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HELENA STOBIŃSKA
DOROTA KREGIEL
HELENA OBERMAN

IMPROVEMENT OF *Schwanniomyces Occidentalis* YEAST STRAINS BY MUTATION AND REGENERATION OF PROTOPLASTS

Institute of Fermentation Technology and Microbiology, Technical University, Łódź

Regeneration of *Schwanniomyces occidentalis* Y671/6 protoplasts led to production of clones boasting a 4-54% greater ability to biosynthesize amylolytic enzymes. Mutation of the protoplasts with N-methyl-N'-nitro-N-nitrosoguanidine also enabled the selection of active *Schw. occidentalis* clones producing up to 16% more biomass and displaying an up to 31% amylolytic activity than the parent strain.

INTRODUCTION

The yeasts *Schw. occidentalis*, displaying a high amylolytic activity, belong to the Saccharomycetaceae family and the Ascomycetes class [1, 3], and are capable of utilizing starch as their only source of carbon. This feature of theirs is due to the presence in their amylolytic complex of enzymes hydrolyzing α-1, 6-glicoside bonds (E.C.3.2.1.1 — α-amylase, E.C.3.2.1.3 — glucoamylase, E.C.3.2.1.9 — pullulanase). Glucoamylase degrades starch, maltotetriose, maltotriose and maltose to glucose, whereas the principal product of starch decomposition by α-amylase is maltose [4, 7, 10, 11, 14, 15]. The investigated yeasts are excellent starting material for developing a high-yield production strain to be used in microbiological synthesis of fodder protein or ethanol, the required material outlays being modest, and the carbohydrate raw material to be used — starch — being one of the most widespread. All this encourages further work on the selection and improvement of active strains and on optimizing methods of this selection and improvement. Among the more popular methods of improving industrial strains is mutation and genetic recombination. Strains may also be improved by protoplasts regeneration [2]. Data contained in the literature [6, 9] concerning the effects of mutagenic factors on protoplasts suggest that possibilities of improving strains by this method are also considerable.

In this research we attempted to obtain improved strains of *Schw. occidentalis* Y671/6 by means of regeneration and mutation of protoplasts.

MATERIAL AND METHODS

1. BIOLOGICAL MATERIAL AND METHODS OF CULTURE

The strain we used, *Schw. occidentalis* Y671/6, was obtained by mutagenization with UV radiation [13]. The biological material was cultured on agar slants (YPG, YPS, malt wort) at +28°C for 48 h, and then stored at +4°C. Table 1 presents the culture media used in this research.

Table 1. Culture media and their use

Medium		Use
YPG [3]:		Multiplication of biological material
peptone	– 0.5%	
yeast extract	– 0.25%	Storage of strains
glucose	– 1.0%	
YPS [3]:		Storage of strains
peptone	– 0.5%	
yeast extract	– 0.25%	
starch	– 1.0%	
Minimal medium M_o [3]:		Study of growth dynamics and amylolytic capabilities
$(NH_4)_2SO_4$	– 0.3%	
KH_2PO_4	– 0.1%	
$MgSO_4$ $7H_2O$	– 0.05%	
yeast extract	– 0.1%	
starch	– 1.0% 3.0% 5.0%	
pH = 5.0-5.5		
Regenerative media according to Jacobsen [8]:		Regeneration of protoplasts
medium I:		
glucose	– 0.5%	
yeast extract	– 0.3%	
agar	– 1.5%	
medium II:		
yeast extract	– 0.3%	
agar	– 1.5%	
Minimal medium M_o (see above) + RBB--starch (Remazol Brilliant Blue Starch)	– 0.2%	Study of amylolytic activity by the plate method

2. DETERMINATION OF KINETIC PARAMETERS OF GROWTH [16]

Determination of specific growth rate

The specific growth rate was calculated from the formula

$$\mu = \frac{\ln x_t - \ln x_0}{t_2 - t_1}$$

where $\ln x_t$ is the natural logarithm of dry mass (g/dm^3) at time t_2, $\ln x_0$ is the natural

logarithm of dry mass (g/dm^3) at time t_1; t_1 is the time of beginning of the logarithmic phase of growth (h), t_2 is th time conclusion of the logarithmic phase of growth (h).

Determination of the period of generation

Generation time was calculated, knowing the specific growth rate μ, from the formula

$$T = \frac{\ln 2}{\mu}$$

3. DETERMINATION OF HYDROLYTIC ENZYMES COMPLEX ACTIVITY IN ACELLULAR POSTCULTURE FLUIDS

Determination of amylolytic complex activity by th indicator method

The indicator method was used to select forms capable of secreting extracellular amylases. Amylolytic capabilities are revealed in th presence of starch introduced into the M_0 growth medium and containing Remazol Brillant Blue R which gives the medium a blue colour (RBB-starch) [12]. When active ··ms are present in the blue-coloured medium, there appear colourless zones of starch hydrolysis. The criterion with which the strains' activity is evaluated in coefficient q, the quotient of the diameter of the hydrolysis zone in the substrate, and the diameter of the colony. This method enables the discovery of extracellular amylases in a relatively short period of time (about 15h).

The activity of α-amylase (E.C.3.2.1.1.) was determined by the Fischer-Stein method [5].

The assumed unit of activity was the amount of enzyme contained in 1 cm^3 of acellular fluid which at 30°C liberates during 1 min 1 μmol of glucose in a 1% solution of soluble starch.

The results reported in this work are arithmetical means from three repetitions.

4. OBTAINING AND REGENERATION OF *Schw. Occidentalis* Y671/6 PROTOPLASTS

The *Schw. occidentalis* Y671/6 protoplasts were obtained and regenerated on suitable substrates according to methods described in Oberman et al. [12].

5. MUTATION OF *Schw. Occidentalis* Y671/6 PROTOPLASTS.
the Scheme of the Mutation Process was as Follows:

1. Obtaining of yeast protoplasts in conditions specified in an earlier stage of research [13].
2. Preparation of the initial protoplasts suspension in phosphate buffer (pH 7.2) in the presence of 0.8 M $MgSO_4$ as osmotic stabilizer.

3. Treatment of protoplasts suspension with nitrosoguanidine (500 and 800 $\mu g/cm^3$ of suspension) for 15, 30 and 60 min.

4. Separation of protoplasts from the mutagenic factor by centrifuging three times at 800 r.p.m.

5. Separation of protoplasts from mycelial cells on G3 filter.

6. Preparation of suspension of mutated protoplasts by suspending them in phoshphate buffer (pH 7.2) stabilized osmotically with 0.8 M $MgSO_4$

7. Quantity spread in Petri dishes with regenerative medium according to Jacobsen [8].

8. 72-h incubation at 30°C.

9. Screening of regenerated clones — inoculations on YPG slants; the obtained material was used in further selections.

6. BIOMASS DETERMINATION

Biomass was determined spectrophotometrically by measuring absorbance at 550 nm wavelenght. Results of the measurements were compared with the standard curve $E = f$(dry mass) representing absorbance as a function of yeast dry mass (Fig. 1). The results (means from three repetition) are expressed in mg/cm^3.

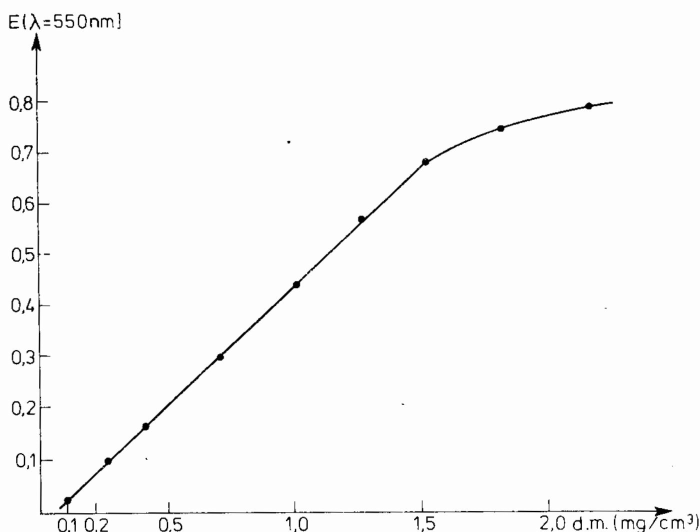


Fig. 1. Growth curve of *Schw. occidentalis* Y671/6, $E = f$ (dry mass)

RESULTS AND DISCUSSION

1. ESTIMATION OF AMYLOLYTIC ACTIVITY OF REGENERATED *Schw. Occidentalis* Y671/6 PROTOPLASTS IN THE PLATE TEST

As can be seen in Table 2, the *Schw. occidentalis* Y671/6 protoplasts regenerated in Jacobsen's medium. Their regeneration capability, ranging from

10.3% to about 72%, depended on the kind of osmotic stabilizer. Protoplasts regenerated most readily (ca. 72%) in the medium stabilized with 0.8 M mannitol (Table 2).

Table 2. Regeneration of *Schw. occidentalis* Y671/6 protoplasts in various media

Regenerative medium	Total number of cells and protoplasts determined microscopically	Number of protoplasts determined microscopically	Protoplastization yield (%)	Number of regenerated protoplasts CFU/cm³ %
Jacobsen's medium I + osmotic stabilizer:	2.69×10^8	2.13×10^8	79.2	
0.8 M $MgSO_4$				3.1×10^7 14.5
0.8 M KCl				2.2×10^7 10.3
Jacobsen's medium II + osmotic stabilizer 0.8 M mannitol	3.07×10^8	2.75×10^8	89.6	1.97×10^8 71.6

Of the 20 considered clones, 31 forming large colonies (4-6 mm in diameter) in the regenerative medium were selected for study. The amylolytic activity of these clones was checked by the plate test in Mo + RBB-starch substrate [12]. The results, collected in Table 3, show that the clones obtained from regenerated protoplasts displayed various amylolytic activities. Two of them were characterized by an enhanced ability to secrete extracellular α-amylase, with their q coefficient being 11-30% higher than in the parent straint in which $q = 4.5$.

Table 3. Amylolytic activity of regenerated *Schw. occidentalis* Y671/6 protoplasts checked by the plate method

Number of studied colonies	Colony diameter mm	Diameter of substrate hydrolysis zone, mm	q*
20	0	0	–
1	2	4	2.0
2	4	11	2.7
3	3	10	3.3
1	3	12	4.0
3	2	10	5.0
1	2	12	6.0
parent strain Y 671/6	2	9	4.5

$$* q = \frac{\text{diameter of starch hydrolysis zone (mm)}}{\text{colony diameter (mm)}}$$

Six of the tested clones had q values lower by 12–55% than in the parent Y671/6 strain, and 61% of the regenerated isolates failed to grow on the test Mo + RBB-starch medium (Table 3).

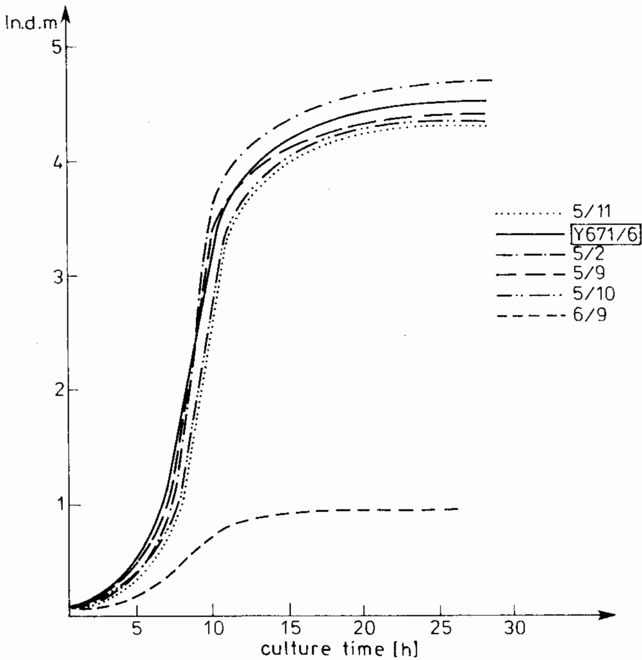


Fig. 2. Growth of the regenerated protoplasts *Schw. occidentalis* Y671/6 in M_0 medium with 1% starch

2. GROWTH AND AMYLOLYTIC ACTIVITY OF REGENERATED Y671/6 PROTOPLASTS IN SHAKER CULTURES

The growth dynamics and amylolytic activity of the four clones (denoted 5/2, 5/9, 5/10 and 5/11) which displayed the high amylolytic activity in the plate test were investigated in shaker cultures in the M_0 substrate in the presence of 1% of starch. The obtained results are collected in Table 4 and illustrated in Fig. 2. In the M_0 + 1% starch substrate that was used, the regenerated protoplasts produced 4.3-4.7 g biomass per dm³. In analogous conditions, the parent strain Y671/6 produced 4.5 g biomass per dm³. The clone which biosynthesized amylolytic enzymes best was 5/10. -amylase activity determined in the 7th hour of culture was 6.8 units, exceeding the amylolytic activity of the parent strain by 54%. The regeneration of protoplasts had a favourable effect on the basic growth parameters of the obtained strains. In the four investigated clones, the specific growth rate μ was found to be 7-38% greater than in the parent Y671/6 strain, which means that the generation time T decreased by 6-28% (Table 4).

3. MUTATION OF *Schw. Occidentalis* Y671/6 PROTOPLASTS

Results concerning the survivability of NTG-treated protoplasts are given in Table 5. Survivability depended on the applied dose of nitrosoguanidine.

Table 4. Basic growth parameters of regenerated *Schw. occidentalis* Y671/6 protoplasts in M_O medium + 1% starch

Strain	Dry mass (g/dm³)	Y_p*	μ (h^{-1})	T (h)	α-amylase activity (U)
parent strain Y671	4.5	0,45	0.490	1.41	4.4
5/2	4.7	0.47	0.675	1.02	4.6
5/9	4.4	0.44	0.596	1.16	4.8
5/10	4.3	0.43	0.555	1.24	6.8
5/11	4.3	0.43	0.523	1.32	4.6

$$* \; Y_p = \frac{\text{yeasts dry mass (mg/cm}^3\text{)}}{\text{starch concentration (mg/cm}^3\text{)}}$$

Tabele 5. Survivability of *Schw. occidentalis* Y671/6 protoplasts after treatment with nitrosoguanidine

NTG (μg/cm³)	Time of NTG action (min)	Number of regenerated clones (CFU/cm³)		Survivability	
		YG medium + 0.8 M mannitol	YPS medium + 0.8 M mannitol	YG medium	YPS medium
0	0	3.45 10⁶	3.45 10⁶	100	100
500	30	8.4 10⁴	9.05 10⁴	2.43	2.62
800	30	1.25 10⁴	1.30 10⁴	0.36	0.37

* YG = 0.3% yeast extract + 0.5% glucose + 1.5% agar
YPS = 0.3% yeast extract + 0.5% peptone + 1.5% agar + 1% starch

When 500 μg NTG was added to 1 cm³ of suspension of *Schw. occidentalis* protoplasts (of 3.45×10^6 density), the survivability of protoplasts after mutation was 2.62%. At 800 μg NTG/cm³, the survivability of regenerated protoplasts dropped to 0.36%.

4. SELECTION OF REGENERATED *Schw. Occidentalis* PROTOPLASTS AFTER MUTATION

The amylolytic ability of regenerated protoplasts was assessed by the plate test in minimal medium Mo with RBB-starch present. The results are given in Table 6.

The effect of nitrosoguanidine on amylolytic activity of the obtained clones varied:

— about 6.0% of the investigated clones displayed a lower ability to hydrolyse starch (q = 1.5) than the population prior to mutation;

— about 8% of the protoplasts regenerated after mutation hydrolysed starch on a level similar to that of the initial protoplasts;

— 86.0% of the mutants displayed amylolytic activities (q = 1.83-2.70) which were greater than in populations subjected to the action of the mutagen NTG (Table 6).

Table 6. Amylolytic activity of regenerated *Schw. occidentalis* protoplasts following treatment with nitrosoguanidine determined by the indicator method

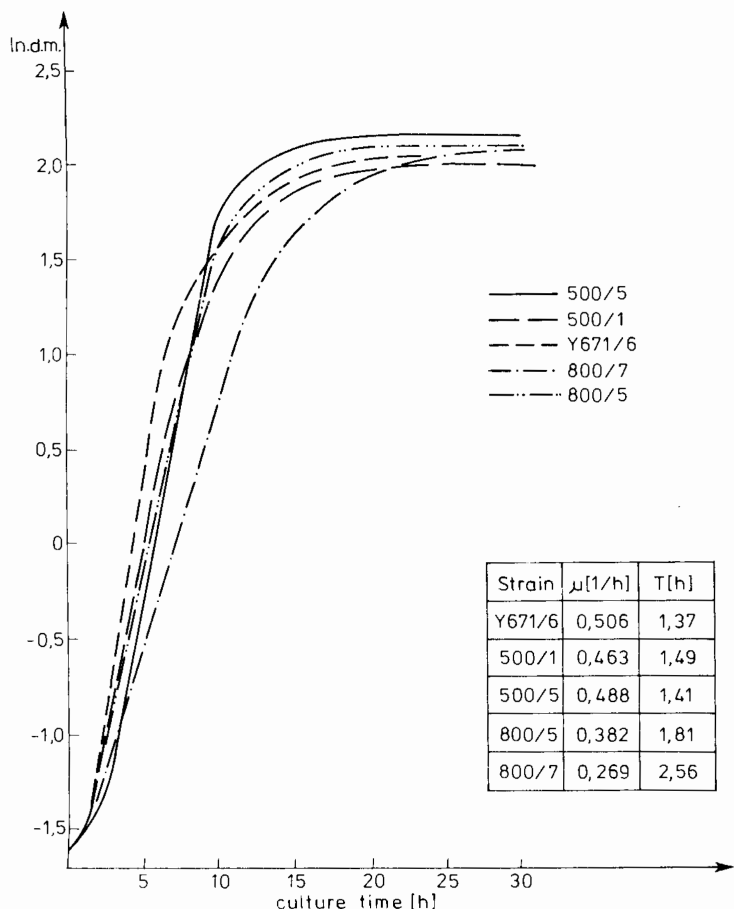
NTG dose ($\mu g/cm^3$)	Number of studied colonies	Diameter of colonies (mm)	Diameter of starch hydrolysis zone (mm)	q^*
500	2	2	3	1.50
	3	3	8	2.70
	6	4	9	2.25
	4	5	12	2.40
800	3	4	7	1.75
	12	5	12	2.40
	7	6	11	1.83
0	4	4	7	1.75
initial suspension of protoplasts	6	5	9	1.80
	5	6	12	2.00

$$q^* = \frac{\text{diameter of starch hydrolysis zone (mm)}}{\text{diameter of colonies (mm)}}$$

5. ESTIMATION OF GROWTH OF REGENERATED *Schw. Occidentalis* PROTOPLASTS FOLLOWING TREATMENT WITH THE MUTAGEN

The four yeast clones selected in the plate test were studied in minimal medium M_0 in the presence of 1, 3 and 5% of starch, with their growth and amylolytic activity (-amylase) being compared in acellular culture fluids. The results of these studies are given in Table 7 and in Figs 3-5.

Compared with the parent strain, the investigated clones were characterized by varied amylolytic activities. The one denoted 800/7 metabolized starch poorly, while the other three proved to be good producers of biomass. With 3% starch in the minimal medium they produced 14.5-17.7% more biomass than the parent strain Y671/6. The coefficients of biomass yield in the applied range of starch concentrations (1, 3 and 5%) were from 0.44 to 0.80. In identical conditions the biomass yield of the parent strain ranged from 0.45 to 0.76. Results characterizing the amylolytic activity of mutants and the parent strain Y671/6 are compared in Table 7. The maximum activity of -amylase in the mutants occurred in the 17th hour of culture, i.e. at the beginning of the stationary state. In some of the mutants the -amylase activity increased by 10-31% as compared with the activity of the parent strain.



Strain	μ[1/h]	T[h]
Y671/6	0,506	1,37
500/1	0,463	1,49
500/5	0,488	1,41
800/5	0,382	1,81
800/7	0,269	2,56

Fig. 3. Growth of *Schw. occidentalis* Y671/6 clones obtained by protoplasts mutation in M_o medium with 1% starch

The presence of starch in the growth medium induced -amylase activity (Table 7). The increasing starch concentration (1-5%) stimulated the biosynthesis of amylases by 24-128%. These results show that the -amylase of *Schw. occidentalis* is an inductive enzyme.

Summing up, we may say that mutagenization of *Schw. occidentalis* protoplasts with nitrosoguanidine can improve some of the biotechnological properties of these strains. Among the effects of the mutagen is increased biomass production in starch media, and increased amylolytic activity.

6. DIFFERENTIATION OF *Schw. Occidentalis* PROTOPLASTS MUTANTS WITHIN A POPULATION — STATISTICAL ANALYSIS

In order to statistically analyse the differentiation of strains (the strain denoted 500/5 obtained by mutation and subsequent regeneration of protop-

Table 7. Biomass produced by *Schw. occidentalis* Y671/6 clones obtained after mutation of protoplasts (M_0 medium + 1,3 or 5% starch)

Clone	Starch concentration (%)	Dry mass (g/dm³)	Biomass increment (%)	Y_p*	-amylase activity (U)**
500/1	1	7.5	− 1.3	0.75	5.80
	3	17.3	+ 17.7	0.58	6.20
	5	24.9	+ 10.7	0.50	7.20
500/5	1	8.8	+ 15.8	0.88	5.80
	3	17.0	+ 15.6	0.57	6.40
	5	22.6	+ 0.4	0.45	7.50
800/5	1	7.9	+ 3.9	0.79	2.10
	3	17.2	+ 17.0	0.57	4.20
	5	22.0	− 2.2	0.44	4.80
800/7	1	7.8	+ 2.2	0.78	4.57
	3	12.0	− 18.4	0.40	6.10
	5	13.3	− 40.9	0.27	6.80
parent	1	7.6	—	0.76	4.44
strain	2	14.7	—	0.49	5.80
Y671/6	5	22.5	—	0.45	6.10

$$* \; Y_p = \frac{\text{yeast dry mas (mg/cm}^3)}{\text{starch concentration (mg/cm}^3)}$$

** Activity measured in the 17th hour of culture; $U = \frac{\mu\text{mol glucose}}{\text{cm}^3/\text{min}}$

plasts, and the parent strain Y671/6) the relevant populations were transferred to plates with minimal medium M_0 containing 1% of starch. 24 clones were isolated from each plate.

The isolates were cultured for 24 h in M_0 medium with 3% of starch on a laboratory shaker (220 cycles/min), following which the biomass yield of the tested strains was determined. Also assessed was the amylolytic activity of the isolated clones. This was done by the indicator method in M_0 medium with RBB-starch, determining coefficient q (quotient of diameter of substrate hydrolysis zone and diameter of colonies). The obtained results were subjected to statistical analysis. We calculated scatter indices, taking as the norm the range of values embracing 95% of the distribution of the given feature of the population [17]. The results of this analysis are given in Table 8.

The statistical analysis revealed that all measurement results remained in the conventional range of values (95%). This means that the studied populations were homogeneous.

Table 9 shows results of assays of amylolytic activity of the *Schw. occidentalis* mutant denoted 500/5 following 15 months of storage on agar slants in laboratory conditions. As can be seen, the mean value of coefficient q (3, 11) dropped by only 2% as compared with the respective figure for the parent strain

Table 8. Results of statistical analysis

Strain	Criterion of evaluation	Range (R)	Standard deviation (S)	Variance (S^2)	Standard deviation of arithmetical mean ($\bar{S}$)	Variance coefficient (W)	Normal range of values	Range of experimental values
500/5	biomass $\bar{x} = 15.30$	10.78	2.78	7.72	0.56	18.2%	9.7-20.9	10.1-20.9
	q^* $\bar{x} = 3.17$	1.25	0.36	0.13	0.07	11.3%	2.5-3.9	2.5-3.75
parent strain Y/671/6	biomass $\bar{x} = 12.46$	5.80	1.53	2.34	0.31	12.3%	9.4-15.5	9.6-15.4
	q^* $\bar{x} = 2.80$	0.50	0.20	0.04	0.04	7.1%	2.4-3.2	2.6-3.1

$$q^* = \frac{\text{diameter of starch hydrolysis zone (mm)}}{\text{diameter of colonies (mm)}}$$

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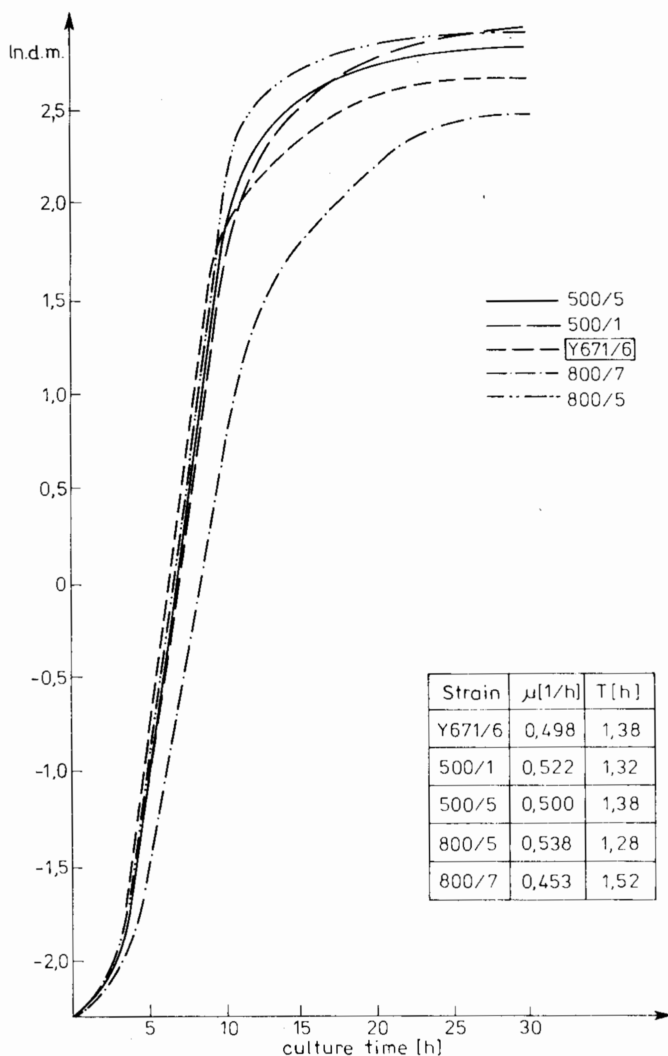
Table 9. Amylolytic activity of *Schw. occidentalis* mutant 500/5 after 15 months of storage in laboratory conditions

Strain	Time of storage (months)	Criterion of evalutaion q^*	Range (R)	Standard deviation of arithmetical mean ($\bar{S}$)	Variance (S^2)	Standard deviatin of arithmetical mean ($\bar{S}$)	Variance coefficient (W)	Normal range of values	Range of experimental values
500/5	0	$\bar{x} = 3.17$	1.25	0.36	0.13	0.07	11.3%	2.5-3.9	2.5-3.75
	15	$\bar{x} = 3.11$	1.22	0.46	0.21	0.10	14.6%	2.2-4.0	2.28-3.5

$q^* =$ diameter of starch hydrolysis zone (mm)



Strain	μ[1/h]	T[h]
Y671/6	0,498	1,38
500/1	0,522	1,32
500/5	0,500	1,38
800/5	0,538	1,28
800/7	0,453	1,52

Fig. 4. Growth of *Schw. occidentalis* Y671/6 clones obtained by protoplasts mutation in M_0 medium with 3% starch

Schw. occidentalis 500/5. This indicates that the studied strain's ability to hydrolyse starch remains stable after 15 months of storage in laboratory conditions. At the same time, after this period of storage there is no negative selection of clones with regard to the amylolytic capabilities of the yeasts.

In conclusion, we may say that mutation and regeneration of protoplasts may produce active *Schw. occidentalis* clones. Both methods may be effectively used to improve the amylolytic activity and biomass production of *Schw. occidentalis* yeasts.

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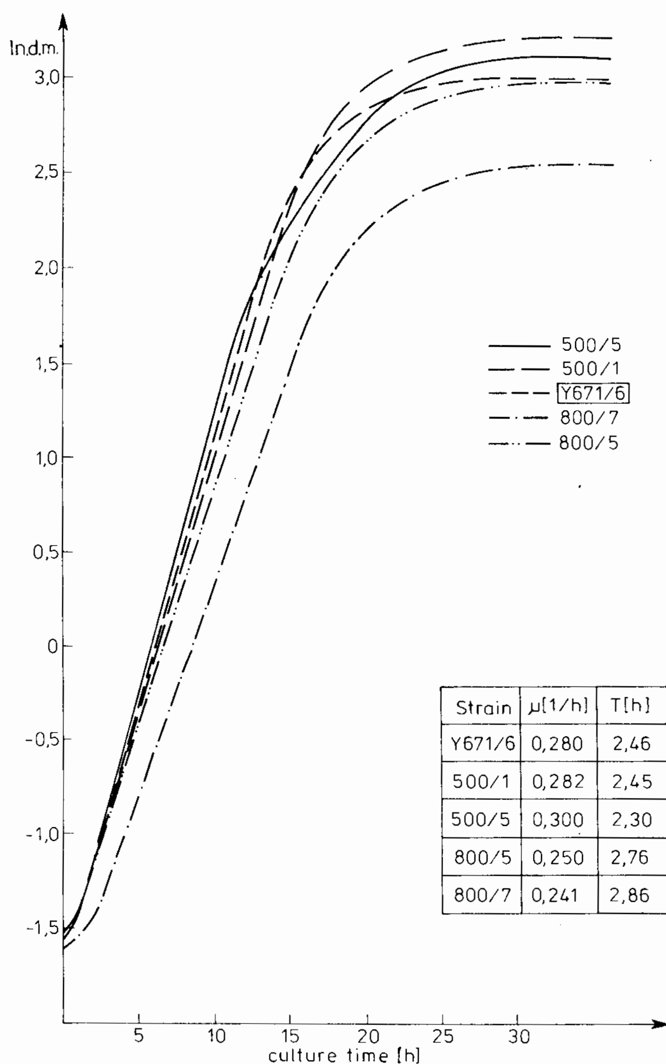

Strain	μ[1/h]	T[h]
Y671/6	0,280	2,46
500/1	0,282	2,45
500/5	0,300	2,30
800/5	0,250	2,76
800/7	0,241	2,86

Fig. 5. Growth of *Schw. occidentalis* Y671 clones obtained by protoplasts mutation in M_o medium with 5% starch

LITERATURE

1. Barnet J.A., Payne R.W., Yarrow D.: Yeast characteristic and identification, Cambridge 1983.
2. Brown J.A., Ogawa K., Wood T.A.: Enzyme Microbiol. Technol., 1986, **9**, 527, 532.
3. Burbianka M., Pliszka A.: Mikrobiologia żywności, PZWL, Warszawa 1981.
4. Farkas V., Liskova M., Biely P.: FEMS Microbiol. Letters 28, 1985, 137.
5. Fisher L.E.H., Stein E.A.: Biochem. Prep., 1961, **8**, 87.
6. Homolka L., Vyskočil P., Pilat P.: Appl. Microbiol. Biotechnol., 1988, **22**, 166.
7. Howard J.J., Wilde P.J., Caster A.T., Kirkham J.A., Best D.J., Lancashize W.W.: XIVth Int. Conf. on Yeast Genetics and Molecular Biology, Helsinki 1988, 467.

8. Jacobsen T., Jensen B., Olsen J., Allerman K.: Can. J. Microbiol., 1983, **31**, 93.
9. Kim K.S., Cho N.Y., Pai H.S., Ryu D.D.Y.: Appl. Environ. Microbiol., 1983, **46**, 689.
10. Lusena C.V., Champagne C.C., Calleja G.B.: Can. J. Biochem. Cell. Biol., 1985, **63**, 366.
11. Oberman H., Stobińska H., Kozanecka E.: XIIth Int. Specjal. Symp. on Yeast, Weimar 1987.
12. Oberman H., Stobińska H., Kręgiel D., Kozanecka E.: Acta Alimentaria Polonica AAP 1991, **17** (1), 57.
13. Oberman H., Stobińska H., Kręgiel D., Kozanecka E.: Sprawozdanie z pracy naukowo-badawczej w ramach programu CPBP 04.11/1.2, Łódź 1986.
14. Sills A.M., Zygora P.S., Stewart G.G. Appl. Microbiol. Technol., 1984, **20** (2), 124.
15. Simoes-Mendes B.: Can. J. Microbiol., 1984, 30, 1163.
16. Kotełko K., Sedlaczek L., Lachowicz T.M.: Biologia bakterii, PWN, Warszawa 1979.
17. Zgirski A., Gondko R.: Obliczenia biochemiczne PWN, Warszawa 1981.

Manuscript received: July, 1989
Authors address: 90-924 Łódź, Stefanowskiego 4/10

H. Stobińska, D. Kręgiel, H. Oberman

OTRZYMYWANIE ULEPSZONYCH SZCZEPÓW DROŻDŻY *Schw. Occidentalis* METODĄ MUTACJI I REGENERACJI PROTOPLASTÓW

Instytut Technologii Fermentacji i Mikrobiologii, Politechnika, Łódź

S t r e s z c z e n i e

Zastosowane metody regeneracji protoplastów *Schw. occidentalis* Y671/6 lub ich mutacji pod wpływem nitrozoguanidyny pozwoliły uzyskać klony o zróżnicowanych aktywnościach amylolitycznych. Po regeneracji protoplastów na podłożu Jacobsena wyizolowano 2 klony oznaczone symbolami: 5/9 i 5/10, które charakteryzowały się wyższą o 9 i 54% aktywnością -amylazy niż szczep wyjściowy. Regeneracja protoplastów wpłynęła korzystnie na wartości parametrów wzrostu u otrzymanych klonów. Nastąpiło obniżenie okresu generacji, a tym samym wzrost właściwej szybkości wzrostu μ o 7 i 38% w porównaniu ze szczepem rodzicielskim. W efekcie przeprowadzonej mutacji protoplastów i ich regeneracji, a następnie selekcji w teście płytkowym na podłożu Mo + RBB-skrobia 86% testowanych klonów charakteryzowało się wyższą zdolnością biosyntezy enzymów anylolitycznych niż szczep rodzicielski Y671/6.

Najaktywniejsze klony, które wyselekcjonowano w teście płytkowym i w hodowlach wstrząsanych w podłożu minimalnym M_0 z dodatkiem skrobi: 1%, 3% i 5% wykazały w stosunku do szczepu rodzicielskiego wyższą o 10-31% aktywność -amylazy. Wzrastające stężenie skrobi w podłożu hodowlanym od 1% do 5% indukowało zdolność biosyntezy pozakomórkowej -amylazy.

Analiza statystyczna wyników tworzenia biomasy i aktywności amylolitycznej klonów *Schw. occidentalis* wykazała, że mieściły się one w umownym przedziale wartości obejmujących 95% rozkładu danej cechy w obrębie populacji. Powyższe dane jednoznacznie potwierdziły jednorodność badanych populacji *Schw. occidentalis*.