ON SOME ISOLATES OF THE GENUS PREUSSIA FUCKEL FROM INDIAN SOILS

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INTRODUCTION

Cain (1961) monographed the little-known genus Preussia Fuckel (Fuckel, 1866) and transferred certain species of genera Perisporium Fries, Sporomia, de Notaris, Muellerella Hepp, Pycnidiothora Clum, Anixiopsis Hansen and Thielavia Zopf to this genus. Cain (1961) observed that for species of Preussia "no adequate means of delimitation is available" and emphasised the need of further work in this direction. He accepted the presence of cleistothecial pseudothecia, "the size and relative shapes of the ascospore segments" and "pigment formation in culture" as the most reliable characteristics of this genus.

This paper embodies our observations on four ascomycetes. Preussia aurantiaca Rai and Tewari spec. nov., P. dispersa (Clum) Cain and P. multispora (Saito and Minoura) Cain are new to India. A form formerly described as Pseudoeurotium globosum Rai and Tewari (Rai and Tewari, 1961) has been renamed as Preussia globosa (Rai and Tewari) Rai and Tewari comb. nov.

Species of genus Preussia described here, except P. globosa comb. nov., are marked by a fairly high rate of saltation and an account of this will be published elsewhere.

MATERIALS AND METHODS

Soil samples were collected under sterile conditions and plated out on Czapek-Dox agar + 0.5% yeast using Soil-plate (Warcup, 1950) and Soil-dilution (Waksman and Fred, 1922) methods.

Cultures listed in Table I were used for comparison.
Table I

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<thead>
<tr>
<th>Name* Source</th>
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<tr>
<td>Pyenidiophora dispersa Clum . . . . ATCC 12436</td>
<td>Pseudeurotium zonatum van Beyma . . . NRRL 2520</td>
<td>P. ovalis Stolk . . . NRRL 2515</td>
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<td>. . . . NRRL 2518</td>
<td>P. multisporum (Saito and Minoura) Stolk . . NRRL 2518</td>
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* Cultures are listed under the names in which they were originally received.

Description

Preussia aurantiaca Rai and Tewari spec. nov.

Coloniae in agaro Czapek-Dox + 0·5% fermenti extracto ad temp. 27 ± 1°C. circulares, mycelio aereo pallide aurantiaco sparso vel nullo, diametrum attingentes ca. 8·5 cm. post dies 10. Pars obversa agari pallide rubra vel pallide aurantiaca. Mycelium submersum hyalinum, septatum, ramosum, 1·0–4·0 µ latum, hyphis tenuioribus saepe plus minusve spiraliter convolutis. Cleistothecia sphaerica, carbonacea, confluentia, efformata irregulariter in superficie vel submersa in medio, parietibus constantibus unica serie cellularum brunnearum, parietibus crassis praeditarum, polygonalium, pseudoparenchymaticarum, absque ulla sutura, 148·0–592·0 µ diam. Ascii ellipsoidae vel pyriformes, evanescentes, efformati ut rami laterales hypharum ascogenarum, octaspori, 9·6–16·0 µ × 7·2–9·6 µ. Ascosporae leves, ovales vel bacilliformes (vulgo bacilliformes) apicibus rotundatis, primo hyalinae, ad maturitatem evadentes bruneae, absque ulla germinationis poro, ut plurimum 1–3 globulis oleaceis ornatae, 2·0–4·8 µ × 1·6–2·4 µ. Pycnidia globosa vel elongata collo protrudente et unum ostiolum monstrante; ut plurimum ostiolum unicum in pycnidio; parietes constantes bina ternave serie cellularum; pycnidia efformata in superficie vel immediate sub ipsa superficie, apparentia ut puncta minuta pallide aurantiaca vel rubreola, praecedentia formationem cleistotheciorum, abundantia etiam in regione intramarginali coloniae, 20·0–56·0 µ alta, 24·0–72·0 µ lata. Pycnosporae hyalinae, globosae vel ovales vel pyriformes, insidentes conidiophoris simplicibus brevibusque, 1·0–3·2 µ × 1·0–1·6 µ.
The Genus Preussia Fuckel from Indian Soils

In solo ad oras maritimas (pH 7.3) sub finem decembris anni 1960 ad Kagh Dweep, in Bengalia occidentali, in India; cultura typica posita in Comm. Myc. Inst. Kew in Anglia sub numero Herb. I.M.I. 86825.

Colonies (Figs. 57, 58) on Czapek-Dox agar + 0.5% yeast extract in dark at 27 ± 1°C. circular with no or little aerial light orange mycelium, attaining a diameter of about 8.5 cm. in 10 days. Reverse agar light red to light orange. Submerged mycelium hyaline, septate, branched, 1.0-4.0 μ in width, the thinner hyphae often more or less spirally coiled. Cleistothecia (Figs. 59-62) confluent, spherical, carbonaceous, formed irregularly on the surface or submerged in the medium, wall (Figs. 1, 62, 63) at maturity composed of a single layer of brown, thick-walled, polygonal, pseudoparenchymatous cells, devoid of any sutures, 148.0-592.0 μ in diameter. Asci (Figs. 2, 62, 64) ellipsoidal to pyriform, evanescent at maturity, no apical structure seen, formed as side branches of the ascogenous hyphae, octasporous, 9.6-16.0 μ × 7.2-9.6 μ. Ascospore segments (Figs. 3, 65) separate out as soon as visible, generally 20-30 segments issuing out from each ascus, smooth, oval to rod-shaped (mostly rod-shaped) with rounded ends, hyaline at first, turning brown at maturity, devoid of any germinal slit, generally with 1-3 oil globules, 2.0-4.8 μ × 1.6-2.4 μ, few ascospores elongate even up to 8.0 μ. Pycnidia (Figs. 28-33, 66, 67) globose to elongate with a protruding neck bearing a single ostiole, generally only one ostiole per pycnidium, wall composed of 2-3 layers of cells, formed on the surface or just below it, appearing as minute light orange to reddish dots to the naked eye, precede the cleistothecial formation, abundant even in the intramarginal region of the colony, generally formed in groups, 20.0-56.0 μ in height and 24.0-72.0 μ in breadth. Pycniospores (Figs. 4, 68) hyaline, globose to oval to pyriform, borne on simple short conidiophores, 1.0-3.2 μ × 1.0-1.6 μ.

P. aurantiaca spec. nov. differs from all the hitherto known species of genus Preussia (except P. dispersa) in having the conidial stage a pycnidium. From P. dispersa it differs mainly in having light orange colonies, production of orange pigment in the medium, presence of cleistothecia which tend to be confluent and in the absence of brown cells in the ostiolar region of the pycnidium. Segments of the ascospores separate out as soon as they are visible. At times the septum formation does not take place and the ascospore segment is about double its normal length. The species is named after the orange colour of its colonies and production of orange pigment in the medium.

Cleistothecial initial (Fig. 13) is in the form of an intercalary segment of swollen cells and there is no indication whatsoever of the presence of a well-defined antheridium or ascogonium. Subsequent development involves
division of these cells in all planes resulting in the formation of a rather compact cleistothecium. The internal tissue differentiates into the ascogenous hyphae and the ground tissue. The paraphyses are very poorly developed. The asci develop as side branches on the ascogenous hyphae. In most cases the development of asci takes place from the centre outwards. Simultaneously, the outer tangential and radial walls of the cells of the outermost layer of the cleistothecia also get thickened. In fully mature cleistothecia the ground tissue disappears and the asci deliquesce to release the ascospores into its cavity. The perfect stage of *P. aurantiaca* spec. nov. is thus a cleistothecial pseudothecium as in other species of this genus (Clum, 1955; Cain, 1961).

In cultures the cleistothecia have a marked tendency to develop in groups. During the later part of development, the walls of cleistothecia abutting each other do not undergo thickening and before the cleistothecia attain maturity, the common unthickened walls disintegrate resulting in the formation of irregular locules (Figs. 59, 60).

The pycnidia also develop (Figs. 18–27) more or less in the same fashion and have similar initials. Clum (1955) also recorded this fact for another species of this genus and remarked that "the pycnidial development is simple meristogenous and that the cleistothecial development is much the same except that the latter primarily develop on submerged hyphae while the pycnidia develop on superficial hyphae".

Percentage ascospore segment germination is fairly high in slide cultures using Czapek-Dox agar + 0.5% yeast-extract after 24 hours' incubation at 27 ± 1° C. The side wall of the ascospore segment bursts open at an undetermined spot (generally lateral), to give way to a single proximally broadened germ-tube (Fig. 5). Similar ascospore germination has been recorded for *Pseudeurotium ovalis* Stolk (Stolk, 1955).

Percentage pycniospore germination is also fairly high. They first swell to about double their volume and then put forth one or two germ-tubes from undetermined spots (Fig. 6) and the length of germ-tubes produced from the two spore types in 24 hours is more or less equal.

Hyphal loops and H-bridges (Figs. 34–35) are quite common in cultures and may be playing an important role in the genetics of this form. These are frequently associated with cleistothecial and pycnidial initials (Figs. 14, 19, 23), a fact also recorded for *Preussia dispersa* by Cain (1961). H-bridges have also been observed in the Peru isolate of *Anixiopsis stercoraria* (Hansen) Hansen by Stolk (1955), a strain which was later described as *A. peruviana* Cain by Cain (1955).
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Colonies (Fig. 69) on Czapek-Dox agar + 0.5% yeast-extract at 27 ± 1°C. in dark circular with a little white aerial mycelium, attaining a diameter of about 7.5 cm. in ten days. Reverse agar uncoloured. Submerged mycelium hyaline, septate, branched, 1.0-4.5 μ in width, the thinner hyphae being more or less coiled. Cleistothecia (Figs. 70-72) sphaerical, carbonaceous, formed irregularly on the surface or submerged in the medium or at times in the aerial mycelium, outer wall (Figs. 7, 72, 73) composed of a single layer of brown thick-walled polygonal, pseudoparenchymatous cells, devoid of any sutures, 370-0-925.0 μ in diameter. Asci (Figs. 8, 72, 74) ellipsoidal to pyriform, evanescent at maturity, devoid of any apical structure, formed as side branches of the ascogenous hyphae, octosporous, each ascus with 12-32 ascospore segments, 9.6-14.4 μ × 8.0-11.2 μ. Ascospore segments separating out as soon as visible (Figs. 9, 75), smooth, oval to rod-shaped (mostly rod-shaped) with rounded ends, at times slightly curved, hyaline at first, turning brown at maturity, devoid of any germinal slits, generally with 1-3 oil-globules, 2.0-8.0 μ × 2.0-3.0 μ. Pycnidia (Figs. 42-48, 76-78) globose to irregularly lobed with a slightly protruding ostiole, one to few ostioles per pycnidium, wall composed of 2-3 layers of cells which along with a few other adjoining hyphae are thick-walled and browned in the neck area, formed on the surface or just below it and also within the aerial mycelium, appearing as minute black dots to the naked eye, precede the cleistothecial formation, abundant even in the intramarginal region of the colony, 30-0-112.0 μ in height, 43-0-128.0 μ in breadth. Pycniospores (Figs. 10, 79) hyaline, globose to oval to pyriform, minute, produced in great abundance, borne on simple short conidiophores, 1.6-4.0 μ × 1.6-2.0 μ.

Isolated from samples of Mangrove mud (pH 7.3 and 7.5) collected in late December 1960 from Kagh Islands, W.B., India. A subculture has been deposited in the Commonwealth Mycological Institute, Kew, England, under reference number I.M.I. 86824.

The development of cleistothecia (Figs. 36-41) and pycnidia (Figs. 49-55) is similar to that described for *P. aurantiaca* spec. nov. except that the cleistothecia are not confluent.

Percentage ascospore segment germination (Fig. 11) using Czapek-Dox + 0.5% yeast-extract in slide cultures is low even after 24 hours' incubation at 27 ± 1°C. This observation is similar to that recorded for *Pseudem'otium ovalis* (Stolk, 1955). On the contrary the pycniospores germinate (Fig. 12)
in good numbers under similar conditions and the length of germ-tubes produced by them is greater than those produced by the ascospores within the same period.

Hyphal anastomoses and H-bridges (Fig. 56) are quite common in cultures.

This form was first described by Clum (1955) as *Pycnidiothora dispersa* who isolated it from *Phlox drummondi* Hook seedlings where it was causing a damping off disease. Cain (1961) later redescribed it as *Preussia dispersa*. The strain of *P. dispersa* described here differs from the type (ATCC 12436) in having larger cleistothecia, in its cultural response on Czapek-Dox agar + 0.5% yeast-extract and certain other minor morphological differences. The type culture of this species produces neither cleistothecia nor pycnidia on Czapek-Dox agar + 0.5% yeast-extract. Pathogenicity experiments on *Phlox drummondi* seedlings with the strain under study were also not successful.

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Colonies on Czapek-Dox agar + 0.5% yeast at 27-30°C. circular with little or abundant aerial mycelium, light pink to light reddish-pink in light and almost white in dark. The strain is marked by a high rate of saltation. Occasional colonies are almost white even in light and from such colonies coloured colony sectors often develop both in dark and in light. In certain cases the whole colony is coloured in dark. On Oatmeal agar very often the colony sectors are light pale-yellow in colour both in dark and in light. Cleistothecia developing very sparsely, carbonaceous, unappendaged, superficial or submerged outer wall pseudoparenchymatous (Fig. 80), devoid of any sutures, with cells having outer tangential and radial walls thickened. Ascii (Fig. 81) embedded in the ground tissue, produced as side branches of the ascogenous hyphae, globose to pyriform, without any apical structure, evanescent at maturity, octasporous, each ascospore with about four segments, 11.2-19.2 μ × 9.6-14.4 μ. Ascospore segments (Fig. 82) smooth, devoid of germinal slits, light brown, cylindrical with rounded ends, sometimes slightly curved, generally with 1-3 guttules, 3.2-5.6 μ × 2.4-4.5 μ rarely even up to 8.0 μ in length. No conidial stage observed.

Isolated from samples of Mangrove mud (pH 7.3) collected in late December, 1960, from Kagh Islands, W.B., India. A sub-culture has been deposited in the Commonwealth Mycological Institute, Kew, England, under reference number I.M.I. 86823.


According to Cain (1961) species of genus Preussia have “ascospores lying parallel in a fascicle or irregularly disposed in a subglobose mass, with three transverse septa, deeply constricted; segments readily separable at maturity (or in some species, as soon as visible)” Rai and Tewari (1962) while describing Pseudoeurotium globosum recorded frequent “ascospores bound together in groups” and concluded that they may be the “resultant of incomplete division in some asci”. These groups (Fig. 83) are generally 2—many celled with cells arranged in a linear, two or three-dimensional fashion and are rather not very clear in situ within the ascus due to intimate crowding of the ascospores. However, in the majority of cases the ascospore segments are readily separable at maturity. Thus in the light of Cain’s (1961) paper, Pseudoeurotium globosum should be included under the genus Preussia. It is renamed as Preussia globosa (Rai and Tewari) Rai and Tewari comb. nov.
Three species of genus *Preussia* Fuckel are recorded for the first time from India. These are: *P. dispersa* (Clum) Cain, *P. multispora* (Saito and Minoura) Cain and *P. aurantiaca* Rai and Tewari spec. nov. A fungus formerly described as *Pseudeurotium globosum* Rai and Tewari has been renamed as *Preussia globosa* (Rai and Tewari) Rai and Tewari comb. nov.

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REFERENCES


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Saito, K. and Minoura, K. .. *J. Fermentation Technol.*, 1948, 26, 47-49.


* Not seen in original.

EXPLANATION OF FIGURES

Figs. 1-12. Figs. 1-6. *Preussia aurantiaca* spec. nov. Fig. 1. Wall of the cleistothecium, ×1,200. Fig. 2. Asci, ×1,200. Fig. 3. Ascospore segments, ×2,500. Fig. 4. *Pyenio-
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*spores, ×2,500. Fig. 5. Stages in germination of ascospore segments, ×1,200. Fig. 6. Stages in germination of pycniospores, ×1,200. Figs. 7-12. *Preussia dispersa*. Fig. 7. Wall of the cleistothecia, ×1,200. Fig. 8. Asci, ×1,200. Fig. 9. Ascospore segments, ×2,500. Fig. 10. Pycniospores, ×2,500. Fig. 11. Stage in germination of the ascospore segment, ×1,200. Fig. 12. Stages in germination of pycniospores, ×1,200.*


*Figs. 80-83. Figs. 80-82. *Preussia multispora*. Fig. 80. Wall of the cleistothecium, ×1,200. Fig. 81. Asci, ×1,200. Ascospore segments, ×1,200. Fig. 83. *Preussia globosa* comb. nov. Ascospore segments, ×1,200.*

**EXPLANATION OF PLATES**

**PLATE I**

*Figs. 57-68. *Preussia aurantiaca* spec. nov. Figs. 57, 58. Ten-day old cultures on Czapek-Dox agar + 0.5% yeast-extract. Note the saltant sectors, ×0.5 approx. Fig. 59. A group of confluent cleistothecia showing unthickened butting walls, ×73.5. Fig. 61. Silhouettes of cleistothecia and pycnidia on Czapek-Dox agar + 0.5% yeast-extract, ×30.5. Fig. 62. Microtome section of the part of a cleistothecium, ×522. Fig. 63. Wall of the cleistothecium, ×522. Fig. 64. Asci, ×522. Fig. 65. Microtome section of ascospores, ×999. Fig. 66. Pycnidia, ×255. Fig. 67. Microtome section of pycnidia, ×291.5. Fig. 68. Pycniospores, ×1,215.*

**PLATE II**

*Figs. 69-79. *Preussia dispersa*. Fig. 69. Ten-day old culture on Czapek-Dox agar + 0.5% yeast-extract, ×0.6 approx. Fig. 70. Microtome section of cleistothecia and pycnidia, ×60.2. Fig. 71. Silhouettes of cleistothecia and pycnidia, on Czapek-Dox agar + 0.5% yeast-extract, ×30.5. Fig. 72. Microtome section of the part of a cleistothecium, ×522. Fig. 73. Wall of the cleistothecium, ×522. Fig. 74. Asci, ×522. Fig. 75. Microtome section of ascospores, ×999. Figs. 76, 77. Pycnidia, ×270. Fig. 78. Microtome section of a pycnidium, ×291.5. Fig. 79. Pycniospores, ×1,215.*