TWO STRAINS OF PULLULARIA PULLULANS (DE BARY), BERKHOUT ISOLATED FROM THE HUMAN SKIN

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Strain 1326 [2], Ashford's collection; 229 Ciferri's collection.
Strain 637, Ashford's collection; 218 Ciferri's collection.

The source of Strain 1326 [2] was a superficial lesion on the skin of the neck, about the size of a twenty-five cent piece in a rather malnourished young woman of twenty years. The case was seen at its very commencement and yielded readily to an ointment of salicylic acid and sulphur. The lesion itself was faintly festooned, dirty gray in color, and covered with fine scales. The underlying tissue was very little infiltrated.

Strain 637 was isolated from extensive and deep-seated lesions of the palms of the hands and the soles of the feet, as well as of chest, arms and legs. The lesions were darkly pigmented and heavily crusted, with deep infiltration of the skin. The patient stated that the eruption began as small vesicles on the toes, these broke and were covered over later by crusts. No pus was seen. The areas affected were of irregular size and shape, were festooned by a scaly red border and caused intense itching, and, at times, considerable pain.

This eruption was extremely obstinate in disappearing and lasted for about three months in spite of the persistent use for one month of a combination of salicylic acid two per cent, ichthyol ten per cent, sulphur twenty per cent in lanolin, and for two weeks thereafter, of ammoniated mercury ointment, ten per cent. What seemed to finally cause its disappearance was intravenous sodium iodide.

We have no proof that these organisms were the cause of the lesions and on the contrary feel that *Pullularia pullulans* is a quite frequent contaminating organism in these tropics. We have isolated it from the most diverse cutaneous diseases and even from the human stomach by means of the Einhorn tube. But this fact should be recognized: the organism creeps into cultures from without by aerial contamination, or actually subsists on animals as a saprophyte, and it may excite the curiosity and waste the time of investigators who seek the etiologic factor in a given lesion, generally of the skin.

For the above-mentioned reasons we have decided to rehearse our personal experience with it.

Strain 637

Cultural Characteristics:

It would be superfluous to recite the cultural characteristics at different periods of development because these cultures are very uniform throughout and because they are already well known.

The young colonies, on whatsoever sugar medium are white, round, and very similar to those of any yeast. The lack of color is sustained for some time without alteration, but generally, when the colonies grow larger, yellowish-tan to brown spots appear which rapidly conceal part or the whole of the colony and which correspond to that variety known as the "fumagoid type". The little white colonies hardly ever exceed 2 to 3 mm. in diameter, but even then the first signs of encysted cells are visible on direct microscopical examination, or the membrane is partly gelatinoid and the cells are partly stuck together.

At first, the colony presents a rapidly darkening aspect, and eventually becomes brilliant black, and tar-like, the black layer covering the entire surface of the colonies. This, however, fails to stop the development of the culture: under the black surface, the colonies grow, taking forms which are different the one from the other, and irregular, becoming at last partially confluent and forming an irregular black crust. When first formed the black surface is shiny but this polish diminishes as the substratum dries; an old colony is black, but its surface is opaque. This type of colony is capable of some unimportant variations, depending upon the composition of the medium; in general, substrata rich in sugars and peptones, or sugared broth are more favorable.

Nevertheless, the fungus adapts itself well to any organic or mineral terrain which is solidified (*Raulin, Raulin-Dierckx, Hayduck,* etc.). Liquid cultures, either decoctions, such as beer or grape-must, or the above-mentioned mineral solutions, have their well-known characteristics: generally a very abundant formation of tufts, which are submerged in the liquid from the surface downward, but not reaching the bottom to form a deposit; these cottony tufts, at first irregularly white, become gray and even greenish. At the surface the culture becomes dark, taking on a maroon color, but without forming a black layer as complete and as thick as in the solid media.

For more details we refer the reader to the numerous studies made of *Pullularia pullulans* to date.

Morphologic Characteristics:

A detailed description of all the numerous morphologic elements found in an investigation of this fungus would be long; on the other hand, the investigators which have preceded us have studied the *Pullularia* so thoroughly that we have little to add to what they have already said. We prefer, therefore, to sum up our observations on the formation and development of the multiple forms seen just as they have appeared to us in hanging drop and micro-culture on solid media. A sowing of a few germinating cells with a relatively thin membrane, the blastospores most commonly found, stimulates in them, rapidly and easily, a true gemmation with the production of one or more daughter-cells, generally one, but not infrequently three or four. Gemmation is uni- or bipolar or it may occur in other parts of the periphery, indifferently. The daughter cell may separate, as it normally does in solid media, in the first few hours of culture, and, itself, reproduce by gemmation. But occasionally, and especially after the first twenty-four hours of development in solid media, or immediately in liquid media, the line of division between the mother and the daughter-cell broadens from the growth of the latter, forming two cells of approximately equal size which are linked as in a chain. Successively, the number of cells constituting the chain rapidly increases in the same manner and axis, the chain lengthening at one or both extremities. At times a double gemmation of a cell causes a bifurcation of the chain which continues to increase in length in each of its branches, which in their turn, may branch. Almost at the same time, all the cells which constitute the chain begin to bud laterally, producing a quantity of blastospores which give to the chain a singular and characteristic appearance, the most typical, or at least, the best known of the Genus *Pullularia*. These lateral blastospores, improperly called "conidia", may remain smaller than the mother-cells, as is usually the case, and may adhere thereto, or become detached and atrophic, or separate and reach the normal size, giving rise to other buds by gemmation, isolated or in groups, or to true, hyaline, mycelial hyphae, which later become yellowish and septate. This last-mentioned type is especially frequent on solid media, the central axis being formed by the chain and its lateral buds, intersected by the hyphae which these produce. These hyphae form at first a filamentous net, and later a little ball the interior structure of which becomes more and more difficult to make out in proportion to the degree of gemmation and the intersecting of mycelial elements. Although the free yeast-budding forms constitute other centers for the diffusion of the fungus, in liquid

media, or in certain little colonies in solid media, the length of the main chain of blastospores does not extend indefinitely but reaches its limit. At this point, generally, a number of the blastospores are seen to possess a transverse septum; much more rarely there may be two septa, both transverse, or one transverse and the other approximately longitudinal. This formation is generally referred to as the *Cladosporium* type. At the same time, a number of blastospores, isolated or connected together in chains, become deeper in color which reaches a yellowish hue, while the external membrane, before apparent but not especially thick, rapidly increases in thickness. They are thus transformed into chlamydospores ("cystic", or, more properly, "chlamysdosporous" stage). This phenomenon, at first isolated here and there, in a few hours or days becomes generalized throughout the superficial layer of the colonies until this layer becomes finally well-nigh completely filled with chlamydospores. The thickness of the layer thus affected varies considerably according to the medium; in liquid cultures the phenomenon is limited to relatively few cells, and these superficial; in solid media, various layers of cells are involved. These chlamydospores rarely germinate at the site of their formation if the conditions are unfavorable. If conditions are favorable they give rise to a scanty number of fine filaments, hyaline or yellowish, similar to those just described. They very rarely branch and very rarely give rise to buds, which are, after all, generally doubtful.

If the conditions continue to be unfavorable, as, for example, through the drying-out of the medium, the membrane of the chlamydospores becomes darker and eventually nearly or quite black. At the same time, the isolated elements become soldered together, as it were, by a partial gelatinizing of their membranes. At first, microscopic examination is still able to reveal the form and structure of the various fused elements. Later on, it is impossible to see more than a blackish or dark-yellowish crust, amorphous, opaque, and, without definite structure, although not homogenous. The majority of the authors denominate this the "fumagoid stage", although it would be more appropriate to call it the "plectenchymatous stage". We should state here that we have never verified any mucilaginous formation, although we have had reason to suspect its presence.

The chains of blastospores are generally quite resistent to parting, although, especially in liquid media, they may be accidentally broken into two or more pieces. The re-sowing of these pieces, if the gelatinization of the walls is not too far advanced, can bring about the formation of new chains of blastospores of the same sort, but shorter

and slower in development; or a formation of lateral blastospores, or the development of septate mycelial hyphae. These are thin and hyaline, later yellowish, and branch and actively bud, despite their evident degradation, with a scanty number of lateral blastospores. Of these blastospores, some attain a normal size and begin to bud, reproducing the chain of budding elements already described. The black plectenchymatous crust, once definitely formed, can only germinate feebly and gives rise to mycelial hyphae which are unbroken but not continuous. But below this crust there are always masses of normal blastospores, and on re-sowing, it is to these that generally we owe new colonies. In solid media, at the margin and under the re-sown crust, we see a compact series of budding cells appear, one overlying the other, while from the crusts spread radially chains of heavy blastospores in a series of budding forms, entangled with thin hyphae and bearing lateral blastospores.

These phenomena, which we have attempted to briefly summarize, are already known and described by almost all of the authors who have studied *Pullularia*. A phenomenon which we do not believe has as yet been pointed out, is a sub-clavate or sub-spherical swelling at the extremity of the thin hyphae, which may be isolated by a septum and function as a blastospore, giving off daughter-cells, either one, or, as is the rule, several arranged like the fingers on the hand. When this phenomenon is repeated on hyphae with a branched extremity, indeed, frequently dichotomously branched, figures are formed which remind one of *Hormodendron* (''hormodendroid stage''), but generally the resemblance limits itself to a mere sketch as we have never found a true *Hormodendron* type. This is in accord with other authors who have found the hormodendroid stage in culturing *Cladosporium,* although not in *Dematium* (Planchon,[2]).

We should say in closing that the main chains of blastospores can become disarticulated, not only in two or more fragments but in as many as there are budding cells, although a few pieces of twos and threes may still remain. Such disarticulation, however, is unusual, and is generally seen in liquid cultures.

Biochemical Characteristics:

The biochemical characteristics were not carefully worked out in view of the scanty interest that the *Pullularia* possesses. This strain ferments none of the sugars, etc.; glucose, levulose, maltose, galactose, lactose, mannose, mannite, dulcite, arabinose, trehalose, raffinose, xylose, inulin, sorbite and dextrin were tested and found negative.

It grows equally well in solutions containing peptone, asparagin, glycocol, ammonium sulphate or nitrate or nitrite of potassium. It also grows well in synthetic media containing, as a source of carbohydrate, glucose, levulose, maltose, dextrin, lactose, two per cent methylic or ethylic alcohol, and two per cent citric, malic, tartaric and acetic acid. All of this is in accord with the observations of Berlese, Laurent, etc. The observations of Cuboni and Pasteur according to whom the *Pullularia pullulans* has the power of fermentation, Berlese(3) and Planchon(2) consider doubtful, as the fungus may not have been sufficiently purified.

Strain 1326 [1]

Cultural Characteristics:

Absolutely the same as those detailed for Strain 637.

Morphologic Characteristics:

They cannot be differentiated from Strain 637 save for a special type of mycelial hyphae, which we have failed to observe in Strain 637, although we cannot be certain that this peculiarity did not fail to attract our attention. This type of mycelium is found only in solid media, and of these, especially in Difco prune agar. It is also noticeable, however, in grape-must agar, in a solution of peptone and glucose, etc. The hyphae are nastriform, stubby and slightly constricted at the site of the septa. The entire hypha is filled with oil drops and refringent bodies, these being normally grouped around one or both of the membranes and extending into the interior of the hypha. It should be noted that the membranes are not cuticulized, gelatinized, or in any way thickened; on the contrary, they are thin, although clearly visible. This type of mycelium, resembling a dead hypha, is not mentioned by Planchon or any of the authors whom we have consulted, although this morphologic aspect of the mycelium, if it really represented nothing more than dead mycelium, would have no importance warranting mention. We do not believe it a dead hypha, however, as we have seen it produce thin mycelial hyphae whose protoplasmic content appeared normal at the free extremities, the part which was actively growing, but which left behind empty and guttulate articles as the hypha grew in length.

Biochemical Characteristics:

These differ in no way from those observed in Strain 637.

Systematic Position

The varied form of its elements, its colonies, and above all, the peculiar character of its complicated budding forms, place this fungus in the Genus *Pullularia* Berkhout (formerly *Dematium*). The Genus *Dematium,* with the type species *D. pullulans,* was created by *De Bary*(1), with a quite brief diagnosis, far from revealing the complex morphology of this fungus. The chief contributions to the knowledge of this organism are due to Loew(6) and Planchon(2). The literature on this fungus since 1900 is abundant, either to point out the complexity and relations of its varied morphologic elements, or, especially, to bring out its relations, apparent or real, to other fungi, principally with the Hyphales Dematiaceae, even with the Ascomycetaseae. We do not propose to rehearse the long history of the Genus but we desire to simply point out certain facts concerning the affinities found between our strains and *Dematium pullulans* De Bary (*Pullularia pullulans* Berkh.)

Let us note that the polymorphism of this fungus does not permit us to differentiate our strain from *P. pullulans,* not only Strain 637 but even Strain 1326 [2] with its peculiar type of nastriform mycelium filled with oil drops. On the other hand, the question as to whether *P. pullulans* represents a well-defined species (elemental species) or a group of separate species (collective species) is still sub-judice, in spite of the arguments in pro and in contra. Berlese(3) thinks that as different strains of these fungi present distinct conditions which vary from each other, it should be considered as a collective group. Planchon(2), with other authors, thinks that it is a quite clearly defined species, in spite of slight variations noted by other authors. It seems to us that until we can clearly establish its connection with perfect fungi, of which it may represent a metagenetic phase, its polymorphism does not permit us to consider it a collective entity. And once these metagenetic connections are proven in a number of cases, *Dematium* would lose all of its specificity and would become only a dematioid phase of higher forms. In brief, we have not sufficient reason to consider *Pullularia pullulans* as a divisible species. In so far as relates to our present investigations, we should diagnose our strains as *Pullularia pullulans* (De Bary) Berkhout.

In closing we should add that forms of *Dematium* and *Fumago* were observed in *Cladosporium Wernecki* Horta, by Horta himself, and by Langeron (see Brumpt(4)) who recovered these from a case of dermatomycosis similar to tinea nigra of Castellani.

It is of interest to note that the fungi that have been isolated

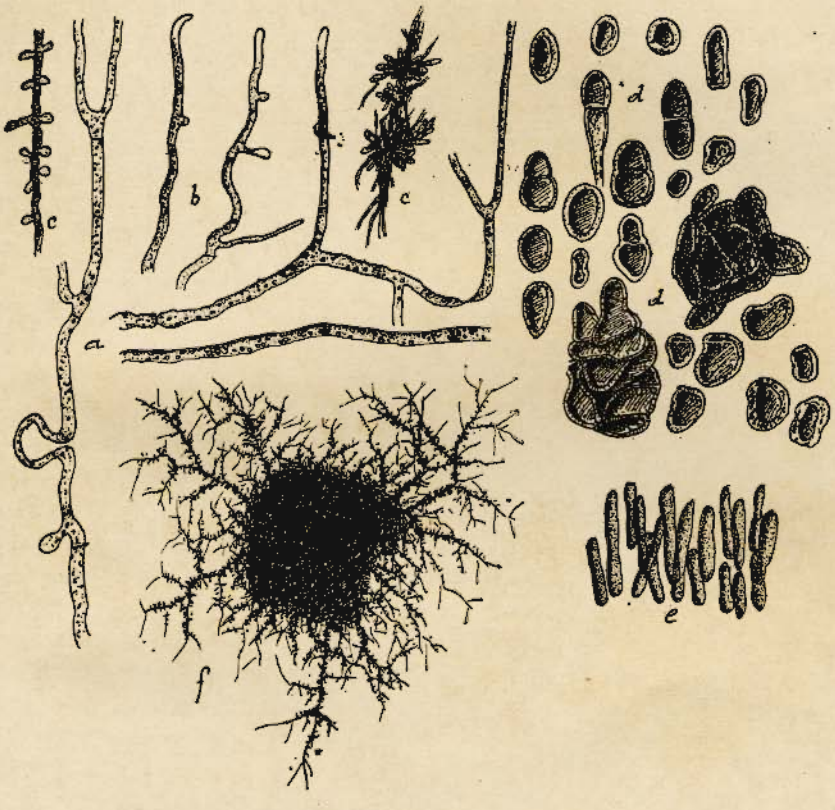

FIGURE I.—PULLULARIA PULLULANS (DE BARY) BERKHOUT.

a. Nastriform mycelium. *b*. Young hyphae producing lateral blastospores. *c*. Same in adult stage. *d*. Blastospores with thick membranes and initial stage of the black crust. *e*. Free adult blastospores. *f*. A mycelial mass with a central black crust, peripheral branches producing blastospores. *g*. A microphotograph of same.

from cases of "pinta" differ greatly among themselves, although they are alike in that they all appear to belong to the Hyphales Dematiaceae. In view of this fact, Castellani created the genus *Montoyella* for pinta nigra, with the species, *M. nigra* Castellani. Ciferri had occasion to investigate a fungus isolated from a case of this disease in Mexico which he defined as *Acrothecium nigrum Ciferri,* and in spite of his inability to identify it precisely as *Montoyella nigra,* he believes that this identification is a fact. Moreover, grouping tinea nigra with "carate", a second species should be added as a cause of this disease, *Cladosporium* Mansoni. This last mentioned relationship is very interesting when we remember that, although *Cladosporium* and *Dematium* are distinct entities, they are intimately related, as far as *Cladosporium* goes, as other Dematiaceae can assume dematioid types (Planchon(2) et.).

Let us remember, finally, that *Montoyella nigra* Ciferri, cultivated in liquid media presents strongly the characteristic dematioid forms.

The results of all these observations is that two fungi, *Montoyella nigra,* and *Cladosporium Mansoni,* recognized as causes of certain pigmented mycotic affections of the skin in the tropics, grouped under the term "carate", can readily be confused with *Pullularia pullulans,* which is either a contaminating organism or which may be a harmless saprophyte. The fact is, that all three may have a common vegetative form, a form which quite probably it does not assume in its parasitic life. This fact speaks without doubt in favor of a real unity, in spite of the mycologic distinction between pathogenic and saprophytic species of these fungi which may be found in the lesions of black pinta.

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