A NEW METHOD FOR HYBRIDIZING YEAST

By Carl C. Lindegren and Gertrude Lindegren*

The Henry Shaw School of Botany, Washington University, St. Louis

Communicated September 13, 1943

Winge and Laustsen[1] hybridized yeasts by placing a haploid ascospore from one strain in close proximity to an ascospore of a second strain by use of the micromanipulator. When all conditions are favorable, the two spores fuse to produce a diploid hybrid cell. We[2] have used this method extensively, and have found that copulation usually fails (1) if the two spores are of the same mating type, (2) if either of the spores germinates directly into a diploid cell (this happens rather frequently, especially in vigorous strains), (3) if either spore is inviable (viability is generally about 50 to 75 per cent). These facts mean that relatively few hybrids are obtained by ascospore to ascospore matings. The method has the further disadvantage that the characteristics of the parents cannot be determined, since the single haploid spore used as a parent is consumed in the mating process. This is particularly important in the yeasts which are extremely heterozygous.

We have developed a new procedure based on the fact that some single ascospores from *Saccharomyces cerevisiae* produce persistently haploid cultures. It is possible to hybridize these with other similarly derived persistently haploid cultures simply by mixing the cells together in an appropriate medium. These mixtures result in copulation if each culture is paired with a complementary type. One test tube can be used for large number of matings. This experiment has given further support to our proposed scheme of alleles controlling copulation in *S. cerevisiae* and other indications prove that a simple allellism obtains. Morphology and biochemical characteristics can be studied previous to matings, and matings made between complementary types result in copulation tubes and diploid zygotes.

The four spores from one single ascus all produced persistently haplophase cultures. The four single spores were designated, *A*, *B*, *C* and *D*, and the haplophase cultures were paired in all combinations. *A* and *D* were found to belong to the same mating type, while *B* and *C* belonged

---

DESCRIPTION OF FIGURE 1

The four stable haplophase single ascospore cultures derived from a single ascus of *Saccharomyces cerevisae* (*A*, *B*, *C* and *D*) and the results of pairing these cultures in all possible combinations. $A \times D$ and $B \times C$ pairings do not result in copulations. The $A \times B$ pairing results in copulation, and the zygotes are much larger than the cells found in either *A* or *B* alone, suggesting that the hybrid is more vigorous than either parent. The same increase in size is observed in the $C \times D$, $A \times C$ and $B \times D$ pairings.

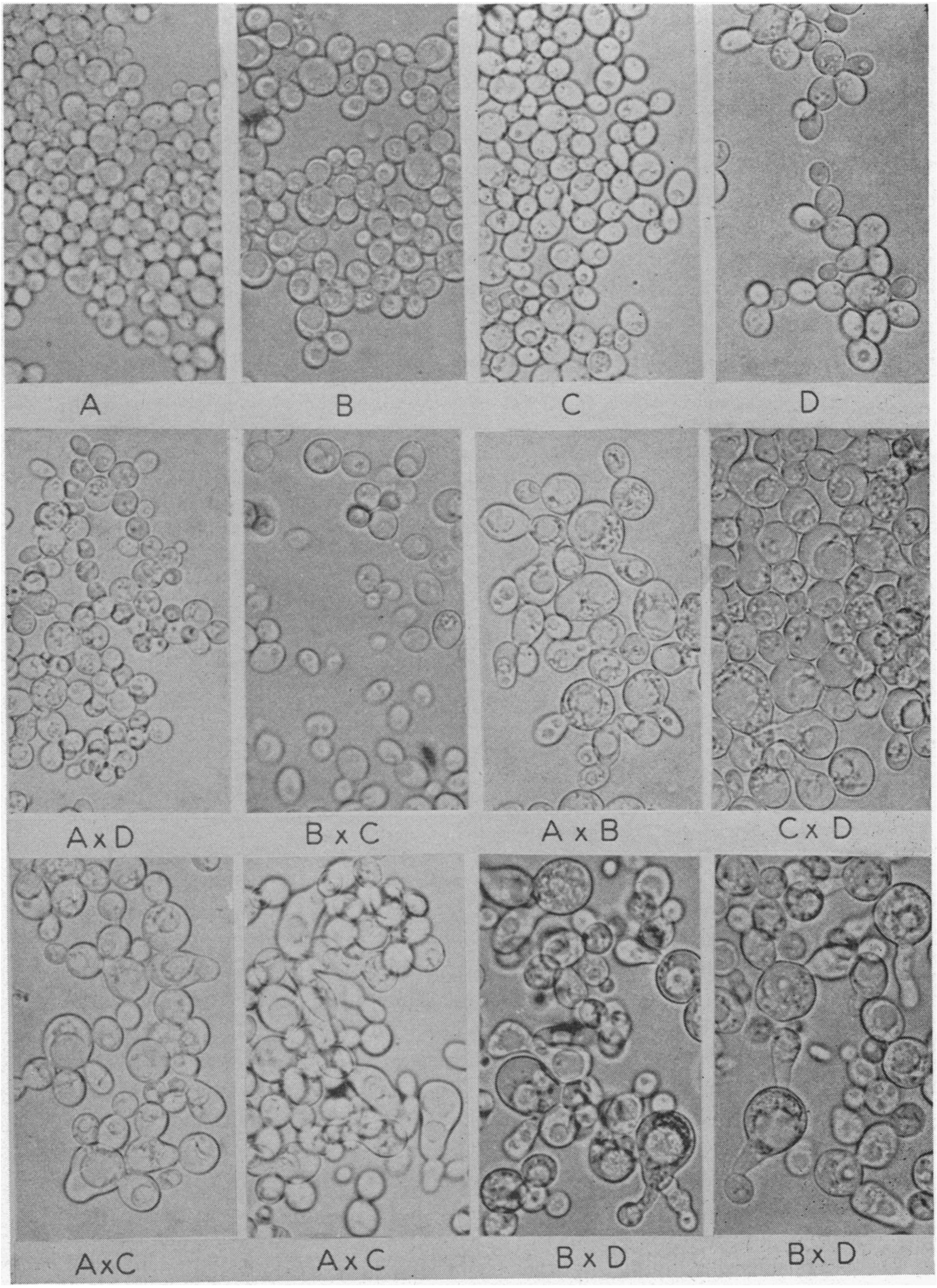

FIGURE 1 (Description on Opposite Page)

to the complementary type. Copulation tubes and zygotes were produced when A and B, A and C, B and D, and C and D were paired. These results are shown in figure 1. When transferred to gypsum,[3] the diploid cells produced 4-spored asci, while the A and D, B and C combinations failed. No spores were obtained from any of the unmated single ascospore cultures.

We have already confirmed the views of Winge[4] and Satava[5] that the round-celled members of the genus *Torula* are imperfect forms derived from the genus *Saccharomyces*. In the present experiment these *Torulae* when properly paired produced copulation tubes according to the pattern of the genus *Zygosaccharomyces*, indicating that it is also identical to *Saccharomyces*.

* This work was supported by a grant from Annheuser-Busch, Inc., St. Louis.

[1] Winge, Ö., and Laustsen, O., *Compt. rend. travaux lab. Carlsberg, série physiologique*, **22**, 337 (1929).

[2] Lindegren, C. C., and Lindegren, G., *Jour. Bact.* (in press) (1943).

[3] Lindegren, C. C., and Lindegren, G., *Bot. Gaz.* (in press) (1943).

[4] Winge, Ö., *Compt. rend. travaux lab. Carlsberg, série physiologique*, **21**, 77 (1935).

[5] Satava, J., *III[e] Congres international technique et chimique des industries agricoles*, Paris, 1934.

SPECIAL INVARIANT SUBGROUPS

BY G. A. MILLER

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF ILLINOIS

Communicated September 30, 1943

The concept of invariant subgroup but not the name thereof was introduced by E. Galois, who considered the subgroups which have the property that when in a finite group G all the elements of the given group are arranged in the form of a rectangle with respect to the subgroup H as the first row, then all the co-sets, or rows, thus obtained are the same independently of whether these elements are arranged in the form of right co-sets or in the form of left co-sets with respect to H. If all these co-sets are the same in these two arrangements then H is now commonly called an invariant subgroup of G, but if not all of them are the same then H is called a non-invariant subgroup of G. E. Galois did not give a special name to these subgroups but he spoke of the decomposition of G with respect to such subgroups and it is on this account that the concept of invariant subgroup is now credited to him.

A considerable number of other terms have been used instead of the term invariant subgroup. Among these are the terms self-conjugate subgroup