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EXPERIMENTAL ARTICLES

Involvement of Microbial Alkyl Hydroxybenzenes in the Regulation of Autolytic Degradation of Yeast Cells

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Abstract—A comparative study was performed of the processes of autolytic degradation of the cells of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* under conditions simulating the phase of cell death in microbial cultures: (1) during autolysis induced by oleic acid, which is the chemical analogue of factors d_2 (autolysis autoinducer), (2) under the effect of extracellular microbial proteinases (enzymatic lysis), and (3) under the concomitant effect of the enzymes of the endogenous autolytic complex and exogenous proteinases (heterolysis). Regulatory mechanisms controlling the rate and profundity of autolysis were elucidated, relying on the stabilization of hydrolytic enzymes and enhancement of their activity in their complexes with a chemical analogue of microbial autoregulatory factors d_1, which belong to alkylhydroxybenzenes and fulfill functions of chemical chaperones. The changes in the activity of proteinases and enzymes of the autolytic complex were shown to be dependent on the concentration of the analogue at the moment of complex formation.

Key words: autolysis, chemical chaperons, enzyme stabilization, stimulation of catalytic activity.

Autolytic degradation of cells is an important stage in the development of microbial cultures, since this process is linked with the formation of resting microbial forms and their maturation [1–3]. On the other hand, autolysis of cells is widely used in biotechnology and is considered to be a most efficient method of obtaining cell-free preparations (autolysates) enriched in readily assimilated proteins and amino acids [4]. Therefore, theoretical and applied investigations in this field are of considerable importance both for understanding the regularities of the development of microbial cultures and for elaboration of procedures for the regulation of hydrolysis of cellular biopolymers.

The process of autolysis occurs in two stages. At the first stage (stage of induction), irreversible impairment of the balance of synthetic and degradation processes occurs, which is caused, among other reasons, by inhibition of energy-yielding reactions [5]. In developing microbial cultures, this inhibition occurs under the action of extracellular autoregulatory factors, described as AMI in myxobacteria [3] and as d_2 factors (autolysis inducers) in a wide range of prokaryotic and eukaryotic microorganisms [6, 7]; in both cases, the active principle in these autoregulatory factors is unsaturated fatty acids. An increase in the concentration of these autoregulators above a certain threshold causes an increase in the fluidity of the plasma membrane to a level over the critical one and, as a consequence, impairment of the membrane structural organization and functional activity [6, 7]. The second stage of autolysis—autolytic cell degradation per se—occurs under the action of cellular depolymerases, primarily hydrolases, and is accompanied by the release of the degradation products of cellular polymers into the incubation medium.

The rate and efficiency of autolysis depend on the parameters of the medium; each of the stages has its specific optima [8, 9]. Thus, acidification of the culture promotes transport of protonated fatty acids into the cell and results in an increase of their intracellular concentration. The intensity of the second stage of autolysis depends on the amount and activity of the enzymes of the hydrolytic complex and on the physicochemical conditions influencing the activity of these enzymes.

It should be emphasized that, in the late stationary phase of microbial cultures, during which autolytic processes occur, not only autolysis autoinducers (d_2 factors) accumulate, but also anabiosis autoinducers, which, in certain bacteria and yeasts, were shown to be represented by acyl-substituted alkylhydroxybenzenes (AHB), occurring in developing cultures as a mixture of isomers and homologs [10–12]. AHB are involved in the control of cell metabolic activities and fulfill, among all, the functions of chemical chaperons, which form complexes with biopolymers (enzymes), modify their conformation, and thus stabilize their structure and modify their catalytic activity. The functioning of one AHB homolog as a chemical chaperon has been demonstrated in model experiments with individual enzymes [13, 14]. The role of AHB in the processes of hydrolytic degradation of cells has not yet been studied.

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At the same time, proceeding from the above, their effect on the rate and intensity of the process should be significant. The investigation of the regulatory role of AHB in the processes of enzymatic degradation of yeast cells was the aim of the present work. In this investigation, we used the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*; this allowed us to combine the theoretical interest in the regulation of autolysis with the elaboration of recommendations on practical application of the regularities revealed.

MATERIALS AND METHODS

Yeast cultures. The subjects of study were the type strain of *Schizosaccharomyces pombe*, obtained from the Magarach Institute of Grapes and Wine and strain *Saccharomyces cerevisiae* 380 (VKM). In the experiments, the yeasts *Sch. pombe* and *S. cerevisiae* were grown on wheat wort (3.5°) at pH 5.5 and 28°C in 2000-ml flasks with 500 ml of medium on shakers (140–160 rpm) to the midexponential phase (18–20 h). To obtain the inoculum, yeasts were grown to the exponential phase under the same conditions but in 250-ml flasks with 50 ml of medium. The inoculum was introduced in an amount resulting in an OD of 0.15–0.20 (Specord spectrophotometer, λ = 600 nm, l = 1 cm). The grown biomass was separated from the culture liquid by centrifugation (5000 *g*, 15 min) and resuspended in water to a concentration of dry matter equal to 10.5 ± 1.7 g/l for *Sch. pombe* and 90.1 ± 2.1 g/l for *S. cerevisiae*.

Lysis Procedures

Autolysis. The obtained suspensions of yeast cells were acidified to pH 3.0–3.5 with a 10% solution of H_3PO_4, supplemented with the inducer (an ethanol solution of oleic acid, $C_{18:1}$) to a concentration of 0.25–4.0 μmol/ml (the content of ethanol in the suspension was 2%), and incubated at 40–45°C for 30 min (the stage of induction). Then, the pH of the suspension was adjusted to 6.8–7.2 with a NaOH solution and the incubation was continued at 50–53°C for 6–8 h; pH during the incubation was maintained at 6.8–7.2 (the stage of autolytic disintegration of cells).

Enzymatic lysis. The pH in yeast cell suspensions was adjusted to 6.8–7.2 with a solution of H_3PO_4 or NaOH. The amount of enzymatic preparation of neutral–alkaline proteinase of microbial origin (*Bacillus licheniformis*, Ende Industrial Co., USA, 440 000 DAPU/g) was 2% of the biomass of dry cells. To this end, solutions of two concentrations were prepared: 45 mg/ml for *S. cerevisiae* and 5 mg/ml for *Sch. pombe*. To 25 ml of yeast solution, 1 ml of a corresponding solution of enzymatic preparation was added, and the mixture was incubated at 50–53°C for 6–8 h; the pH during the incubation was periodically adjusted to the required value.

Heterogeneous lysis. After the induction stage (see the **Autolysis** section), the pH of the suspension was adjusted to pH 6.8–7.2 with an NaOH solution; a necessary amount of the enzymatic preparation (see the **Enzymatic lysis** section) and the suspension was incubated at 50–53°C for 6–8 h; the pH during the incubation was periodically adjusted to the required value.

Stabilization of the autolytic complex enzymes and proteases by using C_7-AHB. In this work, we used an alkylhydroxybenzene homolog, C_7-AHB. C_7-AHB solutions of required concentrations were introduced into yeast suspensions (after adjusting pH to 6.8–7.2) after the induction of autolysis in the above-discussed variants of enzymatic hydrolysis of yeasts.

For microscopic examinations, an Ampleval phase-contrast microscope (Germany) was used.

Biochemical Methods

The soluble fractions of autolysates and hydrolysates were separated from cell walls and cell residues by centrifugation (5000 *g*, 15 min, 4°C); after that, the content of protein was determined by the Lowry method [15]; the content of amine nitrogen was determined with trinintrobenzene sulfuric acid [16]l the content of nucleotides was determined spectrophotometrically [17].

The level of hydrolysis (W, %) was judged from the dry cell mass (DCM) according to the following formula: $W = (1 - m_1/m_2) \times 100$, where m_1 is the mass of dry cells before hydrolysis, mg/ml, m_2 is the mass of dry cells after hydrolysis, mg/ml. The mass of dry cells was determined after their drying at 105°C for 24 h.

The average rate of the accumulation of proteinaceous compounds in the soluble fraction was determined according to the formula: $\mu_{av} = 2.3(\log x_1 - \log x_0)/(t_1 - t_0)$, where x_0 and x_1 are the concentrations of the protein in the soluble fraction in the beginning and the end of the process, mg/g DCM; and $t_1 - t_0$ is the duration of the process, h.

Statistical treatment of the data was performed by calculating the mean square error (σ) of three repetitive experiments. The results were considered reliable if the mean square error, σ, was less than 15%; Student criterion was used, taking a level of $P \le 0.05$ as the level of significance.

RESULTS AND DISCUSSION

The regularities of hydrolytic disintegration of yeast cells were studied under model conditions with yeast cells taken from the exponential phase (such cells are more sensitive to autolysis than cells in the stationary phase [6]). In our experiments, we used cell supensions to prevent the influence of background concentrations

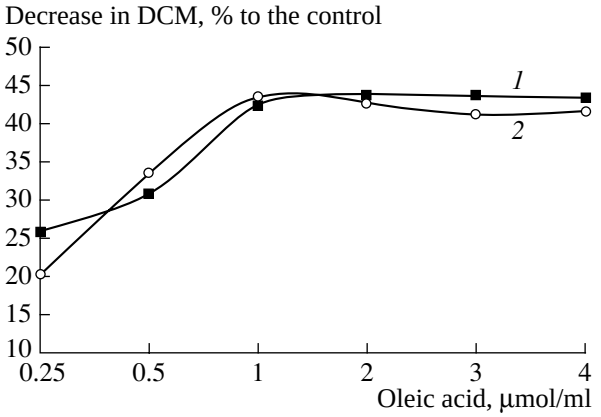

Fig. 1. Effect of the concentration of oleic acid on the level of hydrolysis of the yeast (*1*) *Saccharomyces cerevisiae* and (*2*) *Schizosaccharomyces pombe* (the duration of incubation was 6 h).

of natural autoregulators (d_1 and d_2) present in a developing culture.

To gain a more complete comprehension of the processes occurring during the degradation of *S. cerevisiae* and *Sch. pombe* cells, we used the following characteristics: the level of the cell hydrolysis, including three parameters: the concentration of extracellular protein, amine nitrogen and nucleotides (released from the cells as a result of destruction of cellular polymers), and also the mass of dry cells. The increase in the content of reducing compounds in the medium (which result from hydrolysis of the cell walls) was not determined: the autolysis of yeasts occurs via the endo-type: primarily, the membrane structures are disintegrated, and a weak hydrolysis of cell wall polymers occurs [18].

The autolysis of yeasts, simulating natural process, was induced by the introduction to cell suspensions of *S. cerevisiae* and *Sch. pombe* of a chemical analogue of autolysis autoinducer, namely oleic acid at a concentration of 0.25–4.0 μmol/ml. Acidification of the suspension allowed us to reproduce the conditions in a developing yeast culture; this, as mentioned above, provided for better incorporation of protonated $C_{18:1}$ molecules into cellular membranes, which caused their destabilization and cell autolysis. For both yeast suspensions, the optimal concentration of the inducer was 1 μmol/ml (as judged from the decrease in mass of dry cells, Fig. 1). Monitoring of the dynamics of induced autolysis of yeast cells of both strains revealed a decrease in the rate of cell degradation on the 4–6th hour of the process; this fact can be explained by the inhibition of hydrolases by terminal products of the reactions (Figs. 2a and 2b, curves *3* and *4*).

Since in autolysing suspensions cells and the released oligomers are affected by enzymes released by degraded cells representing the part of the population more sensitive to autolysis inducers, the next series of experiments was staged to reproduce this situation. An

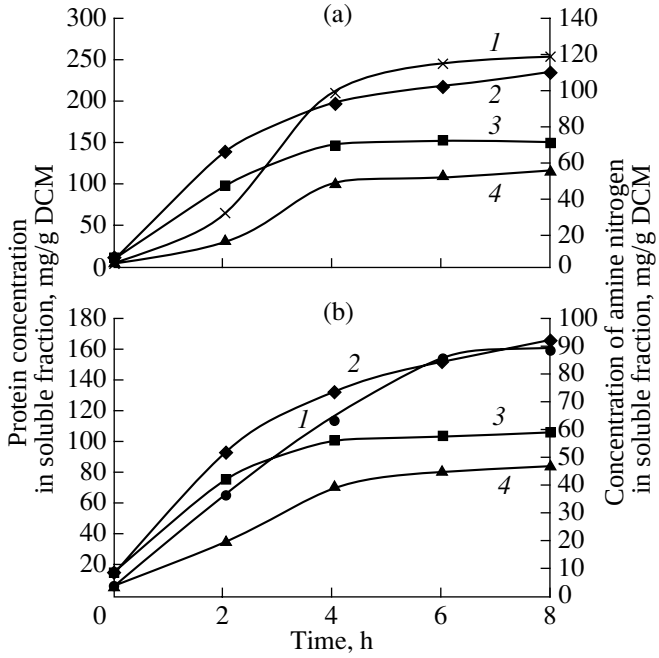

Fig. 2. Dynamics of accumulation of proteinaceous compounds in medium for yeast autolysis: (a) *S. cerevisiae* and (b) *Sch. pombe* after induction with oleic acid (1 μmol/ml) with stabilization of the autolytic complex with C_7-AHB (*1*, amine nitrogen; *2*, protein) and without stabilization: control (*4*, amine nitrogen; *3*, protein). The C_7-AHB concentration was 1.6 g/l for *S. cerevisiae* and 1.2 g/l for *Sch. pombe*.

exogenous microbial neutral–alkaline proteinase was introduced into yeast suspensions. In this experimental variant (enzymatic lysis), extracellular proteinase acted as a specific hydrolase and as an inducer of autolysis [18]. The level of cell hydrolysis (the change in the dry cell mass) in the variant of enzymatic lysis was 20% after 6 h for both yeast species (Table 1). According to all detectable parameters (extracellular protein, amine nitrogen, nucleotides), the process in this variant was less efficient than autolysis induced by the chemical analogue of factor d_2, oleic acid.

In the third series of experiments, both inducers of cell degradation were used (oleic acid and exogenous proteinase). This variant (heterogeneous lysis) is, theoretically, closest to the natural processes of cellular degradation that take place at the stage of dying of microbial cultures. During heterogeneous lysis, the level of cell degradation (according the decrease in the biomass of dry cells) was the highest, comprising 49.1–51.2% (vs. 42.2–43.5% in the course of autolysis induced by oleic acid and 21.9–25.5% during enzymatic lysis, Table 1). It should be mentioned that heterogeneous lysis had an evident advantage with respect to autolysis also in terms of realization of low molecular peptides and amino acids (amine nitrogen increased, on average, twofold for *Sch. pombe* and 1.7-fold for *S. cerevisiae*); as compared to enzymatic lysis, heterogeneous lysis

Table 1. Comparative Characterization of the Methods of Disintegration of the Cells of the Yeasts of *S. cerevisiae* and *Sch. pombe*

Method of cell disintegration	Content in the soluble fraction of			Level of hydrolysis by DCM, %
	protein, mg/g DCM	amine nitrogen, mg/g DCM	nucleotides, mg/g DCM	
Saccharomyces cerevisiae				
Autolysis	137.33 ± 6.55	50.88 ± 7.31	23.17 ± 3.17	42.2 ± 4.5
Enzymatic lysis	100.21 ± 3.72	31.1 ± 2.8	18.13 ± 2.41	25.5 ± 6.3
Heterogeneous lysis	201.65 ± 14.2	86.8 ± 1.5	21.53 ± 4.35	51.2 ± 4.5
Schizosaccharomyces pombe				
Autolysis	102.45 ± 9.85	40.63 ± 9.12	20.15 ± 6.54	43.5 ± 4.4
Enzymatic lysis	59.88 ± 2.21	35.89 ± 14.99	24.94 ± 6.39	21.9 ± 5.6
Heterogeneous lysis	128.21 ± 5.66	80.65 ± 13.09	38.43 ± 4.42	49.1 ± 4.8

was superior in terms of all three parameters (Table 1). Thus, the combined action of oleic acid and exogenous extracellular proteinases considerably increased the level of cellular degradation over 6 h (and, thus, the rate of the process). Another conclusion that can be made is that an increase in the amount of extracellular proteinases increased the efficiency of cell degradation in yeast suspensions undergoing autolysis.

It should be mentioned that the dynamics of cell degradation in all three variants followed the same pattern: a decrease in the intensity of hydrolysis after 4–6 h. At the same time, the rate of cell dying and degradation in natural autolysing suspensions was not subjected to such limits (according to numerous observations). Therefore, the second part of this work was aimed at the determination of the possible mechanisms of the regulation of cell hydrolysis, related not to the effect of the amount of extracellular hydrolases (proteinases) but to the change in their catalytic activity due to modification of the conformation of the enzymes of the autolytic complex.

In earlier investigations, we demonstrated modification of a protein structure by one of the AHB homologs. It is determined by stabilization of enzymes, an increase in their resistance to denaturing effects, and was accompanied by a decrease in their catalytic activity [13, 14]. It was also found that the effect of AHB as chemical chaperones is nonspecific with respect to different enzymes. In the present work, we used another analogue of microbial alkylhydroxybenzenes, namely, C_7-AHB, which have more polar molecules. In the first series of experiments, C_7-AHB were introduced into yeast suspensions undergoing autolysis (induced by oleic acid) immediately after the induction of autolysis (after adjustment of pH to 6.8–7.2); then, we studied the dependence of the efficiency of the process on the concentration of C_7-AHB (Table 2). The AHB analogue that we used caused an increase in the activity of cellular hydrolases in both yeast suspensions, as a result of which the following parameters increased (as compared to autolysis without the introduction of C_7-AHB): the level of cell hydrolysis (a decrease in dry cell mass); the amount of soluble protein and the degree of its hydrolysis (amine nitrogen parameters, Table 2). AHB concentrations optimal for intensification of autolysis of both types of cells were 1.2–1.6 g/l. In the range of supraoptimal concentrations of C_7-AHB, a decrease in the values of all autolysis parameters was observed; this is typical of the action of chemical chaperones and is most probably related to the degree of modification of the protein molecule, resulting in the loss of enzymatic activity.

The use of C_7-AHB for stabilization of enzymes of the autolytic complex in the autolysis variant made it possible to increase the level of cell degradation in the variant of heterolysis (i.e., when autolysis was combined with introduction of exogenous proteinases). The parameters determined in the autolysis + AHB variant (Table 2) were higher than the parameters recorded during heterogeneous lysis (Table 1): the decrease in dry cell mass was 67.1 ± 3.3% vs. 51.2 ± 4.5% for *S. cerevisiae* and 64.3 ± 4.3% vs. 49.1 ± 4.8% for *Sch. pombe*; the values of soluble protein and amine nitrogen were also higher. It should be mentioned that, in addition to more efficient accumulation of the products of hydrolysis of cellular protein in the incubation medium, the amount of soluble nucleotides also increased, indicating the nonspecific effect of C_7-AHB on various enzymes (proteinases, nucleases); this was in accordance with the mechanism of action of chaperons of another structure, e.g., such osmoprotectants as glycine-betaine and ectaine [19]. Since autolysis was carried out under conditions that were close to conditions optimal for the action of proteinases (pH 6.8–7.2), whereas nucleases have a pH optimum at 3.0–3.5, it may be suggested that the C_7-AHB–enzyme complexes have a higher range of catalytic activity than the optimum range (this aspect of the effect of C_7-AHB will be given special attention in our further publications). Since the main effect observed during heterogeneous lysis was an increase in amine nitrogen (also 2-fold as compared to autolysis, Table 1), it should be emphasized that, as a result of stabilization of the enzymes of autolytic complex by C_7-AHB (i.e., without the addi-

Table 2. Effect of C_7-AHB Concentration on the Composition of Autolysates of the Cells of Yeasts *S. cerevisiae* and *Sch. pombe* (the Duration of Autolysis was 6 h)

Concentration of C_7-AHB (g/l) in the incubation medium	Content in the soluble fraction of			Level of hydrolysis by DCM, %
	protein, mg/g DCM	amine nitrogen, mg/g DCM	nucleotides, mg/g DCM	
Saccharomyces cerevisiae				
0.4	166.8 ± 8.34	54.71 ± 2.15	43.32 ± 4.22	43.4 ± 6.5
0.8	196.2 ± 19.62	72.33 ± 6.94	42.59 ± 5.19	55.7 ± 7.2
1.2	202.7 ± 16.2	96.54 ± 5.28	54.77 ± 4.15	55.8 ± 8.1
1.6	233.64 ± 12.7	110.43 ± 7.21	60.83 ± 8.17	67.1 ± 3.3
2.0	212.34 ± 6.71	77.54 ± 3.68	55.42 ± 6.38	65.8 ± 4.2
Control	137.33 ± 6.55	50.88 ± 7.31	23.17 ± 3.17	42.2 ± 4.5
Schizosaccharomyces pombe				
0.4	110.13 ± 9.12	42.17 ± 4.41	38.2 ± 1.72	44.8 ± 5.9
0.8	130.56 ± 11.10	54.39 ± 7.46	46.33 ± 7.13	53.4 ± 6.8
1.2	149.86 ± 2.40	82.43 ± 3.64	50.3 ± 3.72	64.3 ± 4.3
1.6	140.29 ± 9.15	81.32 ± 6.70	48.4 ± 4.65	64.0 ± 5.6
2.0	120.38 ± 6.98	72.82 ± 9.43	40.30 ± 9.10	43.5 ± 4.4
Control	102.45 ± 9.85	40.63 ± 9.12	20.15 ± 6.54	43.5 ± 4.4

tion of exogenous proteinases), the concentration of amine nitrogen in the medium not only reached the level observed during heterogeneous lysis but attained an even higher level (Table 2, cf. Table 1). Along with an increase in the absolute values of amine nitrogen, its ratio to total soluble protein increased, especially for *S. cerevisiae* (from 37 to 47%), indicating a considerable increase in the activity of yeast exopeptidases (Table 3). Thus, it may be suggested that despite the nonspecificity of the action of AHB on enzymes, a certain selectivity does exist with respect to various hydrolase; this evidently requires further investigation.

The study of the time-course of the characteristics of the autolysis of yeast suspensions makes it possible to determine the duration of active development of the process and the rates of biopolymer hydrolysis. In our experiments, we compared the dynamics of cell degradation in two variants: without stabilization of autolytic complex (control variant) and with its stabilization with C_7-AHB (Figs. 2a, 2b). It was found that in the experimental variant not only did the rate of autolysis increase; the duration of intense hydrolysis of biopolymers also increased, resulting in a considerably increased amount of soluble products. In the control variant (without C_7-AHB), hydrolytic reactions terminated in 4 h; the average rates of protein accumulation over this period (0–4 h) were $\mu^1_{av} = 0.679$ and $\mu^2_{av} = 0.474$ (hereafter, μ^1_{av} corresponds to the results obtained for *S. cerevisiae*, and μ^2_{av} corresponds to the results obtained for *Sch. pombe*); the rates of accumulation of amine nitrogen were $\mu^1_{av} = 0.794$ and $\mu^2_{av} = 0.569$. In the autolysis period from 4 to 8 h, no significant accumulation of proteinaceous compounds occurred (for protein, $\mu^1_{av} = 0.004$ and $\mu^2_{av} = 0.014$; for amine nitrogen, $\mu^1_{av} = 0.038$ and $\mu^2_{av} = 0.047$). After stabilization of cellular hydrolases with C_7-AHB, the intensity of autolysis was higher during the whole period of observations: over the first 4 h, $\mu^1_{av} = 0.748$ and $\mu^2_{av} = 0.545$ for protein and $\mu^1_{av} = 0.977$ and $\mu^2_{av} = 0.692$ for amine nitrogen; over the 4–8 h period, $\mu^1_{av} = 0.047$ and

Table 3. Change in the Ratio of Amine Nitrogen to Total Soluble Protein in Various Variants of Cell Disintegration with Stabilization of Hydrolases with C_7-AHB and without Stabilization

Method of cell disintegration	Ratio of amine nitrogen to total soluble protein, %	
	control (without C_7-AHB)	+ C_7-AHB
Saccharomyces cerevisiae		
Autolysis	37.0 ± 6.9	47.3 ± 9.9
Enzymatic lysi	31.0 ± 3.3	46.5 ± 12.8
Heterogeneous lysis	43.0 ± 7.9	44.1 ± 15.8
Schizosaccharomyces pombe		
Autolysis	39.7 ± 9.5	55.0 ± 3.0
Enzymatic lysi	60.0 ± 8.6	57.8 ± 9.2
Heterogeneous lysis	62.9 ± 9.4	47.7 ± 8.4

Table 4. Comparative Characterization of Methods of Destruction of Cells of the Yeasts *S. cerevisiae* and *Sch. pombe* in the Presence of C_7-AHB

Method of Cell disintegration*	Content in the soluble fraction of			Level of hydrolysis by DCM, %
	protein, mg/g DCM	amine nitrogen, mg/g DCM	nucleotides, mg/g DCM	
Saccharomyces cerevisiae				
Autolysis + C_7-AHB	233.64 ± 12.7	110.43 ± 7.21	60.83 ± 8.17	67.1 ± 3.3
Enzymatic lysis + C_7-AHB	184.03 ± 18.4	85.66 ± 7.12	55.74 ± 9.53	54.7 ± 5.4
Heterogeneous lysis + C_7-AHB	261.6 ± 26.1	115.33 ± 5.44	59.13 ± 10.15	76.9 ± 7.1
Schizosaccharomyces pombe				
Autolysis + C_7-AHB	149.86 ± 2.40	82.43 ± 3.64	50.3 ± 3.72	64.3 ± 4.3
Enzymatic lysis + C_7-AHB	130.62 ± 11.10	75.46 ± 7.26	69.53 ± 5.23	50.3 ± 7.6
Heterogeneous lysis + C_7-AHB	205.05 ± 10.13	97.90 ± 6.76	68.43 ± 4..48	75.4 ± 4.8

* The duration of hydrolysis was 6 h; the concentrations of C_7-AHB were 1.6 g/l for *S. cerevisiae* and 1.2 g/l for *Sch. pombe.*

μ^2_{av} = 0.057 for protein and μ^1_{av} = 0.045 and μ^2_{av} = 0.085 for amine nitrogen. Thus the efficiency of the release of cellular proteins into the medium during autolysis in the variant with the stabilization of the enzymes of the autolytic complex with C_7-AHB increased to 50–60%. Most probably, in the control variant, there occurred inhibition of enzymes by reaction products accumulating in the medium; as a result, deceleration of hydrolytic processes occurred, while the formation of the complexes of enzymes with C_7-AHB stabilized the molecules of biopolymers, which, among other consequences, resulted in an increase in the enzyme resistance to the inhibition by terminal products of the hydrolysis of biomacromolecules.

Analogous effects of the intensification of the process were obtained after stabilization of exogenous proteinases and enzymes of the hydrolytic complexes in the variants of enzymatic hydrolysis and heterogeneous lysis. In Table 4, data are presented that characterize the level of cell hydrolysis and the composition of the soluble fractions in these variants. Comparative analysis of three models of hydrolytic degradation of cells (induced autolysis, enzymatic lysis, and heterogeneous lysis) with taking into account stabilization of hydrolases by the analog of microbial d_1 factors (C_7-AHB) showed that for both yeast species the highest results were obtained in the variant heterogeneous lysis + C_7-AHB: 76.9 ± 7.1% of the hydrolysis level (by DCM) for *S. cerevisiae* and 75.4 ± 4.8% for *Sch. pombe* (Table 4). Let us once more pay attention to the decrease in the level of protein hydrolyzation, determined from the amount of amine nitrogen (Table 4), and also to its percent ratio to the soluble protein (Table 3) that were observed in the case of stabilization with AHB in all three variants of cell disintegration. In terms of the absolute parameters, the application of C_7-AHB was most efficient in the variant of enzymatic lysis (Table 4, cf. Table 1). Thus, in the experiments with enzyme stabilization, the yield of protein and amine nitrogen increased for *S. cerevisiae* 1.8- and 2.7-fold, respectively, and 2.1- and 2.2-fold for *Sch. pombe* (as compared to the control). Comparison of the parameters of the soluble products obtained in the processes of stabilized and control hydrolysis in all three variants, showed that the highest effect was exerted by AHB on nucleases. The increase in the products of nucleic acid hydrolysis in experimental variants (as compared with variants without C_7-AHB) amounted, on average, to 150–200%; the increase in the amine nitrogen was 100–150%; and the increase in protein content was 50–80%.

The results obtained demonstrate the significant role of certain homologs of microbial alkylhydroxybenzenes (microbial d_1 factors) in the processes of regulation of the intensity and rate of autolytic degradation of cells in the post-stationary phase. The operation of d_1 factors as chemical chaperons occurs in accord with the operation of d_2 factors (autolysis inducers); and this controls the rate of hydrolytic degradation of cells, thus determining the dynamics of the accumulation of extracellular inducers of anabiosis (d_1 factors, released from newly autolysed cells). This, in turn, serves as an endogenous factor inducing the formation of cyst-like forms by that part of the population that is capable of this process and is most sensitive to the action of factor d_1, as was shown earlier [2]. An analogous autoregulatory mechanism of an additional (to the native concentration) release of autoinducers of sporulation from the autolysed part of the population and an increase in their total amount to a level sufficient for myxospore formation was first described for myxobacteria [1]; probably, this mechanism is universal for the regulation of any resting forms of microorganisms. It should also be emphasized that the discussed regulatory mechanisms of the control of the intensity of dying of microbial populations involves species-nonspecific low-molecular autoregulators. In natural econiches, these compounds

may play an important role in the regulation of the processes of mineralization of organic compounds and also in the balancing of trophic interactions in microbial communities. In the field of biotechnology, the results obtained may be important for enhancing the efficiency of industrial methods of autolysis.

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