Studies on the basidial Formation by Sclerotium rolfsii Sacc XII. A Discussion on the basidial Stage of Sclerotium Rolfsii Sacc. Isolates and its Identity.

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In the earlier papers (Series I to XI, 1965) the authors had already reported the success obtained in getting basidial stage of *Sclerotium rolfsii* Sacc. isolates from potato, *Colocasia* and groundnut (*Arachis hypogea* L.) and media as well as conditions under which these stages could be obtained. It is proposed to review these in this paper and discuss in detail the basidial stage of *S. rolfsii* and its nomenclature. The authors attempted to obtain basidial stage using four isolates, two from potato named as potato isolate 1 and 2 and one each from *Colocasia* and groundnut. Of these isolates, success in getting the basidial stage was obtained by potato isolate 2, *Colocasia* and groundnut. While basidial formation by *S. rolfsii* appears to be difficult either in nature or on the culture media, in some isolates of *S. rolfsii*, particularly in those which Goto (1930—35) had classified under his groups I and II, no much difficulty in getting this stage was encuuntered. Goto (1930) included potato isolate under his group I was easily spore formers, *Colocasia* isolate under group II as spore formers and groundnut isolate under group IV as non-spore forming strains. So far, perfect stage of the potato isolate had been reported by Goto (1930) from Japan, Curzi (1931) from Italy, Barrett (1934) from U.S.A. and by Mundkur (1934) and Misra et al (1960) from India. Goto (1930) also noticed that one of his potato isolates did not produce basidia and hence he classified this isolate under group IV as non-spore forming strain. Except Goto (1930—35) and Altstatt (1941), who reported the perfect stage of *S. rolfsii* from *Colocasia* and groundnut respectively, no report of getting the perfect stage of this fungus from these two hosts is forthcoming.

In India, it appears that no worker had tried to get the basidial stage of this fungus from *Colocasia* and groundnut. While Goto (1930—35) found no difficulty in getting the perfect stage of the fungus from *Colocasia*, in the present studies this difficulty was keenly felt as the media reported by these authors to be the best for promoting the basidial formation, proved to be unsuitable under Indian conditions. However, *Colocasia* isolate could induce perfect stage on a variety of other media newly devised in the present studies.

Since all the attempts to induce the basidial formatoin were unsuccessful in the potato isolate 1, studied now, in the present investigations, it is felt that this isolate should be considered as non-spore forming strain under the group IV of Goto (1930—35). The groundnut isolate, though classified by Goto (1930—35) under his group IV, was found to develop basidia on two culture media, thus confirming the observations of Altstatt (1941). This isolate should find a place under the group II of Goto (1930—35) as spore forming strain. The *Colocasia* isolate, on the other hand behaved similarly, as its basidial stage was obtained on the four media. This isolate should be classified under the group I of Goto (1930—35) since it behaved as easily spore forming strain. The potato isolate 2, however, readily formed basidia on the host as well as on more than one media and thus should be placed under the group I of Goto. Thus Goto's (1930—35) system of grouping *S. rolfsii* was in agreement in the present studies, so far as potato isolates 1 and 2 are considered. *Colocasia* and groundnut isolates, however, behaved quite differently in the present studies. Further, Goto's system was also not in agreement with the findings reported by other workers. This system therefore needs either revision or rejection, since it is felt that this fungus being of a plastic nature, behaves differently under varied conditions of nutrition, temperatur and incubation period.

In the present investigations, potato isolate 1 failed to induce basidial formation on all the media tried. Potato isolate 2 developed basidia on the host, on Lewi's medium with tryptophane and also on uric acid. It developed abortive basidia on extracts of ritha (Soapberry sapindus) raddish (*Raphanus sativus*), Coon's Kirchoff's, and modified Coon's medium containing ammonium sulphate, nitrate and phosphate and on modified Lewi's medium containing Alpha-N-acetic acid and trichloro phenoxy-2-4-5 acetic acid.

The *Colocasia* and groundnut isolates formed basidia on modified Lewi's medium containing vitamine-E and uric acid. *Colocasia* isolate also formed basidia on calcium carbonate with reduced source of carbon and also on onion proteose peptone. These two isolates also developed abortive basidia on modified Lewi's medium containing Alpha-N-acetic acid and trichlorophenoxy-2-4-5 acetic acid. All the three isolates required medium which consisted of nitrogenous compounds mostly in the organic form. For *Colocasia* and groundnut isolates providing vitamine-E in the medium also was felt necessary to get the basidial stage. Besides this, reduced carbon source and increasing alkalinity by the addition of calcium carbonate in the medium also favoured the basidial formation by the *Colocasia* isolate. None of the isolates developed basidia on any of the remaining media tried. It appears, therefore, that organic nitrogen, specially in the form of amino-acids like tryptophane, uric acid or proteose peptone, and also reduced carbon with increased alkalinity are

necessary for the basidial formation. It is interesting to note that all the isolates formed basidia on uric acid which should be considered as the best medium for promoting this stage. The media, on wich the basidial stage of potato isolate 2, *Colocasia* and groundnut isolates of *S. rolfsii* was obtained, were used for the first time in the present investigations. Organic nitrogen, vitamine E, reduced source of carbon and increasing the alkalinity of the medium seem to interefere with normal growth of the fungus, thus retarding the development of sclerotia, in number and size and thereby inducing the fungus to produce the other stage viz. the basidial stage for its survival in nature. The fact that heavy application of nitrogenous fertilisers had beneficial effect in controlling the disease caused by *S. rolfsii* can thus be attributed to the supression of the sclerotial stage of the fungus. Similarly, by providing continuously arid conditions with slight rise in temperature suppressed the sclerotial stage, but induced the basidial stage. It is likely that under heavy nitrogen content of high humid conditions of the soil, the fungus might be tempted to induce the basidial stage. This, ofcourse, appears to be a rare possibility, since the formation of basidial stage by *S. rolfsii* in nature is mostly uncommon. The basidial formation by artificial inoculation of the host has been obtained for the first time.

The temperatures considered to be the optimum for the basidial formation also ranged from 14° to 23° C to 30° to 32° C. In the present studies, incubation at 30° C for 7 days followed by keeping the fungus at 25°—26° C favoured the basidial formation. By providing 28°—30° C and high humidity continuously also induced the basidial formation. By keeping the fungus under low temperatures, no fruitful results were obtained. It appears, therefore, that for the basidial formation, the isolates on hand required a range of temperatures from 25° to 30° C thus, more or less agreeing with the findings of the majority of workers. Further, dim light obtained by storing the petri dishes in the incubators or under the moist cover, appeared to be favourable for the basidial formation as against day light. The period of incubation again appeared to be not definite. Variations in this regard had been reported from 12 to 30 days to 40 to 60 days. In the present studies 12 to 15 days were required for the basidial formation on tryptophane and 35 to 40 days on uric acid or on the host for the potato isolate 2 while for *Colocasia* and groundnut isolates, 50 to 60 days were necessary on vitamine E medium. These isolates also produced this stage in 40 days on uric acid.

Hymenial formation by the potato isolate 2 on the tryptophane or on the host appeared powdery white, while on uric acid it was button like and loose. Basidia were club shaped in all the 3 isolates. In the potato isolate 2, they were stout and thick and measured on the tryptophane medium 16—43 microns $\times$ 5.0—6.4 microns, while on host or uric acid,

they were smaller in size and measured 10—20 microns × 4.5—6.4 microns. In the *Colocasia* and groundnut isolates they were slender, narrow and measured 20—25 microns × 3.5—6.5 microns. Probably nutritional difference had some influence on their formation and size.

Sterigmata were broad at the base, pointed at the tip and varied from 1 to 4 on a basidium. Sterigmata of the potato insolate 2, were long, while those of *Colocasia* and groundnut isolates, were slightly smaller and the smallest respectively. The sterigmata of the potato isolate 2, from the host also were smaller like those of *Colocasia*. Basidiospores were hyaline, smooth, obclavate in all the isolates, but were smaller in the *Colocasia* and the groundnut isolates. Here also, probably nutritional variations might have some influence on their smaller size.

Goto (1930) identified the basidial stage of *S. rolfsii* as *Corticium centrifugum* and rejected the binomial *Hypochnus centrifugus* proposed by Sawada in 1919. Curzi (1931), however, changed it to *Corticium rolfsii* which binomial was subsequently followed by several workers from different countries until in 1945 when West changed it to *Pellicularia rolfsii*. The question now is to find out the identity of the basidial stage of the three isolates under study. The genus *Corticium* included unrelated groups of species and was considered as a collective genus, when Rogers (1935) thought it to segregate from it the genera, *Ceratobasidium* Rogers, *Pellicularia* Cook and *Trechispora* Karst. He attributed the identity of *Pellicularia* to those species of *Corticium, Hypochnus* and *Peniphora,* which possessed areolate hymenium, short celled stout hyphae, right angle branching of the mycelium, stout basidia and mucedinoid texture. In the light of this description, which closely agrees with the findings of West (1945) the identity of his fungus *Pellicularia rolfsii* on *F. pumila* appears to tbe justifiable. The discription given above for the genus *Pellicularia* also agrees with the findings in all the three isolates of *S. rolfsii* now under study, thus placing them under the genus *Pellicularia.* Before assigning specific names to these isolates a study of the comparative statement denoting measurements of basidia, sterigmata and basidiospores as given in the Table 1 appears to be essential.

It will be seen from the Table 1 that though variations in the measurements of basidia, sterigmata, and basidiospores exist in the isolates under study, when compared among themselves as well as among the isolates reported by other workers, it appears that further delimitation within the species is not warranted as these measurements were not recorded from the same nutritional medium. Thus the principle of „nomina conservanda" appears to be justifiable in giving the binomial *Pellicularia rolfsii* West to the basidial stage of all the three isolates of *S. rolfsii* from potato, *Colocasia* and groundnut, now studied in the present investigations.

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Table 1.

Comparative statement denoting measurements of basidia, sterigmata and basidiospores of the three isolates of *S. rolfsii*.

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Table 1. Comparative statement denoting measurements of basidia, sterigmata and basidiospores of the three isolates of *S. rolfsii*

Author	Name of host.	Basidia length	Basidia Breadth in microns	Sterigmata length	Sterigmata Breadth in microns	Basidiospores length	Basidiospores Breadth in microns	Hymenium size colour in mm		Other characters Mycelium	Other characters incubation period	Binomial.
1	2	3	4	5	6	7	8	9	10	11	12	13
Goto (1930) Japan	*Solanum tuberosum*	21.82 to 32.85	4.38 to 7.30	2.19 to 9,49	0.88 to 0,19	5.11 to 12.41	2.90 to 8.03		Pure white powdery appearance	Primary and secondary	60 days	*Corticium centrifugum* (Lev) Bres.
Curzi (1931) Italy						4.5 to 6.75	3.5 to 4.5			Densely intricate, branched hyphae	12–15 days	*Corticium rolfsii*
Mundkur (1934) India	-do-	16.5 to 39.0	4.2 to 6.1	3–5		4.9 to 9.4	2.6 to 7.1	6–12	Aerial dendroid white	Primary and secondary	40–45 days	*Corticium rolfsii* Curzi
Misra and Haque (1960) India	-do-	Details not available									33–39 days	*Pellicularia rolfsii* West.
Isolate under study	-do-	16–42	5–6.4	4–7.2	1.5–2	4.2 to 9.6	2–6.2	3–6	white powdery growth	Primary and secondary	15–15 days	*Pellicularia rolfsii* West.

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1	2	3	4	5	6	7	8	9	10	11	12	13
Altstatt G. E. (1941) USA	*Arachis hypogea*					3.9 to 5.4	1.0 to 3.6	Details not available				
Isolate under study	-do-	10—25	3.5 to 5.0	1.5 to 3.0	1—2	5.0 to 6.5	2.0 to 3.5	0.20 to 0.50 × 0.75 to 1.0 mm	Pure white buttons loosely formed	Primary and secondary	60 days	*Pellicularia rolfsii* West.
Goto (1930) Japan	*Colocasia antiqorum*	21.82 to 32.00	4.5 to 7.3	2.0 to 9.5	0.8 to 2.1	5.1 to 12.4	2.9 to 8.0	Flattened, resupinate rather loosely formed and confluent, dendroid, attached to aerial hyphae or powdery mildew like		Primary and secondary	15—100 days	*Corticium centrifugum* (Lev.) Bres.
Isolate under study	-do-	18.22	4—6	3.0 to 4.5	1.5 to 2.0	5—7	2—4	White loosely formed button like, not changing colour		Primary and secondary right angled branching	40—60 days	*Pellicularia rolfsii* West.

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