NO INBREEDING DEGENERATION IN A HOMOZYGOUS HUNGARIAN WINE YEAST.

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With 4 Tables in the text.

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The heterothallia long observed in various fungi has been demonstrated in the *Saccharomyces* genus only by recent genetical investigations. Besides the very decided appearance of heterothallia in *Saccharomycodes Ludwigii* (WINGE and LAUSTSEN, 1939b), we must consider its a/α as such, which LINDEGREN (1943a), on the basis of his investigations so far, considers a general phenomenon, and the existence of which is also recognized by WINGE (1948). The consequence of heterothallia is that in the germination of isolated spores of the Saccharomycetes there is inbreeding degeneration in the developed cultures, as the regular spore zygote formation does not occur among the sibling spores. The Zygosaccharomycetes seem exempt from this degeneration (WINGE, 1940).

Though with the exception of the Zygosaccharomycetes LINDEGREN (1944a) considers inbreeding degeneration of general and unavoidable occurrence, there have nevertheless been some cases where no degeneration could be found in the single ascospore cultures. DIETLEVSEN (1944) found no data confirming segregation in *Saccharomyces italicus.* This yeast seemed homozygote and WINGE (1944) considers it a special case where degeneration does not occur but, besides the absence of simple segregation, it is possible that the species has gone through a mutation, or that the material investigated "originally may have been a mixture of different biotypes". These questions remain to be cleared up in future.

To LINDEGREN's (1944a) positive assertion that viable ascospores develop only from such zygotes as are heterozygote for a/α genes, as well as to the hypothesis (LINDEGREN, 1943b) that to assure the production of viable ascospores in the yeast fertilization is necessary, WINGE (1948) summarizes the exceptions thus far found. These in general are the Zygosaccharomycetes, *Saccharomyces validus* and *Saccharomyces italicus.*

In seeking for segregation in a strongly fermenting Hungarian wine yeast we thought to have found a new example among the small number of exceptions to this general rule of inbreeding degeneration (BÁNHIDI, 1948).

The absence of segregation in respect to giant colonies and sporulating capacity in the first generation of this wine yeast make more detailed investigation necessary.

MATERIAL AND METHOD.

The material investigated consisted of the afore-mentioned "Balatonfüred 2" (750 e_1) strain originating from a one-cell culture, as well as the 4 F_1 strains deriving from it and the 16 single ascospore cultures of the F_2 generation. The cultivation, segregation, spore-isolation and sporulation technics were the same as in the work previously mentioned.

During the separations and isolations made with the micromanipulator, on touching the ascus with one of the needles, it sticks to the vapour droplets surrounding it and can thus be drawn on to the cover slip, full of such droplets, to the wort suspension drop. The vapour drops often get bigger as the ascus is drawn along. From this augmented drop, on eliminating the superfluous fluid, we repeatedly drew off the ascus by simple cohesion; in the process it could be entirely cleansed of any small impurities clinging to it, such as mildew spores and bacteria. With this method it was easy to isolate pure one-cell or one-spore cultures, even from cultures strongly infected with bacteria or mildew.

The fermentation experiments were made in 250 ml PASTEUR bottles. 100 ml 22° (KLOSTERNEUBURG) must served as culture medium. The inoculation was made with a platinum hookful of 4 day old must medium from a FREUDENREICH flask. Fermentation took place at 26°C. The weight-loss shown was measured to an exactitude of centigrams.

RESULTS AND DISCUSSION.

In Table I we offer data on the germinating capacity of the isolated spores, i. e., the number of colonies developed in 4 daughter strains of the "Balaton Füred 2" yeast and, for comparison, summarized data on their mother strains (BÁNHIDI, 1948).

TABLE I.

Germinating capacity of isolated spores.

Strain	Total no. of isolated spores	Germinated spores		Total isolated spores in one strain	Total germinating spores in one strain
		Singly	Total		
Z III	8	2	4	24	18
„	8	3	6	24	18
„	8	4	8		
Z III	4	3	3		
„	8	4	8	12	11
Z III	20	4	20	20	20
Z III	4	0	0		
„	12	3	9	20	13
„	4	4	4		
750 e_1	—	—	—	36	22

WINGE (1944), in discussing some of his complicated cases of segregation and crossing over, states that it often happens that all 4 spores of one ascus do not germinate. He considers that if there are differences between the germinated spores the germination of 3 out of 4 spores is sufficient for establishing that the types deriving from the 4 parent spores are not pairwise identical. It is apparent from Table I that if in our investigations we had operated on fewer asci in each strain we might easily have considered the non-germination of one or two spores to be segregation according to a 1:3 or 2:2 formula, supposing the non-germination of one spore to be

due to a regularly appearing lethal factor. Because of the non-germination we continued to operate on asci deriving from a completely identical homozygote and we always obtained an ascus all four isolated spores of which germinated and formed colonies of equal value.

From the 4 daughter strains isolated by micromanipulator from the original mother strain we isolated altogether 16 progeny deriving from the 4 asci, and these again produced asci. In the mother (P), the daughter (F_1) and the granddaughter (F_2) strains we examined a large number of asci as to sporulation and have expressed in percent the distribution of the 1, 2, 3 and 4 spored asci, as follows:

TABLE II.

Distribution in the three generations of asci with different spore numbers.

Generation	one-spored		2-spored		3-spored		4-spored		Total asci separated in one generation.
	a s c i								
P	753	23.31	1561	48.34	739	22.87	180	5.50	3233
F_1	449	15.84	1648	53.16	754	24.32	207	6.69	3100
F_2	237	5.26	2914	64.67	1128	24.89	223	4.97	4502
Total	—		—		—		—		10835
Extent of % ual variation		18.05		16.35		2.02		1.72	

The number of asci counted is sufficient to show with complete certainty that there is no significant difference between the different generations as to production of asci with more than two spores, and this fact corresponds completely with the data published by WINGE (1940 and 1948) on the absence of inbreeding degeneration. Although we examined only three generations it must be remarked that the percentual ratio of one-spore asci decreases successively to about a fourth of those present in the mother strain and parallel with this there is a consecutive increase in the percentual ratio of 2-spored asci during the three generations. LINDEGREN's (1994a) observation that a greater number of 2-spored asci arise from the sporulation of one-spored cultures cannot be left completely out of account, for in the three generations we also found 64.67% 2-spored asci, but without a

decrease in the percentage of 4-spored due to degeneration. The fact that simultaneously with the increase in 2-spored, the number of one-spored asci decreased gave rise to the suspicion that the reduced number of spores, instead of indicating a degeneration, represents a tendency towards equilibrium in the course of breeding pure single ascospore cultures in a homozygote strain free from degeneration, like the material under investigation — which perhaps to a certain extent combats inbreeding degeneration.

We selected 4 cultures from the F_2 progeny each deriving from a different F_1 strain, and investigated the germinating capacity of the different-spored asci formed from them. We could thus be convinced that the one-spore cultures of our initial strains did not degenerate even in the F_2 generation, because, as can be seen in Table III, there was no change in the germinating capacity of the spores in this generation as compared with the mother strain.

TABLE III.

Germinating capacity of the spores in the third generation.

Strain	1-spored asci		2-spored asci		3-spored asci		4-spored asci		Total	
	Separ.	Germ.	Separ.	Germ.	Separ.	Germ.	Separ.	Germ.	Separ.	Germ.
Z III. α_1	1	1	8	8	6	6	5	5	20	20
Z III. β_2	5	5	10	8	3	3	2	1	20	17
Z III. γ_4	4	4	8	8	5	5	2	2	19	19
2 III. δ_3	2	0	10	6	2	1	3	3	17	10
Total	12	10	36	30	16	15	12	11	76	66

In investigating fermentative capacity we set out from WINGE's (1994b) hypothesis that though in respect to the qualitative appearance of certain specific enzymes the yeasts in general seem homozygote, this does not exclude the possibility that in cases of segregation quantitative differences might not arise in the production of the enzyme in question and thus also in its fermentative capacity. In our investigation of fermentative capacity carried out in the way mentioned, there were larger differences in weight-loss between the different strains the first 6 days, so we repeated the investigations in 5 series

and, taking their arithmetical mean values for 16 days, obtained the results shown in Table IV.

TABLE IV.

Mean values in fermentative weight-loss in gs in 100 ml culture medium in 5 parallel series.

Strain	No. of days. 2	3	4	5	6	7	9	10	11	16
750 e_1	0.95	4.35	6.26	7.25	8.17	8.83	9.35	9.77	9,88	10,57
Z III α	0.42	3.78	5.89	6.99	8.19	8.72	9.54	9.76	9,93	10,60
Z III β	0.60	3.87	5.93	6.99	7.96	8.72	9.44	9.69	9,80	10,50
Z III γ	0.61	4.32	6.23	7.33	8.13	8.78	9.42	10.04	10,18	10,83
Z III δ	0.74	3.47	5.37	6.60	7.70	8.36	9.16	9.43	9,56	10,59

We sought for segregation possibilities in wine yeast because several strains of standard wine yeast of one-cell origin were the first bases of our research, and genetical research so far has shown that if a culture derives from one single vegetative yeast cell we have no guarantee that it will give a pure culture. WINGE and LAUSTSEN (1937) see proof of this finding of theirs in the fact that by haploid spore formation the yeast cells are capable of segregating new types and this spore formation can also occur in cases which could not be in the least controlled — e. g., on the glass sides of FREUDENREICH flasks for preserving, containing a 10% sucrose solution. The present investigations have shown that if degeneration does not occur in the course of stabilization of derivatives of 4-spored asci in the spore formation of homozygote sister strains, then there will be no appreciable difference in their fermentative capacity. This relation in the preparation of pure yeast cultures gives reassuring data, by control of the remaining sporulating capacity, in respect to the fermentative usefulness of the strain in question.

SUMMARY.

We prepared single ascospore cultures from the 16 progeny of 4 daughter strains of the "Balaton Füred 2" strain of Hungarian wine yeast and found a successive increase in the percentual ratio of 2-

spored asci at the expense of the one-spored, as opposed to the proportions maintained in the mother strain and the F_1 generation. The ratio of 3 and 4 spored asci did not change during the three generations. The germinating capacity of the asci did not diminish even in the third generation. Investigation of fermentative capacity showed no differences between the mother and daughter strains.

The data from this investigation establish that the material examined is free from the inbreeding degeneration which usually occurs.

In none of the three generations of spores isolated by micromanipulator are colonies regularly formed in all cases, but in operating on many asci we could always find 4-spored ones all the isolated spores of which did form colonies. From this we point out that the non-germination of one or two sister spores deriving from one 4-spored ascus cannot always be taken as a sign of heterozygosis.

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