Studies on the basidial formation by Sclerotum rolfsii Sacc.-XI. Attempts made to induce the basidial stage on culture media.

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Introduction

While working on the basidial formation by *Sclerotium rolfsii* Sacc. the authors could get success in getting this stage on a variety media by the isolates of this fungus from potato, *Colocasia* and groundnut. However, this success could only be obtained when several attempts by using a variety of media, there modifications and changing the conditions of growth had to be made. It will be interesting to note how these attemps were made and also the media and the conditions under which this stage was obtained by the authors. This account is abridged in this paper.

a) Use of culture media already tried

Several authors reported basidial stage of the fungus on a variety of media. An attempt was therefore made to grow the four isolates of *S. rolfsii* on hand on these media. Thus, Goto's (1930) onion, carrot and apricot extract agars and P. D. A. with his temperature ranges, Curzi's (1931) potato glucose agar at 12° C to 28° C, Mundkur's (1934) onion aspargine proteose peptone agar keeping cultures at 30° C—31° C, Milthorpe's (1941) onion proteose peptone agar and Brown's agar used by Misra et al. (1960) at 20° C were initially tried. These media were prepared, sterilized, poured in the plates, inoculated in the centre with one sclerotium of the fungus and incubated at temperatures tried by the author for a medium. Periodical observations were taken both macroscopically as well as microscopically to observe the basidial stage. Except on Milthorpe's (1941) onion proteose peptone agar, no fruitful results in getting the stage on the rest of the media were obtained even after incubation of the cultures for over 80 days. *Colocasia* isolate developed white, aerial, velvety mycelial growth on onion proteose peptone agar in 40 days, which on microscopic examinations revealed typical hymenial formation bearing

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basidia and basidiospores. Basidia were stout, erect with 1 to 4 sterigmata bearing basidiospores, one on each sterigma. On careful isolation basidiospores yielded typical culture of *S. rolfsii* bearing sclerotia only. Most of these media, therefore, were not found suitable for inducing basidia of *S. rolfsii* on hand.

b) Use of the vegetable extracts.

Since Goto (1930), Mundkur (1934), Milthorpe (1941) and Venkatkrishnayaah (1946) incorporated extracts of onion as one of the ingredients in their media for obtaining the basidial stage of *S. rolfsii,* it was thought worthwhile to use extracts of such vegetables which had spicy contents. For this purpose, extracts of onion (*Allium cepa,* L.) 30 gm. in 100 cc water, garlic (*Allium sativum,* L.) 6 gm. in 100 cc water, soyabean (*Glycine max,* Merr.) 20 gm. in 100 cc water, *bajri* (*Pennisetum typhoideum,* Stapf.) 20 gm. with *jaggery* 1 gm. in 100 cc water, methi (*Trigonella foenum-graecum* L.) 10 gm. in 100 cc water, raddish (*Raphanus sativus,* L.) 10 gm. in 100 cc water, cinnamon (*Cinnamonum zeylanicum* Blume.) 6 gm. in 100 cc water, *ritha* (*Soapberry sapindus*) 6 gm. in 100 cc water, clove (*Eugenis caryophyllata*) 2gm. in 100 cc water, nut meg scales (*Mace myristica*) 6 gm. in 100 cc water, chinese root 6 gm. in 100 cc water, white gunj (*Lathyrus inconspicum*) 6 gm. in 100 cc water and ginger (*Zinziber officinale,* Rose.) 10 gm. in 100 cc water were prepared by boiling them for one hour, decanted and required quantity of agar was added. The media, thus prepared were sterilised and poured in petri dishes. Slants were also prepared. Poured plates and slants were inoculated with the sclerotium along with a bit of mycelium of the fungus and incubated at room temperature (25°C to 26° C). Observations were recorded daily and continued for 100 days. On ritha *(S. sapindus)* and raddish (*R. sativum*) extracts, whitish, slightly powdery and aerial growth was observed only in the potato isolate-2, after 70 days. On critical microscopic examinations this growth consisted of hymenia with stout, thick and slightly elongated roundish structures indicating the ability of this isolate to induce abortive basidia. The plate cultures and slants were further incubated for 120 days. The whitish growth which yielded abortive basidia subsequently disappeared. Microscopic observations at periodical intervals did not show indications of the basidial formation any more. It appears, therefore, that vegetable and seed extracts did not favour the basidial formation by *S. rolfsii* isolates on hand.

c) Use of the vegetable and fruit pieces.

Pieces of fruits and vegetables constitute an excellent medium for the growth and sporulation of many fungi. Such media are known to induce perfect stages in *Phytophthora, Collectotrichum* and also in

the several other genera. No report on the formation of basidia by *S. rolfsii* on vegetable pieces has so far been made although, Goto (1930) and other workers used extracts of some vegetables and fruits. The present investigations were, therefore, undertaken to see, whether pieces of vegetables and fruits could promote basidial formation by the *S. rolfsii* isolates on hand. For this purpose, pieces of suitable size of raddish (*R. sativus*), carrot (*Daucus carrota*, L.) raw *papaya* (*Carica papaya*, L.), onion (*A. Cepa*), potato (*Solanum tuberosum*, L.) cabbage (*Bassica oleracea*, L.), smooth goured (*Luffa aegyptica*) and of ficus (*Ficus sp.* L.) fruits, were cut and disinfected in ethyl alcohol for 1 minute. These pieces were then transferred to the sterile petri dishes, heated in oven for a while to remove the traces of alcohol. In the other set of the experiment these pieces were placed in the petri dishes and sterilised in autoclave at 15 lbs. pressure for 15 minutes. The pieces were then inoculated with a sclerotium along with a bit of the mycelium of the fungus and stored at 30° C. Observations recorded daily for locating the whitish, dendroid, aerial, powdery growth, were continued over 120 days. No basidia could be formed by any of the isolates. The fungus formed abundant sclerotia only in both the cases. It appears therefore, that vegetable pieces did not induce the basidial formation by *S. rolfsii* isolates on hand.

d) Use of the standard laboratory media.

The following media were prepared according to their standard methods.

1) Coon's with 0.7 sugar content,
2) Czapeck's,
3) Richards',
4) Kirchoff's with 25% sugar content,
5) Uric acid with 50% reduced sugars, (uric acid 1 gm.; dextose 1 gm.; water 100 cc.) — A new medium for getting the basidial stage.
6) Brown's I and II,
7) Leonian's,
8) Yeast chalk glucose,
9) nutrient nitrate,
10) Sabourads,
11) proteose peptone,
12) glutanic acid, and
13) oatmeal agars.

These above media were poured in plates inoculated with the fungus and incubated at 30° C for 7days to induce maximum vegetative growth and then transferred to room temperature (25° to 26° C.).

Periodical observations were recorded and continued upto 100 days. Of these media, fungus could develop whitish growth on Coon's and Kirchoff's agars. On actual microscopic examinations, hymenial development with the formation of sterile basidia was noticed in the potato isolate 2 only.

On 40th day, plates poured with uric acid developed white, velvety mycelial growth in all the isolates of *S. rolfsii* except potato 1. This growth was found developed either in the centre or on the sides of the petri dish. This growth was given out from the creeping mycelial strands and not from the mycelium usually given out by sclerotia. On microscopic observations, this growth consisted of hymenia having typical fertile basidia bearing basidiospores. The basidia were club shaped, stout, erect and had 1—4 sterigmata. On each sterigma one truncate or pear shaped, hyaline and smooth walled basidiospore was found developed. No author has so far reported on the use of uric acid for getting the basidial stage. This medium, now used in the present investigations is new and tried for the first time for promoting the basidial stage of *S. rolfsii* from potato, *Colocasia* and groundnut successfully. Except Altstatt (1941), no worker obtained basidia of *S. rolfsii* from groundnut. Similarly Goto (1930) alone obtained basidia of *S. rolfsii* from *Colocasia*. These two authors obtained the basidia on altogether different media. In India also no report on this stage in *Colocasia* and groundnut isolates of *S. rolfsii* is forthcoming.

As Cooh's, Kirchoff's and uric acid media used in the present study had less percentage of sugar, it is felt that probably less carbon content in the medium might be conductive to the formation of the basidial stage. The observations of Misra et al. (1960) corroborated this view as they also did not get the basidial stage of *S. rolfsii* isolate from potato on media containing best concentration of carbon.

e) Effect of carbon compounds.

In order to note exactly whether carbon compounds had any effect on the basidial formation by *S. rolfsii*, the following experiment was undertaken. Modified Coon's medium without maltose was prepared and 1% of the following carbon compounds were added to it, 1) glucose, 2) sucrose, 3) dextrose, 4) galactose, 5) raffinose, 6) mannitol, 7) dextrin, 8) lactose, 9) arabinose, 10) dulcitol and 11) control without carbon source. Media thus prepared, were sterilised and poured in the plates. These plates were inoculated and incubated at 30° C for 7 days, after which they were stored at room temperature (25° C. to 26° C.) Observations were recorded periodically and continued for over 100 days. All the carbon compounds supported very good vegetative growth and also abundant sclerotial formation in all the isolates, but did not induce basidia. It may, therefore, be stated that carbon compounds had no

effect on promoting the basidial stage of *S. rolfsii*, thus confirming the observations made by Misra et al. (1960).

f) Use of calcium carbonate.
(A new medium for getting basidial stage.)

Gaining the experience obtained in the above experiments attempt was made to formulate a new medium incorporating 1% calicum carbonate and adding 1% dextrose as a carbon source. As usual, this medium was prepared, sterilised, poured in the plates, inoculated and incubated for 7 days at 30° C and thereafter at room temperature. On this medium surprisingly *Colocasia* isolate developed, white, aerial, velvety growth consisting of hymenia, basidia and basidiospores in 40 days. Basidia were stout, erect and short with 1 to 4 sterigmata. No report on the use of calcium carbonate in promoting the basidial stage is known so far. This medium, therefore, appears to be a new one for inducing basidial formation by *S. rolfii*. On isolation from basidiospore, typical culture of *S. rolfsii* with abundant sclerotia was obtained.

g) Effect of inorganic nitrogen.

Modified Coon's medium (without KNO 3) was prepared, distributed in 100 ml flasks and one percent of the following nitrogen compounds were added, 1) calcium nitrate, 2) magnesium nitrate, 3) barium nitrate, 4) sodium nitrate, 5) ammonium sulphate, 6) ammonium tartarate, 7) amonium nitrate, 8) ammonium phosphate and 9) control (without nitrogen source). The media were sterilised and poured in the plates. The plates were inoculated and incubated for 7 days at 30° C after which they were kept at room temperature (25° C to 26° C). Periodical observations were recorded and continued over 100 days. On ammonium nitrate, ammonium sulphate, ammonium phosphate, whitish powdery growth could be observed in the isolates of potato 2 and *Colocasia* after 20 to 40 days.

On actual microscopic observations this growth consisted of only hymenial structures without any basidia. These plates were further kept under observations but even after 100 days no basidial formation was observed.

h) Effect of organic nitrogen.

Organic nitrogen compounds when incorporated into the medium induced the basimial formation. Thus, Mundkur (1943) used aspergine proteose peptone (1941) and Venkatkrishnayaah (1946) incorporated proteose peptone, while Misra et al. (1960) tried aspargine in their media and obtained the basidial stage of *S. rolfsii*. In order to note exactly the ability of organic nitrogenous compounds in

inducing the basidial stage of *S. rolfsii* isolates on hand, the following experiment was undertaken.

A basal medium advocated by Lewis (1952—57) with reduction of 25% glucose contents and addition of greater quantities of trace elements in micro-element solution was prepared as under:

KH_2PO_4	2.0 gm.
$MgSO_4$	0.5 gm.
NaCl	0.1 gm.
$CaCl_2$	0.1 gm.
Micro-element solution	1.0 cc.
Glucose	15.0 gm.
Water	1000 cc.

The micro-element solution was prepared as follows with increased quantities of trace elements over those proposed by Lewis.

Micro-elements Adcovated by Lewis.	Used now in the experiment.	
1) Bo as Boric acid	0.01 mg./L	10 mg./L
2) Cu as Copper sulphate	0.10 mg./L	10 mg./L
3) Fe as Ferric chloride	0.20 mg./L	10 mg./L
4) Mn as Manganese sulphate	0.02 mg./L	2 mg./L
5) Mo as Molybdic acid	0.02 mg./L	2 mg./L
6) Zn as Zink sulphate	2.00 mg./L	2 mg./L

The stock solution was prepared and distributed in 100 cc lots in 250 ml flasks. To each flasks, 0.75 gm./100 ml. of each of the organic nitrogen compounds *viz.* aspargine, creatine, D L tryptophane (a new medium for getting basidial stage) glycocyamine, DL serine, DL cystine, taurine, DL aminobutyric acid, leucine, arbutine, DL methionine and DL valine was added. The media were then sterisised as usual, poured in the plates, inoculated and incubated at 30° C for 7 days after which the plates were kept at room temperature (25° C to 26° C). Periodical observations were recorded after 10 days. On 12th day plates poured with tryptophane and inoculated with potato isolate 2 developed mycelium which grew out in the sub-centre of the plate, from the creeping mycelial strands and not from the mycelium usually given out by sclerotia. These mycelial strands, two days later assumed white, velvety apperance, which on microscopic examinations revealed hymenium having typical basidia with 1 to 4 sterigmata. Numerous spear shaped, truncate smooth walled and hyaline basidiospores were also found detached from the basidia. Use of the tryptophane in the medium on which the basidial formation was obtained, has been done for the first time. On aspargine and leucine, inoculated with the potato isolate 2, aerial whitish growth

was noticed. On microscopic examinations this growth consisted of only hymenial structures without any basidia. It will thus, appear that some of the organic nitrogenous compounds, preferably amino acids are necessary for inducing the basidial stage of the isolates of *S. rolfsii*, thus, confirming the observations made by Mundkur (1934) Milthorpe (1941), Venkatkrishnayaah (1946) and Misra et al. (1960).

i) Use of vitamins.

This experiment was undertaken with the object of finding out the role of vitamins in inducing the basidial formation. Coon's agar was selected as a basal medium (with reduction of 25% sugar), to which vitamins as shown below were added, Mathew (1954).

1)	Pyridoxine	250 mgs./L
2)	Nicotinic acid	250 mgs./L
3)	Pantothenic acid	250 mgs./L
4)	Ascorbic acid	250 mgs./L
5)	Vidahlyn (Vit. ABCD)	100 mgs./L
6)	Trigol (Wheat germ oil Vit. E) (a new medium for getting basidial stage)	250 mgs./L

The stock solution was distributed in 100 cc quantities in 250 cc Erlenmeyer flasks to which the required quantity of vitamins was added. The media, thus prepared, were sterilised, inoculated and incubated at 30° C for 7 days and thereafter at room temperature (25° C to 26° C) for 100 days. Periodical observations were recorded after 10 days. It is interesting to note that *S. rolfsii* isolates from groundnut and *Colocasia* developed the basidial stage in 50 to 60 days in the plates poured with trigol (Wheat germ oil containing vitamine E). Outwardly, by microscopic observations the basidial formation in the groundnut isolate was found developed on one side of the petri dish, while in *Colocalia* isolate it was at the sub-centre, This growth appeared as white, aerial, velvety, loosely formed and roundish, attaching itself to the growing mecelial strand and measuring 0.5 mm. to 1 mm. in diameter. Potato isolates 1 and 2 did not form basidia on this medium. Use of vitamins in a medium for inducing the basidial stage of *S. rolfsii* has not been reported so far. This also therefore, appears to be the first report on this. Moreover, as stated earlier, except Altstatt (1941) in U.S.A. no worker obtained basidia of *S. rolfsii* from groundnut. Similarly, Goto (1930) alone could get basidia of *S. rolfsii* from *Colocasia*. Both these workers had used different media for getting this stage. No other workers from India had reported the basidial

formation by *S. rolfsii* isolates from groundnut and *Colocasis*. The present investigations yielded basidia of *S. rolfsii* from groundnut and *Colocasia* for the first time in India and that too on media hitherto unreported by any worker.

j) Use of hormones.

In order to note the effect of hormones for inducing the basidial stage of *S. rolfsii* the basal medium advocated by Lewis (1952) and reported earlier was prepared and the following hormones were added to it.

1) Cholchicin	10 mgs./1000 c.c.
2) N-Acetic acid Alpha	10 mgs./1000 c.c.
3) Gibberallin	10 mgs./1000 c..c
5) Trichlorophenoxy 2-4-5 Acetic acid	10 mgs./1000 c.c.
6) Maleic hydrazide 40	10 mgs./1000 c.c.

The media were sterilised, poured inoculated and incubated at 30° C for the first 7 days and then at room temperature (25° C to 26° C). Periodical observations were recorded and continued upto 100 days. No basidial formation was observed in any of the hormones tried. However, there were indications of hymenial development in 40 days on Alpha-N-acetic acid and trichlorophenoxy-2-4-5 acetic acid. These hymenia were subsided without any development subsequently. In the other experiment, these harmones were added after the sterilization of the medium. The media with the hormones were then poured, inoculated and incubated, as above. In this experiment also no basidial formation was observed even after keeping the plates for 100 days.

k) Use of sex stimulants.

In the Ayurvedic, Unani and Allopathic systems, certain medicines are specifically prescribed as sex stimulants. Since the basidial formation is considered as the perfect stage of *S. rolfsii*, it was thought worthwhile to study whether addition of sex stimulants prescribed in the Ayurvedic. Unani and Allopathic systems of medicines could induce the basidial formation by *S. rolfsii* isolates, on hand. The following sex stimulants were used.

i) Ayurvedic and Unani sex stimulants.

(1) Chinese root extract	6 gms/100 cc.
(2) Beegband	6 gms/100 cc.
(3) White Gunj	6 gms/100 cc.
(4) Nutmeg	6 gms/100 cc.
(5) Raddish seed extract	6 gms/100 cc.

These ingredients were boiled in 100 cc. water for one hour, filtered and to this extract 2 grams of agar was added. The media thus prepared, were sterilised, poured, inoculated and incubated at 30° C for 7 days and then kept at room temperature (25° C to 26° C). Periodical observations were recorded and continued for 100 days. No basidial formation was noticed on any of the stimulants used.

(ii) Allopathic sex stimulants.

(1) *Nux vomica*	1 minim/100 cc.
(2) Na. phosphate	1 gm /100 cc.
(3) Ca. phosphate	1 gm /100 cc.
(4) Liquid arsenicalus	1 minim/100 cc.
(5) Gold chloride	1 gm /100 cc.

These ingredients were added to the Coon's medium which was sterilised, poured inoculated and incubated at 30° C for 7 days. The plates were then removed to room temperature and kept in a closed cabinate. Periodical observations were recorded upto 100 days but no basidial formation was obtained.

Summary:

An account of several attemps made by the authors to induce basidial stage by *S. rolfsii* has been given. Basidial stage could be obtained on a variety of media incorporating organic nitrogen, vitamin E and calcium carbonate.

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