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OBTAINING OF SPORE CULTURES AND HYBRIDS OF TOP-FERMENTING BREWERS' YEAST

Key words: brewers' yeast top fermentic hybrids, yeast hybrids

The possibility of applying the "mass spore" isolation and "mass mating" technique for the production of yeast hybrids, suitable for brewery technology, was revealed. A series of stable hybrids, including bakers' and brewers' ones were obtained with a considerably shorter time of generation than that of monospore populations from which they were obtained. The hybrids, displayed no flocculence, all varieties were powder type.

The review of available literature in the field of industrial microbiology points to the increasing interest of microbiological industry in the possibilities of strains improvement. It is strictly connected with the achievements in genetics because any improvement of the properties of microorganisms involves genetic factors.

The best method of yeast improvement for the needs of the brewery industry seems to be hybridization i.e. obtaining of hybrids. When the genetic properties of the initial material are known, the above method gives possibilities of obtaining yeasts with the desired properties without any hazard of changes brought by mutation. Reports concerning this way of improving the bakers' distillers' and brewers' yeasts and also yeasts used in derivative processes, are fairly numerous [2, 11, 13].

The combination of yeast strains in order to strengthen the properties required in the production of beer, is however difficult because most of the technological parameters of the particular strains is not a result of the activity of individual genes but it consists of joint action of many inherent factors [22]. Certain species of yeasts sporulate weakly or they do not sporulate at all which makes impossible production of hybrids. The produced spores have often a low survival rate or weakened ability to mate with other mating spores.

It may be also accompanied by other difficulties, connected e.g. with the isolation of zygotes, their growth, etc. One of the main problems is also the fact that Poland is lacking a set of initial industrial strains, characterized and suitable for hybridization purposes. In connection with this fact, the production of hybrids had to be preceeded by a general characteristic of home strains for the isolation of monospore, haploid populations, suitable for the discussed application.

The aim of the present study was to carry out the characteristics of home strains of brewers' yeasts from the viewpoint of their applicability in hybridization and obtaining hybrids of beer yeasts with technological parameters mainly suitable for production of top fermented beer. The purpose was also to produce a set of characterized, sexually active, varieties of brewery yeasts for obtaining of new, technologically suitable varieties.

MATERIAL AND METHODS

The biological material comprized 24 strains of brewers' yeasts:

a) top fermenting strains

no. no. 2, 3, 4 and 16 — deriving from the Institute of Fermentation Industry in Warsaw

no. 14 and no. 15 — deriving from the Center of Pure Cultures of the Technical University in Łódź

no. no. 21, 22, 23, 24, 25, 26, 27, 28 and 29 — deriving from the Experimental Plant of the Institute of Fermentation Industry in Biskupiec

b) bottom fermentation strains

no. no. 5, 6, 7, 8, 9, 10, 11, 12 and 13 — deriving from the Center of Pure Cultures of the Technical University in Łódź.

Additionally, 8 haploidal populations (7C, 9A, 10B, 11D, 14C, 14D, 15C and 18C) of bakers' yeasts Ja-64 [16] characterized by a good biomass yield and good growth rate, were included.

The following specific cultural media and reagents were used:

— pre-sporulating and sporulating media acc. to Clayton [2]
— media and buffer for determination of flocculation acc. to Greenshields et al. [5].

The monospore populations of initial yeasts were obtained in two stages:

1. Sporulation was induced on media acc. to the Clayton method [2].

2. Spores and monospore populations were isolated acc. to Fowell [4]. This procedure covered digestion of spore-forming walls of yeast cells with an enzymatic preparation from Helix pomatia, inactivation of

vegetative cells by the influence of temperature, seeding of a mixture of spores and residues of vegetative cells on agar medium and determination of mating type of the spore-nonforming populations obtained [15].

Hybrids were obtained by a combination of two monospore populations having opposite sex signs [4].

Zygotes were isolated using a micromanipulator and after their germination on a plate with nutritive medium, the capacity of spore-forming in the population was examined. This feature was considered to be a preliminary property informing about the production of a hybrid because the monospore clones did not sporulate.

The curves of yeasts growth were determined in malt wort 10°Blg at 28°C in shaken cultures. The samples were taken every three hours and the dry substance of yeasts was determined in weight. The time of generation was calculated from the formula:

$$T = \frac{t \cdot \ln 2}{\ln X_t - \ln X_0}$$

where:

T — period of generation,

t — time of duration of logarythmic growth pase,

X_t — dry substance of yeasts in culture after time t,

X_0 — dry substance of yeasts in culture at the beginning of the logarythmic phase,

The capabilities of strains for flocculation were checked, acc. to the method of Greenshields et al. [5], investigating the sedimentation of yeasts cells suspended in buffer with pH = 4.5.

The count of yeast cells was determined under a microscope, using Thom chamber. Fermentation capabilities were examined in relation to glucose, maltose, raffinose, arabinose and galactose, using Durham test [11].

RESULTS

Numerous repeated trials with the aim to induce sporulation in the initial strains of top fermenting yeasts revealed that besides strains 4, 14, 25 and 26 which did not produce spores, the percentage of spore-forming cells in the total cell count varied from below 0.1% (for strains 23 and 29) to 37% (for strain 28).

Yeasts of bottom fermentation sporulated very weakly- spores were obtained only for three strains (5,9 and 10).

Trrials aimed at an isolation of spores "in mass" repeated many times, resulted in a production of 10 monospore populations from 4 strains of top fermenting yeasts (strains no. no. 3, 15, 16 and 18; (Table); no monospore population was obtained from bottom fermenting yeasts. 40% of the monosporous populations obtained possessed mating type "α" (3-5, 15-11, 28-9, 28-19), the remaining ones — "a".

Table The results of mass spore isolation of brewers' yeasts

Strains	Count of isolated colonies	Count of growing clones	Count of clones having sex signs	Percentage of the obtained monosporular populations
Top fermentation				
2	10	1	0	0
3	20	5	2	10
15	15	2	2	15
16	15	2	2	15
18	20	6	4	10
21	10	9	0	0
22	85	18	0	0
23	10	6	0	0
24	117	9	0	0
27	120	6	0	0
29	12	2	0	0
Bottom fermentation				
5	15	4	0	0
9	10	1	0	0
10	10	1	0	0

The obtained monospore populations formed zygotes with the control "a" or "α" tester strains, so they seemed to be suitable for hybrides production.

Due to the fact that relatively few populations suitable for matching were obtained, all possible combinations of cells, in order to produce hybrides, were utilized.

In numerous trials the cells produced zygotes in 75% of trials only. Using a micromanipulator, 117 zygotes were isolated from which 60% germinated. 25% of populations obtained from the germinated zygotes formed spores, thus the coupling of non-sporeforming initial spore populations, was confirmed. Among the 17 obtained populations, 12 hybrids of brewers' yeasts and 5 hybrids of brewers' and bakers' yeasts, were further characterized.

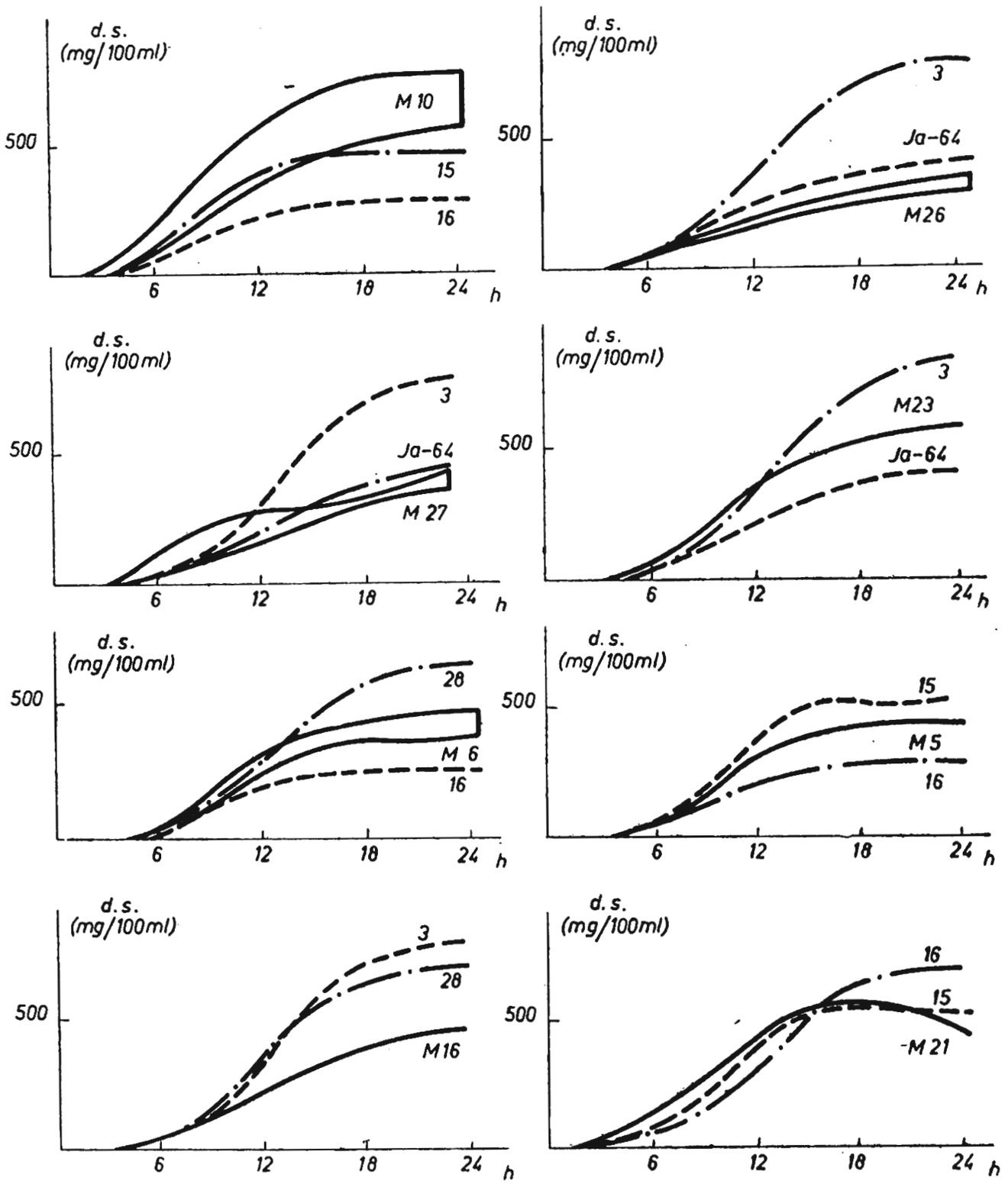

Fig. 1. Comparison of growth curves for hybrids and spore populations: bakers' hybrids — M26, M27 and M23, brewers' hybrids — M10, M6, M5, M16 and M21

Fig. 2. Comparison of growth curves for hybrids and initial strains; M26, M27, M23 — bakers' hybrids, M5, M6, M10, M16 and M21 — brewers' hybrids

Fig. 1, 2 and 3 illustrate the growth curves for the initial yeasts and hybrids, and generation periods of these strains. Among 10 hybrids obtained by a combination of monosporous populations of brewers' origin, six were characterized by a growth rate higher than the parental strains and the remaining hybrids possessed an intermediate growth rate or one equalling the growth rate of the parental strain.

From 5 hybrids, obtained by a combination of monocultures of brewers' yeasts and bakers' yeasts, only one population of hybrid grew slower than

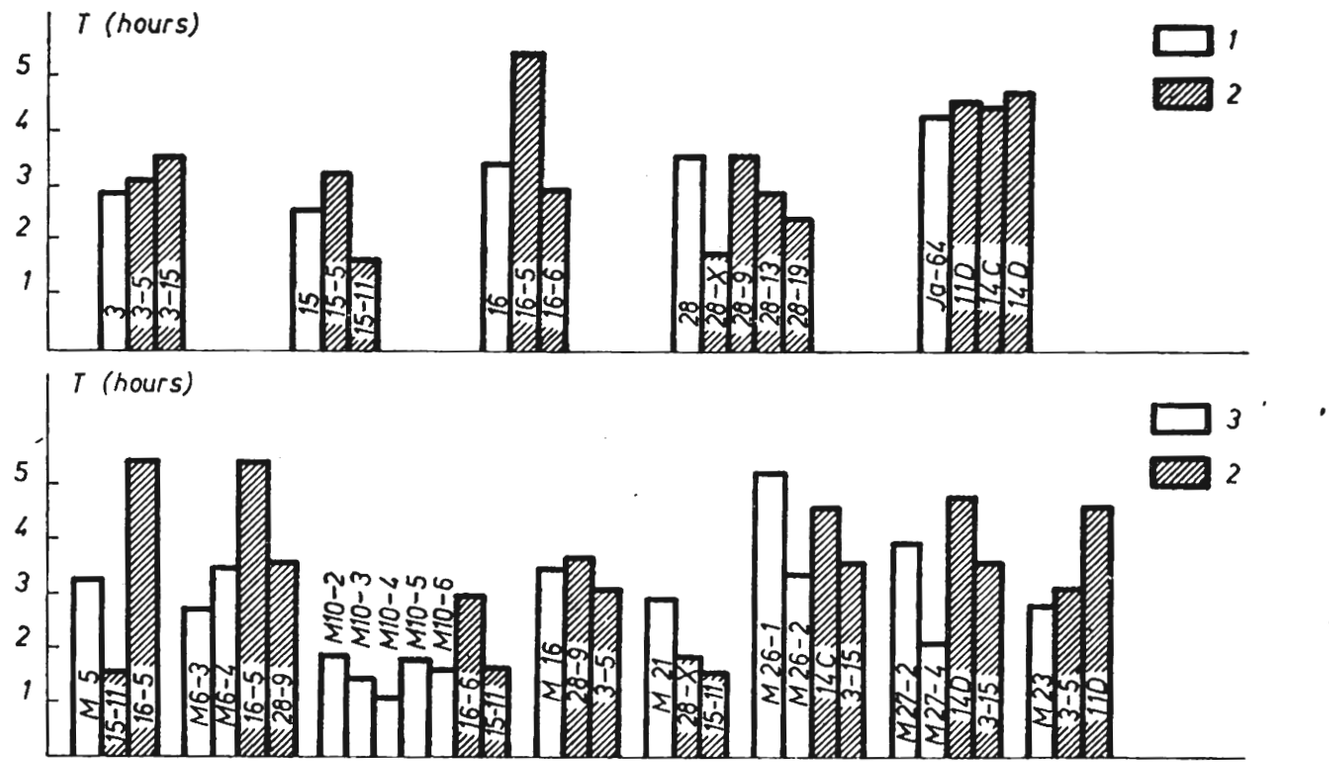

Fig. 3. Comparison of generation periods of initial strains of spore populations and hybrids; 1 — initial strains, 2 — spore populations, 3 — hybrids

the respective parental strains. Hybrids did not give any increased yield of biomass (except for M-10). The time of duration of the logarythmic growth phase was considerably differentiated and in this respect no regularity was achieved after production of hybrids.

The comparative results, characterizing the flocculation of the compared strains revealed that hybrids should be considered as powdery yeasts (Fig. 4), the only exception was strain no 28, showing on intermediate character between the typically powdery varieties and typically flocculating ones (Fig. 5).

The ability of sugar fermentation by hybrids, monosporous populations and intial strains showed that all strains were varieties of top brewers' yeasts. Raffinose was fermented by them in one-third, with the exception of monosporous clone 28-x which did not posses the abilities of sugar fermentation.

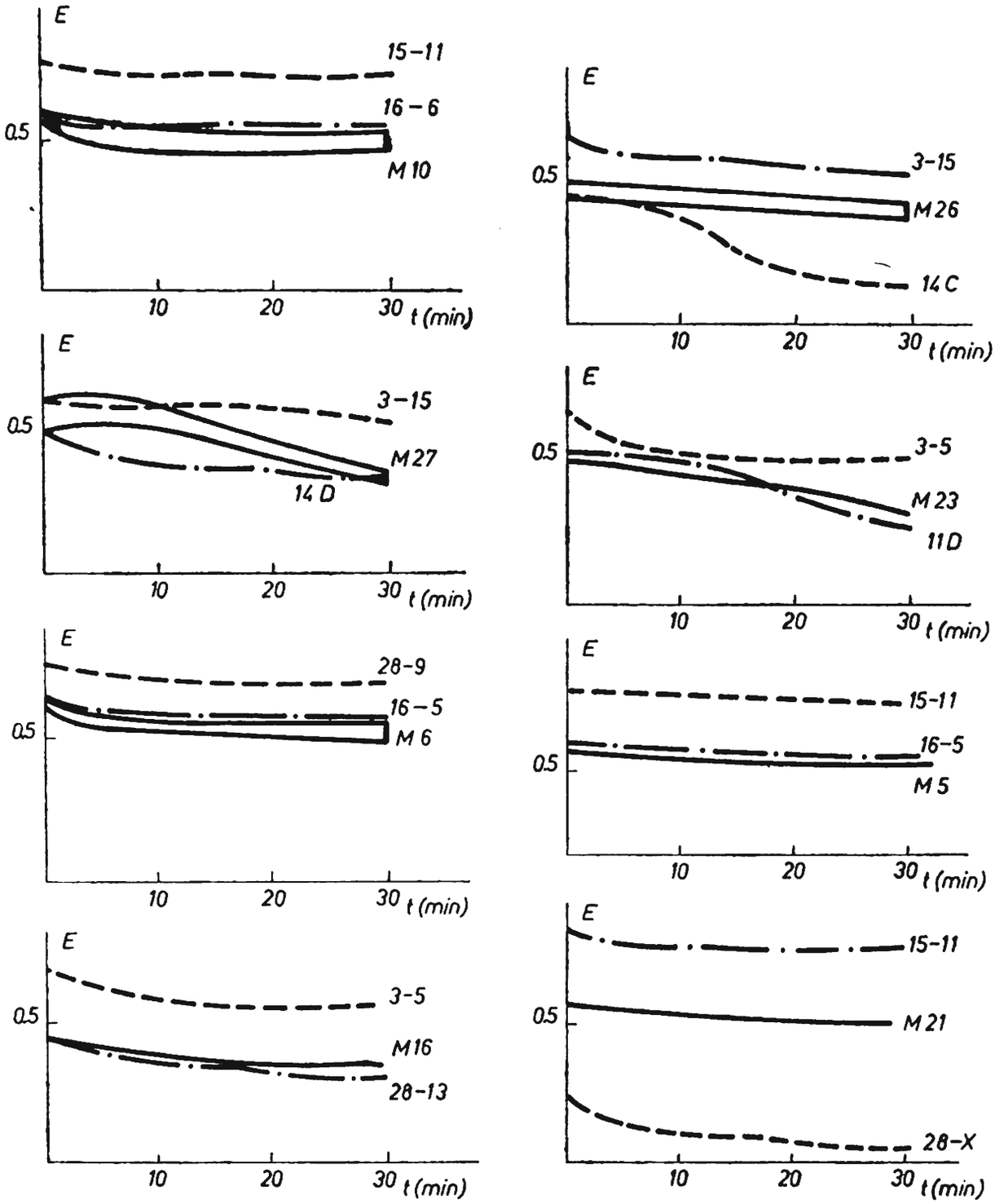

Fig. 4. Comparison of flocculating capability of hybrids and spore populations; extinctions were determined at 660 nm, the full line means hybrids, dotted line means spore populations

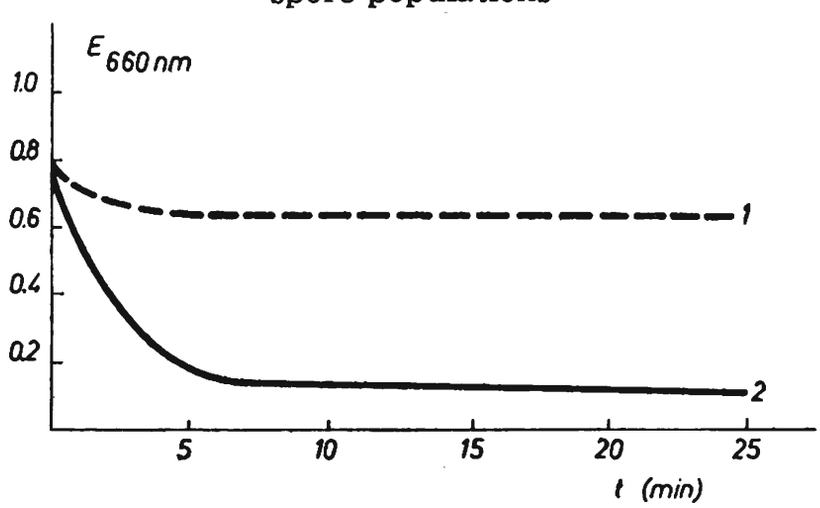

Fig. 5. Tendencies of flocculation, typical of top fermenting strains: powdery and flocculating ones; 1 — powdery strains, 2 — flocculating strain

DISCUSSION

The examined brewers' yeasts were difficult for production of hybrids, due to their biological properties as well as the technological exigeance. The difficulties connected with their production begin already at the stage of sporulation. In order to cope with this problem, pre-sporulating and sporulation-inducing media were utilized in the present work [2, 4, 6]. However, the application of optimal conditions for this process did not ensure satisfactory results. The effects of sporulation were considerably worse than those cited in literature.

Only 40% strains of bottom fermenting yeasts formed spores and only sporadically; 30% of top fermenting yeasts did not sporulate in any series of trials. The remaining strains of this group of yeasts sporulated with a small efficiency and the percentage of sporulation in the population did not exceed 20%.

The confrontation of these results with the literature data shows that this feature is considerably differentiated. There is no regularity in this respect. Johnston [9], Clayton [2], Roth and Halvorson [17] obtained divergent data, showing sporulation abilities from 5% to 32% of the examined varieties.

The effectiveness os spores isolation obtained by us expressed as a ratio between the amount of the obtained monosporous populations and the amount of isolated spores is approximal to the data by Johnston [8] and amounts to 14%.

The coupling of spores by "mass mating" technique was carried ont according to Johnston [9] and Fowell [4] who considered that this method of obtaining hybrids of brewers' yeasts is more effective when compared to other types. The effectiveness of obtaining hybrids in the present study is by approx. 40% lower than that obtained by Clayton [2]. This author's suggestions were confirmed, i.e. that the powderiness of the monosporous population of bakers' yeasts is a dominating feature in hybrids in comparison with flocculating ability.

The strains of brewers' yeasts of both top and bottom fermentation differ considerably in flocculation strength, defined by Jörgensen [12] as the capability of yeasts to form small, macroscopically visible floccules, falling down the bottom of a vessel during fermentation.

This work makes use of a method of discovering the flocculation capabilities based on a turbidimetric measurements of cell sedimentation in buffer with pH = 4.5, suggested by Greenshields et al. [5] and Calley and Johnston [1] who have compared two methods: macroscopic and colorimetric and stated that both methods are good but the colorimetric one is easier and more convenient for routine work.

Most of the strains examined in the present work revealed a typically

powdery nature. Among the monosporous populations obtained there is a lack of typical flocculating strain which might be used for crosses; this makes the assessment of powder flocculation-transmitting ability more complicated and undoubtedly it should be the subject of more detailed studies [11].

CONCLUSIONS

1. The home strains of brewers' yeasts sporulate to a very weak extent. Among the 24 examined strains, only about 30% formed spores.

2. The application of method of spores isolation "in mass", resulted in 10 monospore populations of top fermentation brewery yeasts with differentiated sex signs, suitable for production of hybrids.

3. No sexually active monospore population of bottom fermentation brewers' yeasts was obtained.

4. As a results of numerous crosses of the obtained spore populations of brewers' and bakers' yeasts, 17 stable hybrids were obtained, including 7 resulting from a combination of bakers' and brewers' yeasts strains. Hybrids constituted only about 15% of all isolated zygotes.

5. The obtained hybrids were characterized by differentiated periods of generation in the range 1.1-5.3 hours. About 50% of hybrids had a shorter generation period than the spore populations from which they derived. Only among some of them, a longer period of generation time was stated when compared to mother strains. These data form the basis for obtaining varieties of brewers' yeasts with considerably shorter periods of generation time which may contribute to a significant shortening of the technological process.

6. As a result of strains coupling, exclusively a population of powdery yeasts was obtained. The flocculation property was not revealed in the hybrids obtained.

7. The obtained monosporous populations of brewery yeasts and their hybrids point to the possibility of applying the method of hybridization in production of hybrids of brewers' yeasts for brewery technology — especially for new types of beers and technological processes.

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OTRZYMYWANIE POPULACJI MONOSPOROWYCH I HYBRYDÓW DROŻDŻY PIWOWARSKICH GÓRNEJ FERMENTACJI

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Streszczenie

Spośród 24 krajowych szczepów drożdży piwowarskich jedynie 30% wytworzyło zarodniki. Metodą izolacji spor „w masie" otrzymano z nich monosporowe populacje o zróżnicowanych znakach płciowych nadające się do tworzenia mieszańców. Nie uzyskano żadnej aktywnej płciowo populacji monosporowej drożdży piwnych dolnej fermentacji.

W wyniku licznych kojarzeń populacji monosporowych drożdży piwowarskich i piekarskich uzyskano 17 trwałych odmian, w tym 7 z kojarzenia drożdży piekarskich i piwowarskich. Mieszańce stanowiły tylko 15% ogółu zygot wyizolowanych metodą mikromanipulacji. Miały one zróżnicowany okres generacji w przedziale T = 1.1 do 5.3 h. W tym ok. 50% szczepów miało okres generacji krótszy niż populacje monosporowe, z których pochodziły.

Zasługuje na podkreślenie fakt, iż w otrzymanych hybrydach nie ujawniła się cecha kłaczkowacenia. Wszystkie nowe odmiany należały do odmian pylistych.

W pracy wykazano możliwość stosowania techniki izolacji i łączenia spor „w masie" do konstrukcji mieszańców drożdży przydatnych dla technologii piwowarskiej.