A Proposed Revision of the Genus *Pullularia*

BY E. S. WYNNE AND CORA L. GOTT

Section of Experimental Pathology, The University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas, U.S.A.

SUMMARY: Morphological and physiological studies were made of 12 strains of the genus *Pullularia*. Although inability to ferment carbohydrates has been reported characteristic of this genus, 10 of the strains produced acid from one or more carbohydrates. It is proposed that the genus be emended to include the new species *P. fermentans*.

Lymph nodes in Hodgkin's disease have been the source of a variety of micro-organisms, including members of the genera *Bacillus*, *Brucella*, *Candida*, *Clostridium*, *Corynebacterium*, *Escherichia*, *Staphylococcus* and *Torula* (Hoster, Dratman, Craver & Rolnick, 1948; Haythorn, Robinson & Johnson, 1932). An organism recently isolated in our laboratory from a case of Hodgkin's granuloma appeared morphologically to be a *Pullularia* sp. However, this isolate produced acid from a number of carbohydrates; whereas the genus *Pullularia* has been described as not fermenting carbohydrates (Berkhout, 1923; Dodge, 1935). From culture collections there were obtained 11 additional strains designated as *Pullularia*. Of these, all but 2 fermented one or more carbohydrates. On the basis of morphological and physiological studies of these fungi to be reported in this paper, it is suggested that the genus *Pullularia* be emended to include fermenting forms as members of the proposed new species *P. fermentans*.

METHODS

The organism isolated in our laboratory was obtained from a mid-jugular lymph node of an 11-year-old boy with Hodgkin's granuloma. Paraffin sections stained with Gridley's fungus stain (Gridley, 1953) showed occasional conidia. A portion of the node was ground with sterile sand in a mortar, and the following media were inoculated: Sabouraud glucose agar (Difco), Lowenstein-Jensen medium (Difco), fluid thioglycollate medium (BBL), brain heart infusion broth (Difco) and blood agar. Organisms isolated were a Gram-negative rod, a staphylococcus and a black yeast-like fungus identified on morphological considerations as a *Pullularia* sp. However, the organism produced acid from a number of carbohydrates; whereas the genus *Pullularia* has been described as devoid of fermentative powers (Berkhout, 1923; Dodge, 1935). Therefore, a detailed morphological and physiological study was made of 11 strains of *Pullularia* obtained from culture collections. Two strains were secured from the American Type Culture Collection, Washington, and the other 9 from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. The organisms are listed with their sources in Table 1.

Table 1. Summary of biochemical and physiological reactions of 12 *Pullularia* strains

Source	Designation as received	Proposed designation	Fermentation of carbohydrates										Pigment			
			Glucose	Mannose	Fructose	Maltose	Sucrose	Raffinose	Xylose	Galactose	Rhamnose	Arabinose	Lactose	Growth at 37°	Nutrient agar	Anaerobic
CvS*	<i>P. pullulans</i> (de Bary) Berkhout strain Boedijn	<i>P. pullulans</i>	—	—	—	—	—	—	—	—	—	—	—	+	+	+
CvS	<i>P. werneckii</i> (Horta) de Vries strain da Fonseca	<i>P. werneckii</i>	—	—	—	—	—	—	—	—	—	—	—	+	—	—
Lymph node from Hodgkin's granuloma	Isolate	<i>P. fermentans</i> var. <i>fermentans</i>	+	+	+	+	+	+	—	—	—	—	—	+	—	—
CvS	<i>P. pullulans</i> (de Bary) Berkh. strain Margadent	<i>P. fermentans</i> var. <i>fermentans</i>	+	+	+	+	+	+	—	—	—	—	—	—	—	—
CvS	<i>P. pullulans</i> (de Bary) Berkh. var. <i>fusca</i> (Browne) Berkh. strain Church	<i>P. fermentans</i> var. <i>fusca</i>	+	+	+	+	+	+	+	—	—	—	—	+	—	—
ATCC†	<i>P. pullulans</i> (de Bary) Berkh. 9348	<i>P. fermentans</i> var. <i>fusca</i>	+	+	+	+	+	+	+	—	—	—	—	—	+	—
ATCC	<i>P. pullulans</i> (de Bary) Berkh. 9349	<i>P. fermentans</i> var. <i>melinii</i>	+	+	+	+	+	+	+	—	—	—	—	+	—	—
CvS	<i>P. pullulans</i> (de Bary) Berkh. strain Melin	<i>P. fermentans</i> var. <i>leaoi</i>	+	+	+	—	+	+	—	—	—	—	—	+	—	—
CvS	<i>P. werneckii</i> (Horta) de Vries strain Leão	<i>P. fermentans</i> var. <i>leaoi</i>	+	+	+	+	+	+	+	—	—	—	—	+	—	—
CvS	<i>P. pullulans</i> (de Bary) Berkh. strain Schoen	<i>P. fermentans</i> var. <i>schoenii</i>	+	+	+	+	+	+	—	—	—	—	—	+	—	—
CvS	<i>P. pullulans</i> (de Bary) Berkh. strain Benedek	<i>P. fermentans</i> var. <i>benedekii</i>	+	+	—	—	—	—	—	—	—	—	—	+	—	—
CvS	<i>P. pullulans</i> (de Bary) Berkh. strain Castellani	<i>P. fermentans</i> var. <i>castellani</i>	+	—	—	—	—	—	—	—	—	—	—	+	—	—

* Centraalbureau voor Schimmelcultures, Baarn (Netherlands).

† American Type Culture Collection, Washington, D.C.

Cultures were made on Littman oxgall agar (Difco), nutrient agar (Difco), Sabouraud glucose agar (Difco) and corn meal agar (Difco). These media were incubated at 25° and 37°, both aerobically and in an atmosphere of natural gas. Microscopic studies included examination of growth *in situ* on solid media, of temporary preparations consisting of growth from solid media suspended in water, and of slide cultures made from corn meal or Sabouraud agar. Tests for production of acid from carbohydrate were carried out in 10 ml. of phenol red broth base (Difco) to which a filtered solution of sugar had been added to a final concentration of 4%. After incubation for 4 weeks at a temperature of approximately 25°, the pH values of all tubes were determined with a Beckman Model G pH meter. Tubes with a pH value of 6·7 or lower were then titrated to pH 7·3 with 0·05 N-NaOH. A carbohydrate was considered to be fermented only when the final pH value was 6·6 or less, and 0·4 ml. or more of NaOH was required to adjust the pH to 7·3.

RESULTS AND DISCUSSION

On the basis of morphological considerations, all of the 12 cultures studied were considered as members of the genus *Pullularia*. Morphology of the strain isolated in our laboratory is illustrated in Pl. 1, and is representative of the group.

Of the 12 cultures which morphologically were essentially identical, 10 (including the isolated strain) produced acid from one or more carbohydrates, and therefore did not conform to published descriptions of the genus (Berkhout, 1923; Ciferri & Ashford, 1929; Dodge, 1935). Considerable variation was noted in the fermentation patterns of the various organisms. Variation was also noted in ability to grow at 37°, and in the production of pigment on nutrient agar or under anaerobic conditions (atmosphere of natural gas). The principal biochemical and physiological findings are summarized in Table 1.

To the authors' knowledge only two species are at present recognized in the genus *Pullularia*: *P. pullulans* (de Bary) Berkhout (Berkhout, 1923; Ciferri & Ashford, 1929; Dodge, 1935) and *P. werneckii* (Horta) de Vries (Horta, 1921; de Vries, 1952). On the basis of the physiological characteristics reported in this paper, it is proposed to emend the genus to include fermenting forms in a new species, *P. fermentans*, with 7 varieties (Table 1). The organism isolated in the present investigation is designated as the type for the species. Six of the 7 varieties are new, and the names of these were derived from the name of the investigator listed by the Centraalbureau voor Schimmelcultures as having furnished the strain. The seventh variety, *P. fermentans* var. *fusca*, represents a new combination, since the designation *P. pullulans* var. *fusca* (Browne) was used earlier by Berkhout (1923). The two strains from the American Type Culture Collection appear to belong to this variety.

The proposed revision of the genus *Pullularia* is admittedly based almost entirely on considerations of a physiological rather than a morphological nature. In this connexion it is of interest to note the recent emphasis upon physiological criteria in speciation of genera such as *Streptomyces* (Hesseltine,

Benedict & Pridham, 1954) and *Candida* (Lodder & Kreger-van Rij, 1952). Furthermore, it is recognized that strains of a single species such as *Penicillium chrysogenum* may exhibit pronounced morphological variation (Stauffer & Backus, 1954).

CLASSIFICATION

Pullularia Berkhout emend.

Pullularia Berkhout (1923)

= *Hormonema* Lagerberg, Lundberg & Melin (1927)

Type species *Pullularia pullulans* (de Bary) Berkhout (1923)

Colonies usually black, at first yeast-like but later velvety or woolly, margins often lighter than centres. Hyphae dark on most media. Mycelium at edges of colonies usually formed by lateral budding. Older portions of mycelium fragment into dark thick-walled arthrospores which 'sprout' to form blastospores. Thick-walled chlamydospores may also arise along the mycelium and are particularly numerous in old cultures. Uniseptate or cladosporium forms usually present. Pigment production varies with medium and oxygen tension. Most strains grow at 37°. Acid production from carbohydrates variable.

Key to *Pullularia*

I. No acid from glucose.

- A. Growth predominantly yeast-like, pigment on nutrient agar. *P. pullulans*
- B. Growth predominantly mould-like, no pigment on nutrient agar. *P. werneckii*

II. Acid from glucose.

- A. No acid from mannose. *P. fermentans* var. *castellanii*
- B. Acid from mannose.

- 1. No acid from sucrose. *P. fermentans* var. *benedekii*

2. Acid from sucrose.

- a. No acid from fructose or maltose. *P. fermentans* var. *leaoi*

- b. Acid from fructose and maltose.

(1) No acid from xylose.

- (a) No acid from raffinose, growth at 37°. *P. fermentans* var. *schoenii*

- (b) Acid from raffinose, no growth at 37°. *P. fermentans* var. *fermentans*

(2) Acid from xylose.

- (a) No acid from rhamnose, no pigment on nutrient agar.

P. fermentans var. *fusca*

- (b) Acid from rhamnose, pigment on nutrient agar.

P. fermentans var. *melinii*

Descriptions of species

Pullularia pullulans (de Bary) Berkhout (1923)

≡ *Dematium pullulans* de Bary (in Loew, 1868)

Blastospores 3–4 × 7–11 μ., chlamydospores 14 μ., arthrospores 3–4 × 7–10 μ., cladosporium forms 3–5 × 12–14 μ. Small black colonies with greenish black surface and aerial mycelium on Sabouraud, corn meal, nutrient and Littman agar. Pigment produced only anaerobically. Growth at 37°. Pellicle in broth with slight sediment. Carbohydrates not fermented.

Pullularia werneckii (Horta) de Vries (1952)= *Caraté noir* Montoya y Florez (1898)= *Montoyella nigra* Castellani & Chalmers (1913)≡ *Cladosporium wernecki* Horta (1921)≡ *Dematium wernecki* (Horta) Dodge (1935)

Blastospores 3×7 – 10μ ., chlamydospores 6 – 7μ ., arthrospores 3 – 4×7 – 11μ ., cladosporium forms 3 – $4 \times 10 \mu$. Black colonies with dark and light aerial mycelium on Sabouraud, corn meal and Littman agar. Mould type growth. Pigment produced aerobically or anaerobically, but not on nutrient agar. Growth at 37° . Carbohydrates not fermented.

***Pullularia fermentans* sp.nov. (Pl. 1)**

Blastospores 3 – 4×7 – 10μ ., chlamydospores 13 – 15μ ., arthrospores 5 – 7×10 – 14μ ., cladosporium format $9 \times 17 \mu$. Coloniae nigrae cum mycelio albo in superficie in agaro Sabouraudi et agaro farinae frumenti. Pigmentum solum cum oxygenio effectum, variabile in agaro Littmani et absens in agaro nutriente. Conformatio mycelii aerii variabilis. Non crescit cum 37° . Glucosum, mannosum, fructosum, maltosum, sucrosum et raffinose fermentat.

Blastospores 3 – 4×7 – 10μ ., chlamydospores 13 – 15μ ., arthrospores 5 – 7×10 – 14μ ., cladosporium forms $9 \times 17 \mu$. Black colonies with white surface mycelium on Sabouraud and corn meal agar. Pigment produced only aerobically, variable on Littman agar and absent on nutrient agar. Production of aerial mycelium variable. No growth at 37° . Ferments glucose, mannose, fructose, maltose, sucrose and raffinose.

This type strain was isolated by the authors in 1954 from a mid-jugular lymph node of an 11-year-old boy with Hodgkin's granuloma.

Pullularia fermentans* Wynne et Gott var. *fermentans

Characters as for *P. fermentans*

***Pullularia fermentans* Wynne et Gott var. *fusca* (Browne) comb.nov.**≡ *Monilia fusca* Browne (1918)≡ *Pullularia pullulans* (de Bary) Berkhout var. *fusca* (Browne) Berkhout (1923)

Blastospores 3 – 4×11 – 13μ ., chlamydospores 13 – 16μ ., arthrospores 3 – 6×8 – 12μ ., cladosporium format 4 – $6 \times 14 \mu$. Coloniae nigrae mucosae cum mycelio nigro in superficie in agaro Sabouraudi, farina frumenti et in agaro Littmani. Pigmentum variabile in conditionibus sine oxygenio, absens in agaro nutriente. Mycelium aerium non observatum est. Crescit cum 37° . Circuli pellicularum in decocto. Glucosum, mannosum, fructosum, maltosum, sucrosum, raffinose et xylosum fermentat.

Blastospores 3 – 4×11 – 13μ ., chlamydospores 13 – 16μ ., arthrospores 3 – 6×8 – 12μ ., cladosporium forms 4 – $6 \times 14 \mu$. Black mucoid colonies with black surface mycelium on Sabouraud, corn meal and Littman agar. Pigment variable under anaerobic conditions, absent on nutrient agar. Aerial mycelium not observed. Growth at 37° . Rings of pellicles in broth. Ferments glucose, mannose, fructose, maltose, sucrose, raffinose and xylose.

Type strain obtained from Centraalbureau voor Schimmelcultures as *Pullularia pullulans* (de Bary) Berkhout var. *fusca* (Browne) Berkhout (1923).

***Pullularia fermentans* Wynne et Gott var. *melinii* var.nov.**

Blastosporae $3-5 \times 7-13 \mu$., chlamydosporae $13-16 \mu$., arthrospora $3 \times 14 \mu$., cladosporium format $5-8 \times 10-17 \mu$. Coloniae nigrae mucosae cum mycelio nigro in agar Sabouraudi, farina frumenti et agaro nutriente. Nullum pigmentum sine oxygenio. Nullum mycelium aerium. Crescit cum 37° . Circuli pellicularum in decocto. Glucosum, mannosum, fructosum, maltosum, sucrosus, xylosus, galactosus et rhamnosus fermentat.

Blastospores $3-5 \times 7-13 \mu$., chlamydospores $13-16 \mu$., arthrospores $3 \times 14 \mu$., cladosporium forms $5-8 \times 10-17 \mu$. Black mucoid colonies with black mycelium on Sabouraud, corn meal and nutrient agar. No pigment anaerobically. No aerial mycelium. Growth at 37° . Rings of pellicles in broth. Ferments glucose, mannose, fructose, maltose, sucrose, xylose, galactose and rhamnose.

Obtained from Centraalbureau voor Schimmelcultures as *Pullularia pullulans* (de Bary) Berkhout strain Melin. Received by the Centraalbureau in 1929 as *Hormonema dematioides*.

***Pullularia fermentans* Wynne et Gott var. *leaoi* var.nov.**

Blastosporae $3-4 \times 7-10 \mu$., chlamydosporae 12μ ., arthrospora $4 \times 4-7 \mu$., cladosporium format $3 \times 11-14 \mu$. Coloniae subvirides nigrae cum mycelio aereo in agar Sabouraudi et agar farinae frumenti, coloniae nigrae in agar Littmani. Pigmentum cum aut sine oxygenio, absens in agaro nutriente. Non crescit cum 37° . Crescit in decocto solum qualis pellicula. Glucosum, mannosum, sucrosus et raffinosis fermentat.

Blastospores $3-4 \times 7-10 \mu$., chlamydospores 12μ ., arthrospores $4 \times 4-7 \mu$., cladosporium forms $3 \times 11-14 \mu$. Greenish black colonies with aerial mycelium on Sabouraud and corn meal agar, black colonies on Littman agar. Pigment aerobically or anaerobically, absent on nutrient agar. No growth at 37° . Growth in broth as pellicle only. Ferments glucose, mannose, sucrose and raffinose.

Obtained from Centraalbureau voor Schimmelcultures as *Pullularia werneckii* (Horta) de Vries strain Leão. Received by the Centraalbureau in 1948 from Leão and Cury, who isolated it from a case of tinea nigra (keratomycosis nigricans palmaris).

***Pullularia fermentans* Wynne et Gott var. *schoenii* var.nov.**

Blastosporae $3-4 \times 7-12 \mu$., chlamydosporae $13-14 \mu$., arthrospora $8-11 \times 10-13 \mu$., cladosporium format $7 \times 14-17 \mu$. Coloniae nigrae viscosae cum mycelio nigro in superficie et mycelio viridi aereo in agar Sabouraudi et agar farinae frumenti, coloniae nigrae scabrae cum mycelio albo aereo in agar Littmani. Pigmentum solum cum oxygenio effectum, absens in agaro nutriente. Crescit cum 37° . Circuli pellicularum in decocto. Glucosum, mannosum, fructosum, maltosum et sucrosus fermentat.

Blastospores $3-4 \times 7-12 \mu$., chlamydospores $13-14 \mu$., arthrospores $8-11 \times 10-13 \mu$., cladosporium forms $7 \times 14-17 \mu$. Viscous black colonies with black surface mycelium and green aerial mycelium on Sabouraud and corn meal agar, rough black colonies with white aerial mycelium on Littman agar. Pigment

produced only aerobically, absent on nutrient agar. Growth at 37°. Rings of pellicles in broth. Ferments glucose, mannose, fructose, maltose and sucrose.

Obtained from Centraalbureau voor Schimmelcultures as *Pullularia pullulans* (de Bary) Berkhout strain Schoen. Received by the Centraalbureau in 1937 as *Torula schoenii* Roukhelman.

***Pullularia fermentans* Wynne et Gott var. *benedekii* var. nov.**

Blastospores $3-5 \times 7 \mu$., chlamydospores 12μ ., arthrospores $3-5 \times 5-7 \mu$., cladosporium format $3 \times 10 \mu$. Coloniae nigrae cum mycelio aereo cinereo aut mycelio nigro in superficie in agaro Sabouraudi, farina frumenti, nutrimento et agaro Littmani, tantum sine oxygenio quantum cum oxygenio. Crescit cum 37°. Parum formata pellicula et cristae incrementi per totam substantiam liquidam. Glucosum et mannosum fermentat.

Blastospores $3-5 \times 7 \mu$., chlamydospores 12μ ., arthrospores $3-5 \times 5-7 \mu$., cladosporium forms $3 \times 10 \mu$. Black colonies with grey aerial mycelium or black surface mycelium on Sabouraud, corn meal, nutrient and Littman agar, both anaerobically and aerobically. Growth at 37°. Poorly formed pellicle and tufts of growth throughout liquid medium. Ferments glucose and mannose.

Obtained from Centraalbureau voor Schimmelcultures as *Pullularia pullulans* (de Bary) Berkhout strain Benedek. Received by the Centraalbureau in 1933 as *Torula lecanii corni*, isolated from *Lecanium corni*.

***Pullularia fermentans* Wynne et Gott var. *castellanii* var. nov.**

Blastospores $3-4 \times 7-10 \mu$., chlamydospores $12-13 \mu$., arthrospores $3 \times 10-13 \mu$., cladosporium format $3 \times 11 \mu$. Coloniae nigrae cum mycelio viridi aereo et in superficie agaro Sabouraudi, farina frumenti, nutrimento et agaro Littmani. Nullum pigmentum sine oxygenio. Non crescit cum 37°. Nulla pellicula in decocto; coloniae cristatae per totam substantiam. Glucosum solum fermentat.

Blastospores $3-4 \times 7-10 \mu$., chlamydospores $12-13 \mu$., arthrospores $3 \times 10-13 \mu$., cladosporium forms $3 \times 11 \mu$. Black colonies with green aerial and surface mycelium on Sabouraud, corn meal, nutrient and Littman agar. No pigment anaerobically. No growth at 37°. No pellicle in broth; tufted colonies throughout medium. Ferments glucose only.

Obtained from Centraalbureau voor Schimmelcultures, who received it in 1935 as *Cryptococcus metaniger*. Isolated by Castellani (1927) from a case of trichomycosis nigra.

Cultures of the type strain of *Pullularia fermentans* and the type variety and the type strain of each of its other 6 varieties have been dispatched to the American Type Culture Collection, The Commonwealth Mycological Institute Collection of Fungus Cultures, and the Centraalbureau voor Schimmelcultures.

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E. S. WYNNE AND C. L. GOTT—PROPOSED REVISION OF PULLULARIA. PLATE 1

(Facing p. 519)

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EXPLANATION OF PLATE 1

Pullularia fermentans sp.n. isolated from cervical lymph node of a case of Hodgkin's granuloma. All photomicrographs at approximately $\times 300$.

- Fig. 1. Colonies on corn meal agar after 12 days at 25°.
- Fig. 2. Young mycelium with early blastospores from edge of colony on corn meal agar. Portions of the mycelium and some of the blastospores are dark because of beginning pigment production.
- Fig. 3. Older mycelium completely fragmented into dark arthrospores, some of which exhibit characteristic 'sprouting'.
- Fig. 4. Masses of blastospores resulting from sprouting of arthrospores.
- Fig. 5. Formation of double-walled chlamydospores.
- Fig. 6. Arthrospores, blastospores and chlamydospores in a single hyphal strand.

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