Studies on Choanephoraceae III. Effect of sulphur compounds on the growth and sporulation

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Sulphur is also one of the important constituents of a suitable nutrient medium for fungi, but it is required in much smaller quantity in comparison to other essential elements. It plays a vital role in their metabolism; it enters into the composition of the mycelium and the spores of many fungi. It also takes part in protein synthesis, respiration and other biochemical processes. The importance of sulphur compounds with special reference to their role in biological methylation by fungi, has been reviewed by Challenger (1953). Inspite of its importance there are certain fungi that grow without sulphur (Steinberg, 1941; Srivastava, 1951; Agarwal, 1955). There is considerable variation as regards the response of various fungi to sulphur compounds (Armstrong, 1921; Saksena et al. 1952; Agarwal, 1955; Agnihotri, 1962; Kumar, 1962). Raistrick and Vincent (1948), have made an extensive study on the utilization of sulphur compounds and they reported, that their fungi converted essentially all the sulphate sulphur into organic compounds. Amoung sulphur compounds sulphates have mostly been reported to be good sources for the growth of various fungi by several workers (Mosher et al, 1936; Armstrong, 1921; Tandon, 1950). On the other hand, Volkonsky (1933, 34) reported, that members of Saprolegniaceae were unable to utilize sulphur as sulphate.

Volkonsky (1933) classified fungi into two categories on the basis of their sulphur requirements (I) Parathiotrophs-organisms utilizing only reduced form of sulphur (II) Euthiotrophs- organisms utilizing sulphate and other oxidised sulphur.

Since practically no work has been done on the sulphur requirements of the genera *Blakeslea* and *Choanephora*, an attempt was made to investigate the sulphur need of all the recognized species.

Materials and Methods

The cultures used throughout this investigation were: *Blakeslea trispora* Thaxter, *Choanephora cucurbitarum* (Berk. & Rav.) Thaxter, *C. infundibulifera* (Currey) Saccardo, *C. conjuncta* Couch, *C. hetero-*

Effect of variaus sulphur sources on growth and sporulation of Choanephoraceae*.

Sulphur sources	*B. trispora*			*C. cucurbitarum*			*C. infundibulifera*			*C. conjuncta*			*C. heterospora*			*C. circinans*		
	G	S	Final pH	G	S	Final pH	G	S	Final pH	G	S	Final pH	G	S	Final pH	G	S	Final pH
1. Sodium sulphide	16.9	+++	7.0	13.6	+	7.3	20.3	+++	7.2	17.4	—	7.2	15.2	—	7.0	12.8	+	7.2
2. Potassium sulphite	5.2	—	5.5	15.5	—	7.3	13.2	—	5.8	7.2	—	6.0	2.7	—	5.5	15.3	—	7.5
3. Magnesium sulphate	22.8	+	7.6	23.6	++	7.4	28.0	++	7.6	25.2	—	7.8	26.1	+++	7.6	24.3	—	7.0
4. Potassium sulphate	22.5	++++	7.4	21.1	+++	7.8	18.5	++++	7.8	16.3	—	7.4	9.2	—	5.8	14.4	—	7.9
5. Calcium sulphate	25.2	++++	7.4	22.2	—	7.4	24.9	++++	7.3	21.3	+	7.3	16.0	—	7.3	20.5	+	7.3
6. Ammonium sulphate	17.4	++++	7.3	18.4	+	7.6	12.5	++	7.2	14.6	—	7.2	15.2	—	7.2	18.5	—	7.3
7. Sodium thiosulphate	15.3	++	7.8	9.3	—	7.4	14.2	+++	7.8	14.5	—	7.5	14.2	+	7.5	14.2	—	7.5
8. Pot, pyrosulphite	3.2	—	6.0	3.5	—	5.6	3.3	—	5.7	9.6	—	7.2	3.1	—	5.6	8.4	—	5.5
9. Cystine	8.3	—	7.7	12.2	—	7.4	20.2	+	7.1	5.4	—	7.0	8.1	—	7.4	10.1	—	7.4
10. Methionine	17.4	++	7.4	14.5	+	7.4	14.3	+	7.0	13.6	—	6.8	15.1	—	7.4	10.6	—	7.4
11. Control	4.0	—	5.5	2.8	—	5.5	2.5	—	5.5	3.4	—	5.5	3.3	—	5.5	3.3	—	5.5

Key*: G = Growth in milligrams; S = Sporulation; — = No sporulation;
+ = Poor sporulation; ++ = Fair sporulation; +++ = Good sporulation;
++++ = Excellent sporulation.

Summary of dry weight results and conclusions at 5% level.

1. *Blakeslea trispora*
   S. E. = 1.35; C. D. = ± 2.8
   5 3 4 〉6 10 1 7〉 9 〉 2 11 8

2. *Choanephora cucurbitarum*
   S. E. = 1.46; C. D. = ± 3.0
   3 5 4 6 2 10 1 9 7〉 8 11

3. *Choanephora infundibulifera*
   S. E. = 1.33; C. D. = ± 2.7
   3 〉 5 〉 1 9 4 〉 10 7 2 6 〉 8 11

4. *Choanephora conjuncta*
   S. E. = 1.32; C. D. = ± 2.7
   3 〉 5 〉 1 4 6 7 10 〉 8 2 9 11

5. *Choanephora heterospora*
   S. E. = 1.3; C. D. = ± 2.7
   3 〉 5 1 6 10 7 〉 4 9 〉 11 8 2

6. *Choanephora circinans*
   S. E. = 1.22; C. D. = ± 2.5
   3 〉 5 6 〉 2 4 7 1 10 9 8 〉 11

spora Mehrotra and Mehrotra and *C. circinans* (Naganishi & Kawakami) Hesseltine and Benjamin.

The basal medium consisted of glucose, 3gm; KH_2PO_4, 1gm, asparagine, 1gm; thiamine hydrochloride, 100µg; distilled water, 1 liter. The following sulphur compounds were used for this experiment.

1. *Inorganic compounds;*

Magnesium sulphate, ammonium sulphate, calcium sulphate, sodium thiosulphate, potassium sulphate, potassium sulphite, potassium pyrosulphite and sodium sulphide.

2. Organic compounds:

Cystine and methionine.

The various sulphur compounds were added singly in quantities so as to supply the amount of sulphur present in 0.5 gm of magnesium sulphate. The pH of the medium in each case was adjusted to 5.4 before autoclaving. All the experiments were run in triplicate. The different media thus prepared were poured in 150 ml Erlenmeyer Pyrex flasks, and each flask received 25 ml of the medium. The flasks were sterilized for 15 minutes at 15 lb pressure. The flasks after inoculation were incubated for 15 days at 25° C ± 1.

At the end of the incubation period the contents of the flasks were filtered, washed, dried and weighed for the determination of the dry weigth. Dry weight was used as the quantitative measure of growth. Records of sporulation and changes in pH were also maintained in each case.

Results

The records of the results given in Tables 1 and 2 show that all the species provided negligible growth in medium lacking sulphur. Magnesium sulphate and calcium sulphate were found to support good growth of all the species. However, ammonium sulphate and potassium sulphate behaved differently with the present fungi. Ammonium sulphate proved to be a good source for *Choanephora cucurbitarum, C. heterospora, C. circinans* and *Blakeslea trispora,* moderate and poor for *C. conjuncta* and *C. infundibulifera* respectively. Potassium sulphate supported good growth of *C. infundibulifera, C. cucurbitarum, C. conjuncta* and *B. trispora,* and proved to be a moderate source for the remaining species.

Potassium sulphite was a moderate source for *C. infundibulifera, C. cucurbitarum* and *C. circinans,* poor for *C. conjuncta* and *B. trispora,* and useless source for *C. heterospora.* Cystine, an organic sulphur compound supported good growth of *C. infundibulifera,* moderate of *C. cucurbitarum* and poor of the rest of the species. Similarly methionine, another organic sulphur compound, behaved differently. It was found to be a good source for *C. heterospora* and *B. trispora,* moderate for *C. cucurbitarum, C. infundibulifera* and *C. conjuncta* and poor for *C. circinans.*

From the present results it is clear, that the different species differed in the degree of sporulation produced on different sulphur compounds. Amongst the inorganic sulphur sources, potassium pyrosulphite and potassium sulphite failed to induce sporulation in all the species. Sodium sulphite supported good sporulation of *B. trispora* and *C. infundibulifera* and poor of *C. circinans* and *C. cucurbitarum.* The other two species failed to sporulate on this compound. Magnesium sulphate was found to induce good sporulation in *C. heterospora,* fair in *C. infundibulifera* and *C. cucurbitarum* and poor in *B. trispora.* The remaining two species could not sporulate on this compound. Potassium sulphate induced excellent sporulation in *B. trispora* and *C. infundibulifera* and good in *C. cucurbitarum.* The other three species failed to sporulate on this compound. Calcium sulphate supported excellent sporulation of *B. trispora* and *C. infundibulifera* and poor of *C. conjuncta.* It failed to induce spore production in the rest of the species. Ammonium sulphate induced excellent sporulation in *B. trispora,* fair in *C. infundibulifera* and poor in *C. circinans* and *C. cucurbitarum.* The remaining two species failed to sporulate on this compound. Sodium thiosulphate proved to be a good source for the sporulation of *C. infundibulifera,* fair for

B. trispora and poor for *C. heterospora,* while the remaining three species failed to sporulate.

Of the organic sulphur compounds tested, cystine induced sporulation only in *C. infundibulifera.* Methionine, another organic sulphur compound, supported fair sporulation of *B. trispora* and poor of *C. infundibulifera* and *C. cucurbitarum.* The other three species failed to sporulate on this sulphur source.

Discussion

The negligible growth of the present species in the control medium shows the indispensibility of sulphur for the growth of the present fungi. No growth in media lacking sulphur has been reported by several workers for their fungi (Rabinovitz, 1933; Mehrotra, 1949; Grewal, 1954; Kumar, 1962; Agnihotri, 1962). On the other hand, growth of some fungi has been reported by some workers (Steinberg, 1941; Srivastava, 1955; Grewal, 1954), in the medium in which sulphur was totally absent. It has been shown by Steinberg (1936), Barner and Cantino (1952), and Hungate and Mannell (1952). (Cited from Fruton and Simmonds, 1958), that at a moderate carbohydrate concentration, the sulphur requirement of most of the fungi is about 0.001—0.006 M. As a large part of this amount of sulphur is generally present as impurities even in pure chemicals, small amount of growth of the fungi is generally observed in sulphur free media. The organisms under investigation are able to utilize sulphur from sulphates, therefore, they fall under the category of euthiotrophs of Volkonsky (1933).

Details of sulphate reduction in fungi are not fully known. According to Davis (1955), the available data point to the following pathway: sulphate → sulphite → sulphide or thiosulphate → cystine → methionine. The obviously necessary intermediates are not fully known, nor is it known at what level of reduction inorganic sulphur is converted into organic form. Sulphates have generally been reported to be good sources for fungi (Armstrong, 1921; Mosher et al: 1936; Steinberg, 1941; Hockenhull, 1948; Raistrick and Vincent, 1948).

Sodium thiosulphate supported moderate growth of all the species except *C. cucurbitarum,* for which it proved to be a poor source. Satisfactory utilization of this compound has been reported by Armstrong (1921), Steinberg (1941), Bhargava (1945), Agarwal (1955) for their fungi. Agnihotri (1962), reported it to support good growth of his fungi.

In general, potassium sulphite was not found to be a favorable source for the present fungi. However, satisfactory growth on sulphite has been reported by several workers (Hockenhull, 1948;

Armstrong, 1921; Agnihotri, 1962) for their fungi. Stephered (1958) (Cited by Fruton and Simmonds, 1958) has reported the pathway of utilization of sulphite sulphur in case of *Aspergillus nidulans.* According to him sulphite sulphur is used for the synthesis of sulfinyl-pyruvic acid and from this latter compound, cystine is synthesized.

Lilly and Barnett (1951, p. 92) stated that amongst the organic compounds structure is enormously important and the specific structure of organic sulphur compounds affects their utilization. Both cystine and methionine behaved differently and were good, moderate or poor sources for the present fungi. The former supported good growth of *C. infundiblifera,* moderate of *C. cucurbitarum* and proved to be a poor source for the remaining fungi. Cystine has also been reported to be a good source by Steinberg (1941), for *Aspergillus niger,* Hockenhull (1948), for *Penicillium notatum* and Pontecarvo et al. (1953) (Cited by Cochrane, 1958), for a mutant of *Aspergillus nidulans.* All the species of *Choanephora* and *B. trispora* under study could make use of sulphur from methionine. It supported good growth of *B. trispora* and *C. heterospora,* moderate of *C. infundibulifera, C. cucurbitarum* and *C. conjuncta,* but proved to be a poor source for *C. circinans.* According to Fruton and Simmonds (1958), methionine is an indispensable amino acid, while cystine and cysteine are dispensable and the sulphur of methionine can be used in the biosynthesis of cysteine.

Summary

Amongst the sulphate sulphur sources, magnesium sulphate and calcium sulphate supported good growth of all the species, while others behaved differently. Potassium sulphite was found to be a moderate source for *C. infundibulifera, C. cucurbitarum* and *C. circinans* and poor for the rest of the species. Similarly methionine and cystine, the two organic sulphur compounds tried, behaved differently with the present species. No definite correlation could be established between growth and sporulation of the fungi tested. The different species also differed in the degree of sporulation produced on different sulphur compounds.

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