THE KANSAS UNIVERSITY SCIENCE BULLETIN. VOL. IV, NO. 1, MARCH, 1907 (WHOLE SERIES, VOL. XIV, NO. 1): ON HEREDITY IN CERTAIN MICRO-ORGANISMS

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ON HEREDITY IN CERTAIN MICRO-ORGANISMS.

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With plates I to IV.

THE aim of the work described in this paper has been to conduct with certain micro-organisms investigations on heredity similar to those long practiced with higher plants and animals. From the offspring of single varying cells new races of yeast and bacteria have been obtained, which differ morphologically and physiologically from the type, and this paper is mainly given to a study of the origin and characteristics of these new races.

In order to accomplish this work, involving as it does the selection and isolation of single varying cells lying among thousands of normal ones, a new method of isolation had to be devised, a method which is described in another part of this paper.

The investigations described below have had to do principally with the yeast *Saccharomyces anomalus* and the bacterium *Bacillus coli communis;* though some work was also done with *B. typhosus* and a large, spore-forming bacillus, probably *B. megatherium*. In every case the work was done with absolutely pure cultures, known to be such because known to be the descendants of single isolated normal cells.

In reviewing the literature on this subject, I shall confine myself largely to those relatively few papers which deal with the heredity of new races which have apparently arisen spontaneously from cells varying independently of the environments, and I shall omit the large number of publications

having to do with modifications of micro-organisms induced by altered conditions of culture, unless such modifications are known or supposed to originate from single varying cells.

Hansen, who has been the pioneer in work of this character conducted on yeasts, succeeded in obtaining asporogenous races of *Saccharomyces pastorianus* I, II, and III, *S. cerevisiæ* I, *S. ellipsoideus* I and other normally spore-bearing species by plating in gelatin and testing the offspring of various colonies by placing them on gypsum blocks. He found great variation in the spore-producing power of these colonies, varying from normal to races which have remained asporogenous, though cultivated under diverse conditions for twelve years. He found that some varieties, as *johannesberg* II, could not be made to produce asporogenous forms, except by previously cultivating the yeast at temperatures approaching the optimum temperature of budding, and that in practically all forms the per cent. of asporogenous varieties was increased by this preliminary treatment. Here we have to do with a possible transformation associated with selection; but in the case of those forms which gave asporogenous varieties without this treatment, for example *S. pastorianus* I, which gave five to ten per cent. of asporogenous colonies at the start, we deal with probably spontaneous variations.

Associated with the loss of power of producing spores, Hansen found the loss of power to produce veils. *S. anomalus*, however, did not lose this faculty in asporogenous varieties.

Further, the author found types presenting growth of cheesy character and branching filaments, forms which persisted for a number of generations. Again, cells showing a tendency to extend in an elongated mycelium-like form were found to transmit their peculiarities. In the case of *S. ludwigii* the mycelium-like type returned to the normal form when grown in wort. In type No. I of *carlsberg*, Hansen obtained a strain presenting abnormally elongated cells which preserved its peculiarities during two months' cultivation in wort. This type finally returned to its normal form.

A number of races physiologically different which proceed from the same pure culture are mentioned by Hansen. Among these are types showing an increased power of pro-

ducing alcoholic fermentation. A type of *S. cerevisiæ* produced one to three volumes per cent. more alcohol than the parent form from which it was derived.

Other strains differed from their types in their power of forming invertase and maltase. A race of *S. pastorianus* I for some time failed to impart to beer a bitter taste and disagreeable odor characteristic of the type; and variations of other types having to do with the clarification of beer and other aspects of brewing are mentioned by the author.

These physiological types originated for the most part in changed conditions of cultivation. For instance, Biernacki and, later, Märcker, Haydruck and Effront have found that the addition of small quantities of antiseptics to cultures of certain yeasts may increase their fermentative power. But Hansen is of the opinion that in some of these cases, at least, we have to do with a selection of cells endowed with certain physiological characteristics and not with mere transformation. The relative weight of these two factors in such experiments is a matter hard to determine.

M. W. Beijerinck (1897) isolated from a species of *Schizosaccharomyces* found on fruits of tropical origin two sorts of colonies on wort gelatin. One sort, brown in color, was asporogenous; the other, which was white, produced spores, and intermediate forms occurred. On testing the offspring of these colonies, the white variety was found to give nearly all white colonies; the brown gave brown; while the intermediate types gave both white and brown. Certain morphological and physiological differences were also characteristic of the different races. The brown, for instance, divide without forming the characteristic "yokes." The asporogenous race showed a loss of power of producing trypsin. There was little difference in fermentative power, but the chief fermentation was most marked in the spore-bearing form; the secondary, in the asporogenous type.

In *Schizosaccharomyces pombe* this author found white and brown colonies, one of which was more productive of spores than the other. In *S. asporus* he found white colonies with thick, short cells, and brown colonies with similar cells, but having in addition long, thin ones. He found this same

tendency to split into sporogenous and asporogenous races in other species of budding organisms.

Alfred Jörgensen (1898) has described new races, differing in their qualities with respect to brewing, which may come from the offspring of a single cell. This author has successfully selected races having better clearing powers and others with superior aromatic qualities.

H. Will (1899) noted a variation in the types of colonies produced on gelatin by four bottom beer yeasts experimented on. These types varied in the degree of regularity of the colony nucleus and outline. Long growing on one medium tended to fix the type so that fewer variations of this sort were observed. The author found that irregular forms more often occurred on cultures taken from the pellicle, and he noted a parallelism between the tendency to early formation of pellicles and the early formation of outgrowths in colonies. The pellicle of some forms produced irregular colonies, while the sediment of the same type produced regular, though sometimes irregular also. Repeated transfers in wort and beer gelatin tended to restore regularity to the forms of colonies. The outgrowths of irregular colonies were sometimes composed of elongated cells and sometimes of spherical. Spore formation diminished in forms producing irregular colonies and returned again as the colonies became more regular. Races exhibiting cells of mycelium-like form retained their characteristics during three years of repeated transfers in a favorable medium, and the author regards such types as a sort of generation in the cycle of their life-history. He believes that successive phases or generations occur in these plants, and that the reported formation of new races may be only the inception of new generations.

M. Hartman (1903) found in colonies of *Torula colliculosa* on wort gelatin and agar peculiar colonies having elevations composed of cells larger than the normal. Cultures containing these cells have the power of fermenting maltose, a property not possessed by cultures which lack the large cells. Other sugars tested—raffinose, cane, grape and fruit sugars—were fermented by both type and variation alike.

W. Henneberg (1903) found giant cells in each of two types of distillery yeasts, the large size of which was transmitted to daughter-cells budding from them. The tendency to store glycogen was also found to be hereditary in these types.

W. W. Lepeschkin (1903) found in *Schizosaccharomyces pombe* and *S. melacei* cells which grew out in the form of mycelia, instead of dividing in the usual manner of the genus. Some of these cells were isolated and found to reproduce the new characteristic. When grown under conditions favoring endogenous spore formation, these cells produced an oidium-like growth, and spore formation was rarely observed. When spores were produced and made to germinate, they reproduced the elongated type of growth peculiar to the new race. The author thinks the new form an example of mutation or heterogenesis.

Comparatively little has been done in selection experiments on bacteria where the isolation of single cells is involved.

H. W. Conn (1899) describes a culture of bacteria, isolated by him from milk, which shows great variability, not due, apparently, to the immediate environment. The color of colonies varied from a milk white to deep orange, and from colonies rapidly liquefying the medium to non-liquefying colonies. By selection of colonies in plate cultures made from a pure culture he obtained pure white and pure orange, as well as liquefying and non-liquefying strains.

A. Meyer (1901) found that the proportion of branched cells in *Bacillus cohærans* is greater in that part of gelatin plates where branched cells were sown; and he concludes that there is a tendency for this peculiarity to be transmitted. He is of the opinion that bacteria are descended from fungi with branched mycelia, and that occasional branching is to be regarded as atavism, not as the formation of a new character.

W. W. Lepeschkin (1904) found in *Bacillus berestnewii* certain branched individuals and also small non-septate mycelia. The offspring of isolated branched cells exhibited five to fifteen per cent. of branched cells after only twenty to fifty offspring had been formed, while the offspring of the unbranched showed none until after many generations of cells

had formed. A culture coming from an isolated mycelium soon reverted to the ordinary branched and unbranched forms, though the mycelium type persisted long enough to show a tendency to heredity. The appearance of these mycelia, apparently, does not depend on temperature or the nature of the substratum. Higher temperatures seem to favor the appearance of branched forms. The author holds that these variations represent new characteristics and are not to be referred to atavism.

R. Massim (1906), working with a pure culture of *Bacillus coli mutabilis*, found that colonies remained white on Endo agar, indicating lack of power to ferment lactose. Transplantations of young colonies continually gave white colonies on this medium, but transfers from older colonies sometimes gave a proportion of distinctly red colonies, which remained red on further transplantation. These red colonies he supposes to arise by mutation in the sense of de Vries.

I.—EXPERIMENTS ON YEAST.

In my own work on *Saccharomyces anomalus*, I have made use of a culture kindly furnished me by Professor Freeman, of the University of Minnesota, a culture which originally came from Doctor Barker, of England. My researches were conducted in two directions: First, the selection of cells varying from the normal in size; second, the selection of cells varying in form.

In the first series I attempted to obtain a race exhibiting cells permanently larger than the normal by repeated selection of cells of unusual size. As in all experiments made in the course of this work, the series was begun with a pure culture proceeding from a single isolated normal cell. Cultures were made for the most part in glucose bouillon in hanging drops, and the isolated cells were grown in the same medium. In conducting these experiments, a cell, considerably larger than the normal, was isolated, and, after a considerable number, often hundreds, of offspring had been formed, a second large cell was isolated from these, and so on. A check consisting of unselected cells was frequently compared under similar conditions.

In one series this repeated selection was practiced twelve