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EFFECT OF CARBON AND NITROGEN SOURCES ON YEILD OF OXALIC ACID BY *SCLEROTIUM ROLFSII*

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The effect of different sources of carbon and nitrogen and the different ratios of optimum carbon and nitrogen sources on yeild of oxalic acid by the natural and two X-ray mutated strains (S. r—X_1 and S. r—X_2) of *Sclerotium rolfsii* were evaluated. Sucrose and cystein combination was found to be best for natural and S. r—X_1 straln whereas it was inulin and asparagine for S. r—X_2 strain. The optimum ratios of carbon and nitrogen sources for the yeild of oxalic acid were 25 : 1.5, 15 : 2.5 and 25 : 1 5 for natural, S. r—X_1 and S. r—X_2 strains respectively.

INTRODUCTION

A wide range of organic compounds are being utilized as carbon and nitrogen sources by fungi for the synthesis of structural and functional compounds and as sources of energy. But fungi are rather selective in their requirements.

Similar to individual ones, carbon and nitrogen in combination play an important role on growth and on the metabolic activities of a fungus. Furthermore, the best combination shows a more better result in a definite ratio. Hence C/N ratio of a best-combination (C—N) is taken as another important factor in these regards.

Kodanda Pany and Apparao (1963) have showed that *Sclerotium rolfsii* grow over a wide carbon/nitrogen (C/N) ratio and they have further shown that the absolute quantities of the nutrients being important. Arimura and Gondo (1966) have observed that carbon/nitrogen ratio do not significantly affect the growth rate of *Sclerotium rolfsii* but added nitrogen produced a thicker mycelium in culture. Previous reports reveal no record on the effect of best combination of C/N on yeild ef oxalic acid by *Scleroiium rolfssi.*

In the present investigation, the selectlon of best combination of carbon and nitrogen sources and the effect of their different ratios on the yield of oxalic acid and simultaneously on growth of natural and two mutated strains of *Sclerotium rolfsii* have been recorded.

Test fungus

For the present investigation, *Sclerotium rolfssi* was collected from the agricultural farm of Calcutta University at Baruipur, 24-Parganas. Mutated strains were prepared by Chakranarti and Samajpati (1971). All the strains were subcultured at regular intervals on *potato dextrase agar* medium slants.

Selection of best carbon and nitrogen sources and their ratios

From the experiments on different carbon and nitrogen sources (Chakrabarti and Samajpati, 1978, 1980) the following sources were selected. For natural strain, best carbon and nitrogen sources are sucrose and cysteine respectively. In S. r—X_1 strain, sucrose is the best carbon source whereas cysteine, glycine, phenylalanine and aspartic acid are the better sources of nitrogen. Similarly in strain S. r—X_2, best carbon source is inulin and selected good nitrogen sources are cysteine, aspartic acid and asparagine. To obtain best result in respect of oxalic acid synthesis, different combination of best carbon and nitrogen sources were taken in a definite ratio (carbon—2%, nitrogen—0.02%). For natural strain, modified *potato broth basal medium* was prepared taking sucrose as carbon source and cysteine as a nitrogen source. For strain —X_1, four different types of media were prepared. In all cases, sucrose was used as carbon source instead of dextrose in original medium but only one of the following nitrogen sources, viz., cysteine, glycine, phenylalanine and aspartic acid were added to each set of the medium as 2 g per litre. For S. r—X_2 strain, three types of modified potato broth basal media were prepared taking inulin as carbon source in each set and cysteine or aspartlc acid or asparagine as nitrogen source added separately for each set of combination. After preparation, all the media were taken in Erlenmeyer conical flask (30 ml in each) properly plugged and sterilized in the usual way and inoculated seperately by sclerotia of respective strains. All flasks were then incubated at 35°C for 12 days. The dried weight of mycelia were recorded by the same procedure as stated in previous paper, (Chakrabarti and Samajpati 1971). From the culture filtrate, oxalic acid estimation was done following the method of Maxwell and Bateman (1968).

From the foregoing results, the selected best combination of different strains were taken in differet doses, e. g., carbon source was taken as 15 g (C_1) and 20 g (C_2) and 25 g (C_3) per litre respectively and nitrogen source was taken as 1.5 g (N_1), 2 g (N_2) and 2.5 g (N_3) per litre of media respectively. According to all possible permutation and combination, 9 types (C_1N_1, C_1N_2, C_1N_3, C_2N_1, C_2N_2, C_2N_3, C_3N_1, C_3N_2, C_3N_3) of media were prepared for each strain. After preparation, distribution, sterilization, inoculation incubation, harvesting and estimation of oxalic acid were done in the same way as stated above.

RESULTS AND DISCUSSION

The results obtained during the experimental period are givan in Tables 1 and 2. From Table 1, experimental data reveal that sucrose-cysteine combination exert a better result regarding oxalic acid synthesis by natural and S r-X_1 strain whereas it is inulin-asparagine combination for the strain of S.r-X_2.

The data from the Table 2 reveal the optimum ratio of different strains, i. e., C_3N_1 ratio is optimum combination for production of oxalic acid by natural and S.r-X_2 strain whereas in case of S.r-X_1 strain, it is C_1N_3.

From the tables it is evident that best carbon source together with a best nitrogen source in combination exert a better result increasing a greater yield of oxalic acid regulating the metabolic activities of different strains of *Sclerotium rolfsii*. But the type of combination differ in regards of growth with the yield of oxalic acid, i.e., sucrose-glycine combination is optimum for growth of S.r-X_1 strains whereas sucrose-cysteine combination produces a maximum amount of oxalic acid by the same strains, Similarly inulin-aspartic acid is best combination of carbon and nitrogen sources of S.r-X_2 strain whereas in presence of inulin-asparagine combination, S.r-X_2 strain is able to produce more oxalic acid than other combinations. Cysteins shows a far better yield of oxalic acid when supplied as a nitrogen source than the others and similarly sucrose is also a remarkably good carbon source of natural strain in regards of oxalic acid production. Therefore only sucrose-cysteine combination was taken for natural strain and good result is obtained.

TABLE 1. *Effect of different combination of carbon and nitrogen sources on the growth (mg) and yield of oxalic acid (mg) by parent and two mutated strains of* S. rolfsii *at 12 days of growth*

Combination of carbon and nitrogen	Strains	Growth (mg/30 ml)*	Oxalic acid (mg/30)*
Sucrose-cysteine	Natural	284	282
Sucrose-cysteine	S r-X_1	304	268
Sucrose-glycine	S.r-X_1	604	233
Sucrose-aspartic acid	S.r-X_1	596	192
Sucrose-phenylalanine	S.r-X_1	569	200
Inulin-aspartic acid	S.r-X_2	567	181
Inulin-asparagine	S.r-X_2	554	263
Inulin-cysteine	S.r-X_2	213	227

*Each flasks contains 30 ml of media

Each value represents an average of five separate determinations.

TABLE 2. *Data (mean)* showing the effect of different carbon/nitrogen ratio on the growth (mg) and on yield of oxalic acid (mg) of natural and mutated strains of* Sclerotium rolfsii *at 12 days of growth*

Carbon nitrogen ratio	Growth (Dry weight of mycelium in mg/flask*)			Oxalic acid (mg/flask*)		
	Natural	S.r-X_1	S.r-X_2	Natural	S r-X_1	S.r-X_2
C_1N_1	288	262	553	231	226	239
C_1N_2	344	260	488	284	263	250
C_1N_3	378	309	455	301	274	261
C_2N_1	258	266	591	297	258	253
C_2N_2	284	304	554	282	271	260
C_2N_3	319	252	582	280	238	302
C_3N_1	321	287	672	318	210	319
C_3N_2	342	292	691	279	259	303
C_3N_3	378	353	634	298	272	310

*Each flasks Contains 30 ml of media

Eash value represents an average of five separate deteminations.

C_1 = 15 g of Carbon/litre ; N_1 = 1.5 g of nitrogen/litre

C_2 = 20 g of Carbon/litre N_2 = 2.5 g of nitrogen/litre

C_3 = 25 g of Carbon/litre N_3 = 2.5 g of nitrogen/litre

The data on the C/N ratio play an important role on controlling the rate of synthesis of oxalic acid by the natural and two mutated strains of *Sclerotium rolfsii*. Sucrose-cysteine (2.5% : 0.15%) was the best for yield of oxalic acid by the natural strain. Sucrose-cysteins (1.5% : 0.25%) and inulin-asparagine (2.5% : 0.15%) were the best for the yield of oxalic acid by S.r-X_1 and S.r-X_2 strains respectively. But for the growth, optimum ratios are sucrose-cysteine (1.5% : 0.25%), for natural strain, (2.5% : 25%) for S.r-X_1 strain, and inulin-asparagine (2.5% : 0.20%) for S.r-X_2 strain respectively.

The data of both Table 1 and Table 2 further reveal that there is no relation between growth and yield of oxalic acid by respective strains, which supports the views of Kodanda Pany and Apparao (1963) and Maxwell and Bateman (1968).

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