This substrate had little effect on growth compared to glucose (Fig. 3), and *K. capsulata* absorbed no less than 50% of the total phosphomannan phosphorus.

The findings suggest that the extracellular phosphomannan of *K. capsulata* is a secondary metabolite that can be used by cells as a carbon and phosphorus reserve.

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