Microbiology. — *A new Hanseniospora.* By A. PIJPER.

(Communicated at the meeting of September 29, 1928).

The genus Hanseniospora (ZIKES), syn. Hansenia (LINDNER), has as characteristics: young cells mostly lemon-shaped, sometimes ellipsoid. Young ascospores spherical, older ascospores semi-spherical, and more or less hat-shaped. Germination by budding [1, 2].

This genus so far comprises the species *Hansenia apiculata* (LINDNER), *Hanseniospora apiculata* (ZIKES) [3], which forms one ascospore, and *Hanseniospora valbyensis* (KLÖCKER) [4], which usually forms two. Recently BATSCHINSKAJA has described sixteen forms of Hanseniosporae, some of which form one, and others two ascospores [5].

The following description applies to a third well-defined species of this genus, encountered by me in Pretoria, which forms four ascospores.

From the fingernails of a European woman, resident in Pretoria, who was suffering from onychosis, for which no cause could be found in the ordinary way, scrapings were examined microscopically and culturally. Microscopically oval "spores" were detected in the scrapings after soaking in AMANN's lactophenol and staining with methylenblue. Fifty small pieces of nailsubstance, planted out on SABOURAUD's milieu d'épreuve, in fortyfive instances gave rise to growth of a fungus identified later on as a *Hanseniospora,* whilst from the other five pieces various fungi *(Monilia, Aspergillus)* developed. As the woman was an amateur gardener, at which occupation however she always wore gloves, an examination of the soil in her garden was indicated, but from this source no *Hanseniospora* could be isolated. It must therefore be left undecided whether this *Hanseniospora* is to be regarded as pathogenic. Local and general treatment with iodine brought about slow improvement in the patient's condition.

More interest attaches to the fungus as such. Good growth occurred on all ordinary media, both at roomtemperature and in the incubator at 37°. Slightly acid media furthered growth, and beerwort was found particularly suitable. The characteristic shapes were best seen in fluid media, and are illustrated by Fig. 1. The cells were mostly lemon-shaped or ellipsoid. Occasionally, and especially in old cultures, a tendency towards formation of a simple segmented mycelium became manifest.

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1) A. GUILLIERMOND. Les Levures. Paris. 1912.

2) A. GUILLIERMOND. Clef dichotomique pour la détermination des levures. Paris. 1928.

3) ZIKES. Centralbl. f. Bakt. 1911.

4) KLÖCKER. C. R. Trav. Lab. Carlsberg. 1913.

5) BATSCHINSKAJA. Journ. microb. russe. 1926.

Multiplication, as followed under the microscope, began as a protuberance at one, or occasionally either, end of a cell. This protuberance

Fig. 1.
Hanseniospora guilliermondii × 1000.

remained connected with the cell by a neck for a considerable time. Then followed a constriction of the neck and the new cell at that spot became separated from the old one. As a rule the new cells became detached and took an ellipsoid or lemonlike shape soon after the constriction occurred. The comparative smallness of the cells greatly impeded morphological observations. Young cells measured about 5.2 by 2.4 microns, and dividing cells about 10.4 by 3 microns.

Production of ascospores was easily obtained when the strain was still young. The older the strain became, the more difficult it was get it to produce ascospores. In the beginning growth on blocks of plaster produced enormous numbers, but then numerous spores could also always be found on ordinary media. The number of spores in each cell was nearly always four, and their typical arrangement is shown in Fig. 2. There are two polar and two equatorial spores. The cellwall became invisible whilst the formation of spores was in progress, but the four spores remained united. There was no indication of a sexual process preceding their formation. The germination of the spores was closely followed in numerous instances, and every spore was always seen to germinate by itself, first becoming hat-shaped and then swelling up and becoming spherical, and then emitting a

tube-like protuberance which became separated from the spore and then immediately took on the characters of an ordinary vegetative cell. These

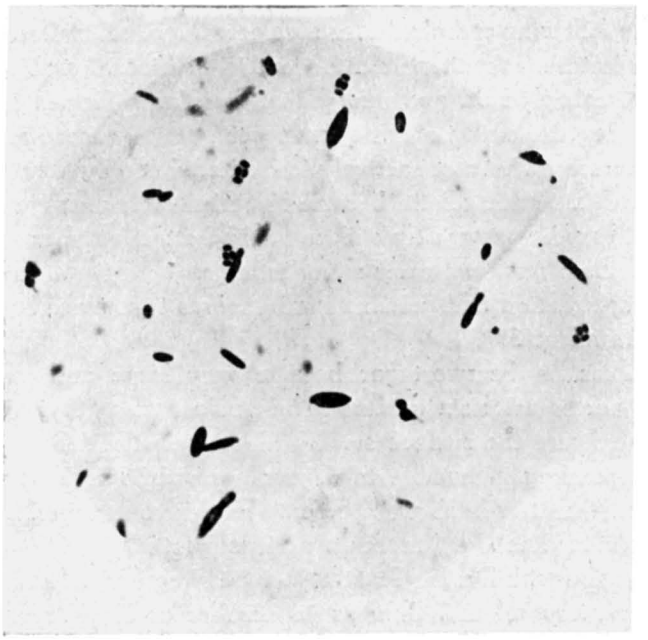

Fig. 2.
Hanseniospora guilliermondii × 1000.

phenomena were best observed, not in a hanging drop preparation, where the slightest current sweeps any particular cell out of sight, but on a solid medium. For this purpose a thin small disc of some agarmedium is sliced off the surface of a slant, and placed in the hollow of an excavated microscope slide. A trace of the material to be examined is deposited in the centre of its upper surface, and a coverglass is gently pressed on until perfect contact is obtained between coverglass and medium. The pressure distributes the cells over the surface and it was always easy to find cells which were sufficiently isolated to allow prolonged observation without interference by others. The edges of the coverglass must be wiped with vaseline.

Although from the method in which the ascospores developed it would appear as if all sexuality had disappeared, there was some evidence that the four ascospores of one cell were not always equivalent. After mordanting with chromic acid, staining with carbol-fuchsin, decolorizing with sulphuric acid, and counterstaining with methylenblue, a striking picture was obtained in some instances : the equatorial spores were acidfast, in contrast to the polar spores which showed up blue.

A difference in resistance of spores and vegetative cells could not be

demonstrated. Alcohol of 45 % killed both spores and cells within four minutes. A temperature of 57 ° was fatal in fifteen minutes for both spores and cells.

The fermentative powers of this *Hanseniospora* were very weak, as tested on the following carbohydrates : glucose, levulose, maltose, galactose, saccharose, lactose, mannite, dulcite, dextrin, raffinose, arabinose, inulin, sorbite, erythrol, glycerin and amygdalin. Acid was formed in raffinose, sorbite and dextrin, and a small quantity of gas in glucose and levulose. Beerwort was somewhat more actively fermented, but only a trace of alcohol was produced. Beerwortgelatin became liquefied after many weeks.

In all fluid media growth took place at the bottom only, no surface-film was ever formed, not even after many months.

Growth, sporulation and germination of spores took place both at room-temperature and at 37°.

Giantcolonies on beerwortagar became very large and measured six centimetres across after six months. Their colour was greyish brown, the edge lobulated and the surface smooth, showing very delicate concentric rings, corresponding in number to days of growth. There were no radial lines visible. A central knob was present from the beginning, and later on secondary similar knobs appeared at various places.

In consultation with Dr. A. GUILLIERMOND it was decided that the fungus was a *Hanseniospora*, and that, as its number of ascospores completely differentiates it from the known forms, it must be regarded as a new species. For this new species I propose the name of *Hanseniospora guilliermondii* PIJPER 1928, in honour of Dr. A. GUILLIERMOND whose work on yeasts is so universally known. I also take this opportunity of thanking Dr. GUILLIERMOND for all the help he has so kindly given me.

Pretoria, June 1928.