# MULTIPLE BUDDING IN SPOROTRICHUM SCHENCKII MATRUCHOT[1, 2]

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In artificial culture, both at room temperature and at 37°C, the mycelial phase of *Sporotrichum Schenckii* predominates, being characterised by thin, septate, branching hyphae which bear small oval to spherical conidia at the ends of short lateral branches and along the sides of undifferentiated hyphae. In the tissues of both naturally and artificially infected animals, on the contrary, the parasite appears as single-celled, cigar-shaped bodies that reproduce by the formation of a single bud at one or both of the cell extremities.

Lutz and Splendore (1, 2, 3) mentioned the transitory appearance of "Hefenformen" in culture on ergot infusion gelatin with 1% glucose and 1:1000 tartaric acid. Although there is no description of the budding process, a drawing shows short chains of the yeast-like cells (1). Beurmann and Gougerot (4) grew the budding phase on Sabouraud's glucose agar slants under a 2–3 mm. layer of liquid, and described briefly the development of buds as occurring typically at one or both ends of the cell. Davis (5) succeeded in culturing the tissue phase in defibrinated blood, and noted that a higher percentage of this form occurred to the exclusion of the mycelium when the cultures were grown under paraffin oil or in contact with sterile animal tissues, such as spleen, liver or kidney. Weise (6) included in his case report a brief statement by Emmons that some spores "budded in a yeast-like manner in old cultures" on Sabouraud's maltose agar, while Weidman (7) included a photograph showing several buds attached to a conidium. Negroni (8) grew the budding cells on 5% rabbit blood agar slants after having first suspended the mycelial inoculum for 24 hours at 37° C. in a sterile solution of 1–2% sodium borate or 0.0005–0.00025% sodium hydroxide. He also described these cells as budding usually at a single pole, and occasionally at two. Campbell (9) briefly reported the conversion of the mycelial to the yeast-like form on Francis' glucose cystine agar at 37°C., while Area Leao and Goto (10) reproduced the budding phase in diluted pus at 37°C.

Thus, the tissue phase appears as a cell or group of cells reproducing typically by the formation of a single bud at one or both ends of the cell, and capable of being grown in the laboratory under special conditions. It is the purpose of this paper to describe (1) the presence of "multiple budding" in the tissue phase and (2) the growth of the tissue form in a fluid peptone medium.

## MATERIALS AND METHODS

The following cultures from human cases of sporotrichosis were obtained from the collection in this laboratory:

*7010 isolated in Oklahoma (1938)
7013 isolated in Wisconsin (1940)

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[2] From the Division of Infectious Diseases, National Institute of Health, Bethesda, Maryland.

7015 isolated in Johannesburg, S. Africa (1942)
7016 isolated in West Virginia (1943)
7017 isolated in Maryland (1944)
7019 isolated in Guatemala (1945)
7020 isolated in Ohio (1946).

These seven strains were maintained in culture on Sabouraud's glucose agar slants at room temperature. Most of the experimental studies were conducted in "YP medium" either in 10 cc. of the medium in 25 x 150 mm tubes or in hanging-drops of YP medium in Van Tieghem cells. YP medium, which was originally devised for the growth of the yeast-like phase of *Histoplasma capsulatum* (11), had the following composition:

| | |
|---|---|
| proteose-peptone | 10 g |
| Neopeptone | 3.25 g |
| bacto-tryptone | 3.25 g |
| bacto-dextrose | 2.0 g |
| sodium chloride | 5.0 g |
| disodium phosphate | 2.5 g |
| bacto-agar | 1.75 g |
| distilled water to make | 1000 cc. |

When necessary, the tubes were sealed with paraffin to prevent evaporation of the fluid medium, especially at 37°C. The hanging-drop cultures were maintained at 37°C. in micro-slide incubators, which fitted onto microscope stages and permitted direct microscopic examination of the budding process.

## EXPERIMENTS AND RESULTS

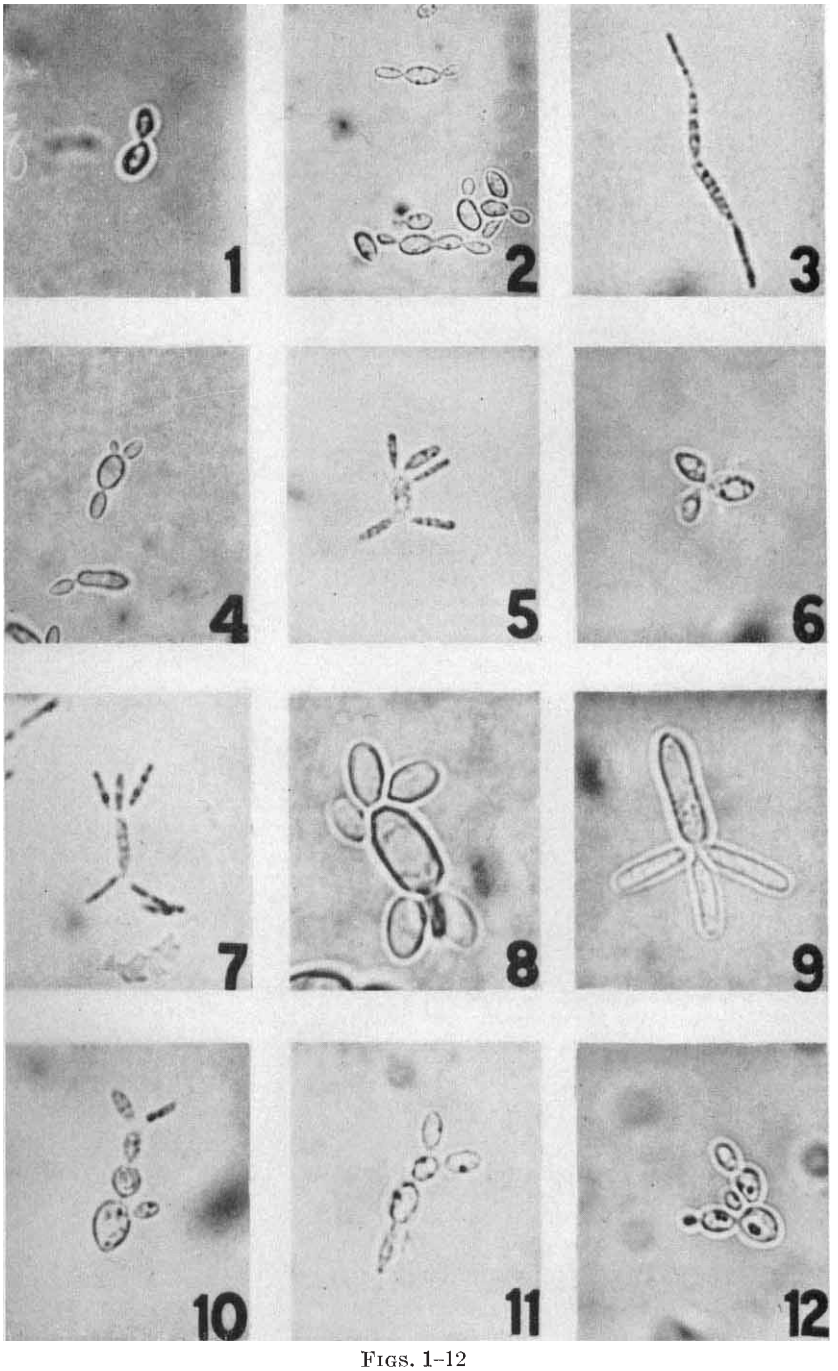
The strains were initially grown for five days in the YP medium at 37°C., at pH 7.3, in sealed tubes. The resulting growth was not pigmented and was confined to the upper half of the medium. Microscopically, both the mycelial and the yeast-like phases were present, with the exact proportion of each varying with each strain of *S. Schenckii*. Although two strains displayed no apparent mycelium, all showed at least a small number of budding cells.

The tissue phase of the seven strains characteristically formed oval to spindle-shaped single buds at either or both the cell poles (figs. 1, 2). These buds did not readily separate from the parent cell, and frequently remained attached to produce short moniliform chains (fig. 3).

In addition, in all the strains studied, many cells displayed a multiple type of budding, in which more than one cell was generated at any single pole. As seen in figures 4–9, one to four buds developed simultaneously or successively at either one or both poles of a cell. These daughter cells frequently remained adherent to the parent cell, formed additional buds, and thus produced arborescent chains of budding organisms (fig. 10–12). The exact shape of both mother and daughter cells varied from spherical to oval to bacilliform, and the size from 1–5 x 2–12 micra, both within a strain and from strain to strain.

Another type of multiple budding was observed (figs. 13–16), wherein the buds were borne not only terminally but seemingly at any point on the cell surface. This type was limited mostly to the spherical and oval cells, being usually absent from the bacilliform cells. When the daughter cells did not become separated, a small mass of connected cells resulted.

The processes of both single and multiple budding were followed in Van

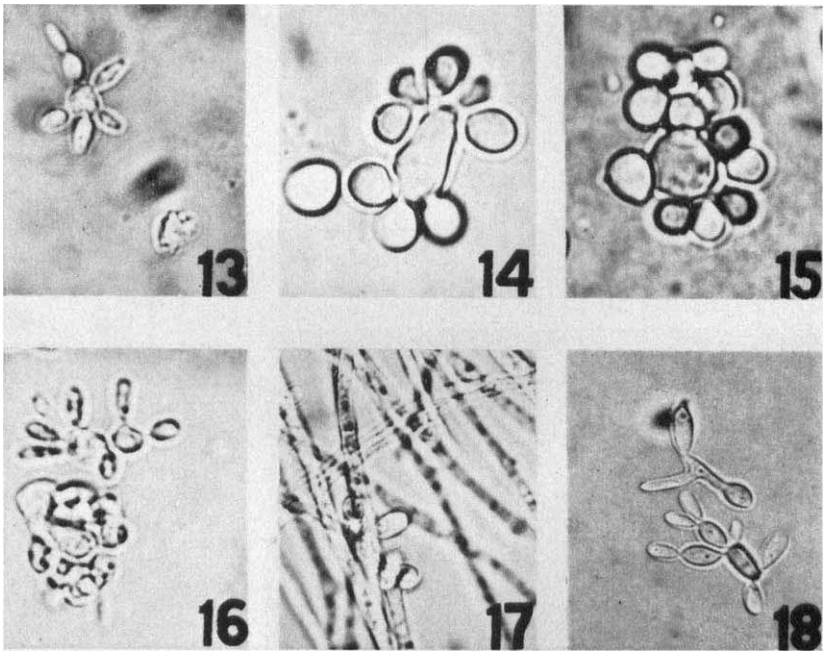



FIGS. 1–12

FIGS. 1–12: Photomicrographs of the budding phase of *Sporotrichum Schenckii*. ✱2, 4 ×1050. ✱1, 3, 5, 6, 7, 10–12 ×1250. ✱8, 9 ×2100.

Tieghem cell preparations in a micro-slide incubator at 37°C. In all cases, the inoculum was the budding phase, and the number of cells limited to 1–5 per hanging drop.

Experiments were conducted in attempts to increase the relative amount of the budding form to the complete elimination of the mycelium. However, although absolute success was not obtained in all the strains, the following conditions favored the development of a maximum quantity of budding cells and a minimum of mycelium.




FIGS. 13–18

FIG. 13–16: Photomicrographs of the budding phase of *Sporotrichum Schenkii*. #13, 16 ×1250. #14, 15 ×2100.
FIG. 17. Photomicrograph of mycelial phase. ×1050.
FIG. 18. Photomicrograph of the abortive-hyphal phase. ×1050.

(1) The temperature should be regulated to approximately 37°C. (2) The percentage of agar in the medium should be between 0.1 and 0.3%, with the optimum at 0.15–0.20%. The absence of agar results in a mycelial growth virtually devoid of the budding phase (fig. 17). (3) The medium should be slightly alkaline (pH 8.2), since in the pH range tested—4.0–9.1—acid conditions produced mycelium and conidia alone, but mild alkalinity decreased the proportion of mycelium and increased that of budding forms. (4) The carbon dioxide tension should be increased, preferably to 60–80%. (5) The amount of protein and carbohydrate should not be excessive, since mycelium then starts to flourish to the detriment of the yeast-like phase.

## DISCUSSION

Multiple budding was probably seen many times by past investigators, but has not been carefully described. Beurmann and Gougerot (4), for example, diagrammed a round body with three small elements attached to it. Davis (5) photographed a group of spindle-shaped cells "arranged in ray-like clusters", which had been growing in pure blood. In Weise's paper (6), a photograph by Emmons showed some cells bearing several buds directly, and others producing short hyphae on which secondary conidia were borne.

Splendore (12) described a new species, *Sporotrichum asteroïdes*, which Beurmann and Gougerot (4) and Talice and MacKinnon (13) later reduced to synonymy with *S. Schenckii*. This form was separated because of the presence in pus of a characteristic asteroid body, 4–12 microns in diameter, apparently with a distinct nucleus, and with a thick wall to which many cylindrical or club-shaped prolongations were attached radially. Although this structure bears a resemblance to the "multiple-budding" phase described in this paper, it differed because of the large number of the radiating prolongations, the thick wall, and the large size.

The pleomorphic qualities of *Sporotrichum Schenckii* are quite pronounced. At least four phases may be recognised, each of which may be readily transformed into the other, depending on the environmental characteristics: (a) the yeast-like or tissue phase with single buds at one or both extremities, which appears characteristically in tissue; (b) the yeast-like phase with multiple buds, which is plentiful in YP medium; (c) the "abortive-hyphal" phase (6, 14, 15), with a spore giving rise to a short conidiophore which in turn bears another group of spores; (Fig. 18) and (d) the mycelial phase, with conidia borne at the tips of conidiophores and laterally on hyphae. The abortive-hyphal phase develops on a variety of media and may be viewed as an abortive response to conditions which favor the conversion of the tissue phase to the mycelial, or vice versa. The yeast-like phase with multiple buds in turn may be considered as intermediate between the phase with single buds and the abortive-hyphal form, since development into either of the latter two occurs readily. Thus, with the additional observation that a mixture of the first three forms may be found together in appropriate media, *S. Schenckii* may be viewed as one of the more pleomorphic of the pathogenic fungi.

*S. Schenckii* is the third pathogenic fungus to be shown to have multiple budding, the others being *Blastomyces brasiliensis* and *B. dermatitidis*. The multiple-budding phase of *B. brasiliensis* predominates both in living tissue and under certain cultural conditions, although cells with single buds also appear to a slight degree (16). *B. dermatitidis*, on the other hand, typically forms only a single large bud both in tissue and under certain cultural conditions, although some multiple budding also occurs (17). Thus, all three species have both single and multiple budding, with the type that predominates depending (a) on the species and (b) on the prevailing growth conditions.

SUMMARY

1. The budding or tissue phase of seven strains of *Sporotrichum Schenckii* was grown in a fluid medium.

2. In addition to the formation of single buds, two types of multiple budding were observed: one in which one to four buds were borne at one or both of the cell extremities; and one in which buds were formed at any point on the cell surface.

3. Optimum conditions for the culture of the tissue phase "in vitro" were studied.

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