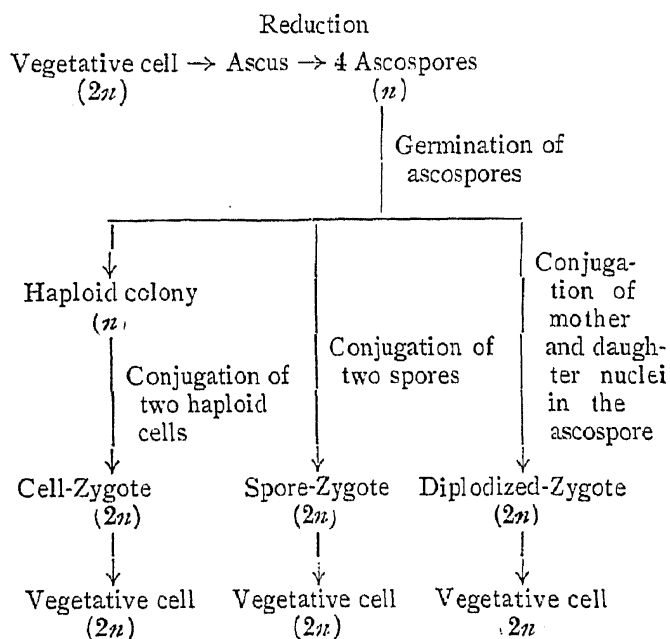


THE CYTOLOGY AND GENETICS OF YEASTS

IN 1935 Prof. O. Winge announced his pioneering researches¹ on the artificial hybridisation of yeasts. Five papers^{2,3,4,5,6} which have since appeared from his laboratories constitute a valuable series of researches on the genetics of yeasts. More than a dozen yeast hybrids, one of them possessing remarkable characteristics of value to the brewing industry, have been evolved by Prof. Winge.

It was generally believed that the vegetative phase in the life-cycle of yeasts was haploid and that the ascospores in the saccharomycetes were parthenogenetically formed. By adopting an elegant microtechnique, Prof. Winge isolated and cultured individually under the microscope, all the four ascospores of an ascus. This technique facilitated the observation of all the cells of a microcolony. As a result of his studies, Prof. Winge showed that the life-cycle among saccharomycetes may be represented schematically as follows^{1,2}:—



The one significant fact which emerges out of this work is that in the genus saccharomycetes, the cells are continuously diploid in the vegetative phase; there is a definite alteration of haploid and diploid generations in the life-cycle. Further, the ascospores germinate and produce the next generation of vegetative cells in any one of the following three ways: (a) The ascospores germinate to produce a haploid colony of limited size whose cells are characteristically round and non-sporulating on plaster block directly on transference. The cells of this colony conjugate to give multiple cell-zygotes which, on germination, give rise to the diploid cells, characteristically elongated, distally budding and chain forming and directly sporulating on plaster block. It is evident that the isolation and germination the cell-zygote produced as a result of cell fusion in the haploid colony, gives a completely homozygous yeast. It is also evident that the fusing cells in the haploid colony are not distinguishable regarding their sex both genotypically and phenotypically. (b) A pair of ascospores conjugate

and give rise to a sporezygote which on germination gives the typically elongated vegetative cells. (c) Direct diplodization of the ascospore nucleus takes place, i.e., the daughter and the mother nuclei fuse in the ascospore itself without giving rise to a bud. On germination, this diplodized zygote gives rise to the vegetative cells directly. It is this behaviour that was responsible for the older conception of the parthenogenetic origin of ascospores in saccharomycetes.

The study of the germination behaviour of ascospores in successive generations of cells, revealed that ascospores from yeasts originating from diplodized zygotes have such a low germinating power as to render the yeast almost sterile. This sterility, according to Prof. Winge and his collaborator is due to the effect of inbreeding on the cytoplasm, particularly, to the maldistribution of chondriosomes amongst the ascospores.⁶

It will be noticed that no work has been done on the chromosome complement in these yeasts. Though the genetical work leaves no doubt as to the reality of the alternation of generations in saccharomycetes; for scientific completeness the chromosome counts in the haploid and diploid phases is necessary.

Winge and Laustsen² demonstrated the genetic segregations in the ascus of a variety of *Saccharomyces ellipsoideus*. Their method consisted in isolating all the four ascospores from an ascus by means of the microtechnique and growing them into giant colonies individually on gelatine. In a typical experiment, two spores germinated forming haploid cells and the remaining two germinated directly into diploid cells. Apparently the latter two ascospores were diplodized zygotes. Of the former two haploid cell microcolonies, one was observed to produce cell zygotes while the other remained sterile, i.e., all attempts to induce zygote formation failed. The cells of this sterile segregate resembled Toruloid yeast. The cell zygotes of the other microcolony behaved normally (i.e., they produced actively budding vegetative cells) and also sporulated immediately on transference to the plaster block, thus strongly recalling the sporulating behaviour of Zygosaccharomycetes. The easily observable macromorphological features of these four segregates on gelatine, three being diploid giant colonies and the remaining one being sterile haploid giant colony, strikingly demonstrated the genetic segregation in the ascus. In this connection, two other points must be stated: the segregating types are not identical from ascus to ascus and there is not always pairwise identity in the four giant colonies. The former fact shows that macromorphological features are multifactorially controlled and the latter fact shows that crossing-over is not infrequent during reduction division.

In two subsequent papers^{3,4} the authors present results of their extensive work on artificial hybridization amongst the members of the genus *Saccharomyces*, resulting in no less than fourteen hybrids, some interspecific and others

being intervarietal hybrids. The method of bringing about the cross was to bring the two spores of the parent yeasts in a droplet of wort and to observe under the microscope the spore zygote formation. This is achieved only after a number of trials, since it is purely a matter of chance that the two spores should germinate simultaneously and effectively conjugate.

The interspecific cross between *Saccharomyces ellipsoideus* (baking yeast) and *S. validus* has been presented by the authors in great detail.^{3,4} An attempt is made to summarise the results in the following table:—

Of pure genetical interest is the author's work⁵ on *Saccharomyces Ludwiggii* Hansen which has been proved to be a balanced-heterozygote involving heterothallism in the haploid phase.

The scientific and economic importance of these researches can hardly be overestimated. Scientifically this work will go a long way in clearing up the taxonomy and phylogeny of the genus *Saccharomyces* and other genera like *Torula* and *Zygosaccharomyces*. For preserving the constancy of type, Hansen's single-cell pure culture must be replaced by single-spore pure cultures. Systematic breeding work for

	<i>Baking yeast</i>	<i>S. validus</i>	<i>Hybrid</i>
1. Cells ..	Oval	Elongated	Oval but distinguishable from cells of the baking yeast
2. Sediment in wort ..	Smooth	Highly granulated	Intermediate to that of parents
3. Giant colony ..	Circular in shape with delicate silky radiate striation	Very irregular outline, rough sculpture of irregular concentric and radiate winding, deep furrows twisted almost like a cerebral	Intermediate to a certain extent, with pronounced concentric and radiate furrows and irregular outline
4. Germinating capacity of the ascospores	68 per cent.	78 per cent.	2 per cent
5. Biochemical character ..	Ferments only $\frac{1}{3}$ of raffinose, <i>i.e.</i> , the yeast produces raffinase but not melibiase	Ferments raffinose completely and hence produces both raffinase and melibiase	Ferments raffinose completely, showing thereby that the presence of an enzyme is dominant over its absence
6. Rate of dry matter production	90 mg. in 72 hours	Variable but definitely at a much lower rate than its mate and the hybrid	98.6 mg. in 72 hours
7. Common method of zygote formation	Spore zygote	Spore zygote	Spore zygotes not formed or formed extremely rarely

The table is to a certain extent self-explanatory; two points may be noted. Firstly the low germinating power of the above hybrid is a general phenomenon common to all interspecific hybrids; in fact, low germinating capacity (0 to 13 per cent.) and high germinating capacity (50 to 94 per cent.) of the various hybrids are taken as indicative of specific differences between the parents. Secondly, specific differences in germinating capacity are accompanied by biochemical differences in fermentation.⁴

obtaining yeasts of desired quality has now been rendered possible.

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¹ Winge, O., *Compt. Rend. Trav. Lab.*, Carlsberg, 1935, **21**, 77.

² — and Laustsen, *ibid.*, 1937, **22**, 99.

³ — —, *ibid.*, 1938, **22**, 235.

⁴ — —, *ibid.*, 1939, **22**, 337.

⁵ — —, *ibid.*, 1939, **22**, 357.

⁶ — —, *ibid.*, 1940, **23**, 17.