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Effect of carbon and nitrogen sources on the mycelial growth and yield of protein by the dikaryon and the protoplast fusant mycelia of *Pleurotus sajor-caju*

RATULA MUKHERJEE
Shree Ramkrishna BT College, Darjeeling 734011, West Bengal

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In order to find out the effect of different carbon and nitrogen sources on the mycelial growth and yield of protein by the dikaryon and the protoplast-fusant mycelia of *Pleurotus sajor-caju.* The respective mycelia were grown separately in basal liquid medium supplemented with different carbon and nitrogen sources separately at 25^{0}C for 15 days. The data on the carbon sources revealed that the glucose was the best carbon sources for the mycelial growth and yield of protein by the dikaryon and the protoplast-fusant mycelia of *Pleurotus sajor-caju* respectively. This was followed by fructose, sucrose, lactose,starch, arabinose,mannitol and sodium acetate. The data on the inorganic, organic and complex nitrogen sources revealed that potassium nitrate was the best source for the mycelial growth and yield of protein by the dikaryon and the protoplast-fusant mycelia of *Pleurotus sajor-caju* respectively. This was followed by ammonium sulphate, asparagine, ammonium nitrate, L-lysine, urea and others.

Key words: *Pleurotus sajor-caju*, dikaryon and protoplast-fusant mycelia, growth, yield of protein

INTRODUCTION

Carbon compounds are important nutritional source of any organism and there are reports of differential utilization of different carbon compounds by mushrooms. Mycelial yields by submerged culture methods was more when xylose, glucose and wheat bran are used in the medium (Styer, 1928). The growth of *Agaricus campestris* in submerged culture with various carbon sources was studied by Humfeld and Sugihara (1952). The evaluation of rate of growth in various media containing the extracts such as citrus press water, orange juice, malt extract, nutrient broth or corn steep liquor was made by Block *et al.* (1953) and they reported that the most efficient results are obtained from the medium containing corn steep liquor. Jennison *et al.* (1955) used malt, glucose, malt extract, malt syrup and molasses as substrates for mushroom culture. Rangaswamy (1956) showed that *Volvariella diplesia* utilizes starch but not cellulose, while according to Atacador- Ramos *et al.* (1967), *Volvariella volvacea* prefers a carbon source at 4% level. They also observed that xylose, fructose and glucose are good sources for growth. Voltz *et al.* (1969) could not identify one single carbon

source to support the growth of the mycelia of Agaricales. Faruta and Yoichiro (1970) described the carbon nutrition of several mushrooms. Guha and Banerjee (1971) reported that the effect of different carbon sources on the growth and yield of protein by *Agaricus campestris* 12 under submerged conditions. Bukhalo *et al.* (1972) observed that few basidiomycetes grew better in a starch medium and others in a maltose medium than in a glucose medium. Safrai and Garibova (1975) found that sugar mixtures (mannitol + glucose, starch + glucose, xylose + glucose) are the optimal carbon sources for the 11 species of the genus *Agaricus* investigated. Ghosh and Sengupta (1977) demonstrated that soluble starch is the best carbon source for the vegetative growth of *Volvariella volvacea*. Sakamoto *et al.* (1978) reported that when glucose is used as the carbon source the mycelia dry yield is 10 g/ l and 15 g/ l for *Lentinus edodes* and *Pleurotus ostreatus* respectively. Brodziak (1980) observed that glucose, mannose, maltose and starch are optimal carbon sources for the vegetative growth of some organisms. Hong *et al.* (1981) reported that glucose and starch increases the mycelial growth of *Agaricus bitorquis* and glucose, fructose and starch increase the mycelial growth of *Pleurotus ostreatus*. Rao (1983) reported that starch, glucose and maltose are the optimal carbon sources for vegetative growth of *Agaricus trisulphuratus*, *Rhodocybe subgliva* and *Agrocybe praecox* respectively. Singh and Verma (1996) studied the effect of monosaccharides, disaccharides, polysaccharides, sugar alcohols and organic acids in the nutrition of *Lentinula lateritia*.

Different nitrogen sources have a pronounced effect on the growth and metabolism of fungi. Styer (1928) reported that different carbon sources gave dense, mycelial yield when ammonium tartarate was used as sole nitrogen source. Also by using different nitrogen sources with glucose and maltose mixture as a carbon source, he concluded that arginine and glycine promote growth more than others. Reports on the utilization of nitrogen sources such as urea (Humfeld and Sugihara, 1948; Humfeld and Sugihara, 1952), nitrates (Block *et al.,* 1953) and ammonium salts (Reusser *et al.*, 1958) by different higher fungi were also been reported. Fries (1955) observed good growth of *Coprinus* sp. on ammonium tartarate to its chelating capacity or dicarboxylic acid qualities. According to Rangaswamy (1956) *Volvariella diplesia* was able to utilize peptone as the best nitrogen source. Nitrates are considered as excellent sources of nitrogen for many fungi, though inability to utilize it also reported in higher basidiomycetes (Cochrane, 1958). Atacador-Ramos *et al.* (1967) found that 0.4% urea as nitrogen source gives best mycelial yield of *Volvariella volvacea.* Voltz and Beneke (1969) used ammonium salts and L-asparagine as nitrogen source for the mycelial growth of Agaricales and concluded that no nitrogen compound is singly useful for mycelial growth. Faruta and Yoichiro (1970) reported the nitrogen requirements for several edible mushrooms. Eger (1970) reported that urea proves to be the best. Michel *et al.* (1971) studied in detail about the utilization of glutamic acid by the mycelium of *Agaricus bisporus.* Bukhalo *et al.* (1972) concluded that among the basidiomycetes studied, all species except *Agaricus bisporus* assimilate nitrate and ammonium nitrate and that some fungi grew better in a medium with ammonium nitrate. It was reported that proline and asparagine are the best nitrogen sources for all the species of Agaricus tested (Safrai and Garibova, 1975). Sattler (1975) observed the correlation between the nitrogen sources with the different temperatures in the growth of the mycelium. Ghosh and Sengupta (1977) reported that *Volvariella volvacea* grows best in a 3 as sole source of nitrogen. Chandra and Purkayastha (1977) compared the nitrogen utilization of various nitrogen compounds in five different species of mushrooms. Nag Choudhury (1977) observed that *Tricholoma gigantium* prefer ammonium tartarate for growth and protein production followed by other ammonium salts. Glutamic acid and peptone were found to be the best organic and complex nitrogen sources respectively. Goto and Noriko (1978) reported the utilization of glutamate as a sole source of nitrogen by the isolates of *Lentinus edodes*. Kaul (1977) studied the biomass production of eight species of *Morchella* with thirty different nitrogen sources, while Duvnjak *et al.* (1980) reported that sodium nitrate, ammonium chloride and ammonium sulphate added to undiluted whey in the growth medium of *Morchella hortensis* stimulate the growth of the fungus. Hong *et al.* (1981) found that among the different nitrogen sources, peptone increased the mycelial growth of both *Agaricus bitorquis* and *Pleurotus ostreatus*. Fermor (1982) found that *Agaricus macrosporus* grew well on complex nitrogen sources and amino acids but ammonium nitrate is the only nitrate source to support more

than minimal growth. Rao (1983) studied the nitrogen sources for best mycelial growth of *Agaricus trisulphuratus* , *Rhodocybe subgliva* and *Agrocybe praecox* and concluded that L-asparagine is the optimal for *Agaricus trisulphuratus* and yeast extract is optimal for both *Rhodocybe subgliva* and *Agrocybe praecox*. Singh and Verma (1996) reported the role of amino acids, amide, ammonium salts, nitrate salts and nitrite salts on the nutrition of *Lentinula lateritia*.

Due to increasing population pressure, high rate of industrialisation and urbanisation, the crisis of food is increasing world over, specially in the developing and under developing countries.
Moreover, undernourishment or malnutrition is a serious problem among the population of developing and under-developing countries. Among the malnutrition the low protein content of the food is one of the most important factors. The animal protein is beyond the reach of majority of Indian population due to low economic condition.

In order to provide adequate amount of protein in the diet, new sources of protein rich foods have to be developed which can be produced economically on a large scale under such socio-economic condition. Cultivation of edible mushroom will be an ideal proposal to counteract the acute protein malnutrition. India is fast gaining its position as a major mushroom producing country in the world. The cultivation of *Pleurotus* has increased significantly in the last few years. Out of the 15 species cultivated in India, *Pleurotus sajor-caju* (Fr.) Singer is cultivated extensively and commercially.

In this study the effect of carbon and nitrogen sources on the growth and yield of protein by the dikaryon and fusant mycelia of *P. sajor-caju* is studied.

MATERIALS AND METHODS

Fresh and healthy basidiocarps of *P. sajor-caju* were collected from different localities of West Bengal during rainy season. The surface of the basidiocarps were cleaned with sterilized and distilled water and with 0.1% mercuric chloride solution and rinsed with sterile distilled water. From the pileus tissue of the freshly collected basidiocarps, tissue cultures were prepared on PDA medium. These PDA cultures were made pure by repeated subculturing and maintained in the laboratory under aseptic condition.

The pH was adjusted to 6.5. The medium thus prepared was transferred to culture tube, properly plugged and sterilized in autoclave at 121°C for 20 minutes.

In order to study the physiological and biochemical aspects, the culture was allowed to grow in Glucose-asparagines medium (Lilly and Barnett, 1951). The pH of the medium was adjusted to pH 6.0.Erlenmeyer flasks (150 ml) cleaned with sulphuric acid and potassium dichromate solution and carefully washed several times with tap water and finally with sterile distilled water, and then dried.

A small portion of growing mycelium from PDA slant of the fungus was aseptically transferred to 150 ml Erlenmeyer flasks containing 30 ml of sterile GA liquid medium. The inoculated flasks were incubated for 7 days with continuous shaking (120 rpm) at 25°C. After harvesting by filtration, the mycelium was washed with sterile distilled water to make them free from the adherent medium and was fragmented into small pieces in a sterile waring blender. Then the fragmented mycelium was suspended in phosphate buffer medium (pH 6.0) for 24 hrs. to overcome the shock encountered during blending. An aliquot of 1ml of this cell suspension was used as inoculum.

The total nitrogen content of the mycelium was determined by the method of Folin and Wu (1919) and Vougel (1961). Crude protein was calculated by using a factor of 4.38 on the basis of 22.83% nitrogen content of mushroom protein (Crisans and Sands, 1978).

The GA basal liquid medium was first prepared without any carbon source. After this , the medium was supplemented with desired carbon sources. Only one source of carbon was added to each set of the medium and was distributed as 30 ml in each Erlenmeyer flask (150 ml).The pH was adjusted to 5.5 with 0.2M phosphate buffer before sterilization. The set of flasks having medium without carbon source was treated as control. The flasks were sterilized at 121°C for 15 minutes. Each set of sterilized flasks with individual carbon source was inoculated separately with 1.0 ml of mycelial suspension of the fungus and incubated at 25°C for 15 days maintaining three replication for each treatment. The carbon sources used in this experiment were : glucose, fructose, sucrose, lactose, arabinose mannitol, starch, sodium acetate.

The GA basal liquid medium was first prepared without any nitrogen source. After this, the medium was supplemented with desired nitrogen sources. Only one source of nitrogen was added to each set of the medium in each Erlenmeyer flask (150 ml). The yeast extract was made vitamin –free by treating with activated charcol powder (5 gl^{-1})before incorporating in the medium.Each source of the nitrogen was added to the medium at the rate of 0.0424 gl^{-1} of nitrogen. The complex sources were added as weight basis. The pH of the medium was adjusted to 5.5 with 2M phosphate buffer and dispensed as 30 ml in each Erlenmeyer flask (150 ml).The flasks were then sterilized at 121°C for 15 minutes. Urea containing medium was sterilized by filtration. The set of flasks having medium without nitrogen source was treated as control. Each set of sterilized flasks with individual nitrogen source was inoculated separately with 1.0 ml mycelial suspension of the fungus and incubated at 25°C for 15 days. Several flasks were inoculated in order to have three replication for each treatment. The Nitrogen sources used were : ammonium sulphate, ammoniumnitrate, potassium nitrite, potassium nitrate, urea, lysine, leucine, isoleucine, arginine, valine, methionine, aspartic acid, glutamic acid, asparagines, casein hydrolysate, yeast extract and peptone.

RESULT AND DISCUSSION

In order to find out the effect of different carbon sources on the growth of mycelia of the mushroom and yield of protein by the mycelia, they were grown in 30 ml basal liquid medium taken in separate conical flasks (150 ml) at 25°C for 15 days with different carbon sources (Table 1).

The data on the effect of carbon sources reveal that glucose is the best carbon source for growth and yield of protein by dikaryon and protoplast-fusant mycelia of *P. sajor-caju*. Both the strains are able to utilize fructose, sucrose, lactose, starch, arabinose, mannitol and sodium acetate. Jandaik and Kapoor (1976) have reported that starch is the best carbon source for *P. sajor-caju* which is followed by maltose, sucrose, glucose and dextrin. This difference might be due to different strains of *P. sajor-caju* or due to different sugar content of the medium used. Hong (1978) has reported that maltose, mannitol and glucose are good sources for *P. ostreatus*. Voltz (1972), Hashimoto

Table 1 : Data showing the effect of carbon sources on the growth of mycelia and yield of protein by the mycelia of *P. sajor-caju* dikaryon and fusant protoplast at 15 days of incubation period under sub-merged condition

	Mycelium of *Pleurotus sajor-caju*			
	Dikaryon		Fusant protoplast	
Carbon source	Dry wt. of mycelium (mg/30ml)	Protein content (%)	Dry wt. of mycelium (mg/30ml)	Protein content (%)
Glucose	92.40±4.80	26.48±1.78	91.80±4.76	26.52±1.82
Fructose	86.20±3.60	22.20±1.72	85.40±4.44	22.50±1.80
Sucrose	78.40±3.60	19.86±1.60	79.40±4.10	19.48±1.64
Lactose	60.10±3.10	18.40±1.60	60.40±3.82	18.48±1.70
Arabinose	44.10±2.48	17.10±1.54	43.20±2.62	17.20±1.60
Mannitol	58.20±2.22	16.84±1.60	37.60±2.30	16.96±1.56
Starch	54.10±2.78	17.48±1.62	53.40±2.64	17.64±1.44
Sodium acetate	28.10±2.10	16.60±1.54	28.36±2.12	16.80±1.52
Control	18.60±2.12	16.40±1.40	18.10±2.10	16.44±1.44

Table 2 : Data showing the effect of inorganic nitrogen sources of growth of mycelium and yield of protein by the mycelium of *P. sajor-caju* (dikaryon and fusant-protoplasts) at 15 days incubation period under submerged conditions

Nitrogen sources	Mycelium of *Pleurotus sajor-caju*			
	Dikaryon		Fusant protoplast	
	Dry wt. of mycelium (mg/30ml)	Protein content %	Dry wt. of mycelium(mg/30ml)	Protein content %
(NH4)2SO4	89.20±4.22	26.10±1.24	89.80±4.20	26.12±1.30
NH4NO3	86.40±4.44	25.98±1.32	86.82±4.42	26.10±1.40
KNO2	68.38±3.68	25.12±1.40	69.10±3.70	25.36±1.32
KNO3	92.60±4.92	26.56±1.86	92.40±4.90	26.60±1.78
Urea	72.40±4.30	25.48±1.64	72.40±4.32	25.60±1.60
Control	38.10±2.80	16.80±1.40	38.26±2.82	16.88±1.44

Table 3 : Data showing the effect of organic nitrogen sources of growth of mycelium and yield of protein by the mycelium of *P. sajor-caju* (dikaryon and fusant-protoplasts) at 15 days incubation period under submerged conditions

Nitrogen sources	Mycelium of *Pleurotus sajor-caju*			
	Dikaryon		Fusant protoplast	
	Dry wt. of mycelium (mg/30ml)	Protein content (%)	Dry wt. of mycelium (mg/30ml)	Protein content (%)
L-Lysine	74.20±2.40	25.62±1.62	74.64±4.12	25.70±1.58
L-Leucine	56.80±2.20	25.12±1.56	56.48±2.80	25.16±1.60
DL-Isoleucine	60.24±2.80	25.10±1.60	60.56±2.78	25.18±1.48
L-Arginine	59.26±2.27	25.10±1.62	60.12±3.12	25.16±1.52
L-Valine	56.26±2.66	25.12±1.48	56.64±2.68	25.18±1.62
L-Methionine	58.16±2.68	25.10±1.50	58.10±2.64	25.14±1.48
DL-AsparticAcid	69.30±2.72	25.48±1.60	69.10±3.20	25.50±1.56
L-Glutamic Acid	68.12±2.80	25.32±1.40	68.42±3.20	25.34±1.62
L-Aspargine	88.60±4.10	26.10±1.92	88.54±4.26	26.18±1.78
Control	38.06±2.10	16.82±1.12	38.10±2.12	16.84±1.14

and Takahashi (1976) and Rypacek (1977) have reported different observations on *P. ostreatus*.

These differences as reported by various researchers about the ability of *Pleurotus* to utilize carbon in nature (Oso, 1979). Regarding the organic acid, there appears to be little difference between the data of the present investigation and that of Sugimori *et al.* (1971) and Hashimoto and Takahashi (1976).

Table 4 : Data showing the effect of complex nitrogen sources of growth of mycelium and yield of protein by the mycelium of *P. sajor-caju* (dikaryon and fusant-protoplasts) at 15days incubation period under submerged conditions.

Nitrogen sources	Mycelium of *Pleurotus sajor-caju*			
	Dikaryon		Fusant protoplast	
	Dry wt. of mycelium (mg/30ml)	Protein content (%)	Dry wt. of mycelium (mg/30ml)	Protein content (%)
Casein	58.44±2.80	25.58±1.80	58.56±3.10	25.62±1.78
hydrolysate Yeast extract	59.12±3.22	25.82±1.82	59.20±3.24	25.88±1.84
(oxid) Peptone (Difco)	57.48±3.10	25.60±1.60	57.60±3.12	25.62±1.72
Control	38.20±2.12	16.78±1.16	38.26±2.14	16.78±1.20

sources appear to be due to physiological differences in the species or of the isolates of the same species (Kurtzman Jr. and Zadrazil, 1982).

The lesser action of all other sources of carbon on the promotion of growth has also been noticed which might be due to the adverse osmotic effects or due to the depletion of other required nutrients or due to the accumulation of unfavorable metabolites (Garraway and Evans, 1984). Similar observations on *Coprinus lagopus* have also been reported by Moore (1969) and Faruta and Youchiro (1970). Glucose, fructose and arabinose are possibly used by non-specific pyranose carrier system which are constitutive in nature (Kotyk and Hanskover, 1968; Cirillo, 1968). Sucrose and mannitol are presumably used by disaccharide carrier system which are known to be inducible in the nature (Gorts, 1969; Kotyk and Michaljanicova, 1979). The capability of utilizing the disaccharides by the mycelia indicate that the responsible hydrolytic enzymes are synthesized and also secreted externally from the mycelia by *P. sajor-caju* (Hankin and Anagnostakis, 1975; Simonson and Liberta, 1975). The ability of the mycelia of *P. sajor-caju* to utilize the starch indicates that the mycelia produce the amylase enzyme but which might be inducible

The data on the effect of nitrogen sources on the growth and yield of protein by the dikaryon and protoplast-fusant mycelia of *P. sajor-caju* reveal that potassium nitrate is the best source of nitrogen. It is being followed by ammonium sulphate, asparagines and others. The data further reveal that there is a wide variation in the responses exhibited by both the strains in utilizing different sources of nitrogen. Similar observations have been reported on *P. sajor-caju* by Jandaik and Kapoor (1976) and on *P. osreaetus*, *P. flabellatus* and others by Srivastava and Bano (1970), Sugimori *et al.* (1971), Voltz (1972), Hashimoto and Takahashi (1976) and Hong (1978).

It appears that nitrates might have entered the cell via simple diffusion by moving slowly through the nitrate concentration gradient which is created by the action of nitrate reductase (Pateman and Kinghorns, 1976; Roldan *et al.* 1982). The utilization of ammonium salts suggests its role as a regulator of many developmental systems (Garraway and Evans, 1984). Ammonia might have been utilized by incorporating the same into amino acid by glutamate dehydrogenase enzyme through reductive amination. Nitrate is first converted to ammonia and utilized. Peptone is possibly used as car-

bon and nitrogen source. Similar observation on *Coprinus cinereus* has been documented by Kalisz *et al.,* (1986). The better effect of asparagine is due to its better buffering capacity. The differential responses exhibited by both the strains against the amino acids might be due to different methods of uptake of the different amino acids (Pateman and Kinghorns, 1976; Whitaker, 1976). The uptake of different amino acids is also controlled by different factors like pH, temperature, energy requirement, transinhibition, concentration of amino acids and due to other nutrients availability (Whitaker and Morton, 1971; Horak *et al.,* 1977).

The data in the Table 1 revealed that glucose was the best carbon source for the mycelial growth (92.40 mg/30 ml) and yield of protein (26.48%) of the dikaryon mycelium of *P. sajor-caju.* It was followed by fructose (86.20 mg/30 ml and 22.20%), sucrose (78.40 mg/30 ml and 19.86%), lactose (60.10 mg/30 ml and 18.40%), starch (54.10 mg/30 ml and 17.48%), arabinose (44.10 mg/30 ml and 17.10%), mannitol (38.20 mg/30 ml and 16.48%) and sodium acetate (28.20 mg/30 ml and 16.60%).

Glucose was also the best carbon source for the mycelial growth (91.80 mg/30 ml) of the mycelia and yield of protein (26.52%) by the mycelia of fusant protoplasts. It was followed by fructose (85.40 mg/30 ml and 22.50%), sucrose (79.40 mg/30 ml and 19.98%), lactose (60.40 mg/30 ml and 18.48%), starch (53.40 mg/30 ml and 17.64%), arabinose (43.20 mg/30 ml and 17.20%), mannitol (34.60 mg/30 ml and 16.96%) and sodium acetate (28.36 mg/30 ml and 16.80%).

Both the mycelia failed to grow appreciably in medium without any carbon source and yield of protein was also very negligible.

In order to find out the effect of different nitrogen sources on the mycelial growth and yield of protein by the dikaryon and fusant protoplasts mycelia, both the mycelia were grown in liquid medium (30 ml) in conical flasks (150 ml) with optimum carbon source and pH at 25°C for 15 days.

The nitrogen sources were added separately in the medium, one in each set of experiment. The conical flask without source of nitrogen was treated as control (Table 2).

The data in the Table 2 revealed that potassium nitrate was the best inorganic source of nitrogen for the mycelial growth and yield of protein by the dikaryon (92.00 mg/30 ml and 26.56%) and fusant protoplasts mycelia (92.40 mg/30 ml and 26.60%).This is followed by ammonium sulphate (89.20 mg/30 ml and 26.10% and 89.80 mg/30 ml and 26.12%), ammonium nitrate (86.40 mg/30 ml and 25.98% and 86.82 mg/30 ml26.10%), urea (72.40 mg/30 ml and 25.48% and 72.40 mg/30 ml and 25.60%) and potassium nitrite (68.38 mg/30 ml and 25.12% and 69.10 mg/30 ml and 25.36%).

The data in Table 3 revealed that asparagine was the best organic source of nitrogen for the mycelial growth and yield of protein by the dikaryon (88.60 mg/30 ml and 26.10%) and fusant protoplasts mycelia (88.54 mg/30 ml and 26.18%). This was followed by lysine, aspartic acid, glutamic acid, isoleucine, arginine, methionine, leucine. The data are revealed that valine was a better source than that of leucine in case of mycelia of fusant protoplast in comparison to dikaryon mycelia.

The data in Table 4 revealed that yeast extract was the best complex source of nitrogen for the mycelial growth of and yield of protein by the dikaryon (59.12 mg/30 ml and 25.82%) and fusant protoplasts mycelia (59.20 mg/30 ml and 25.88%). This was followed by casein hydrolysate (58.44 mg/30 ml and 25.58% and 58.56 mg/30 ml and 25.62%) and peptone 57.48 mg/30 ml and 25.50% and 57.50 mg/30 ml and 25.62%).

From the data of the Tables 2-4, it was further revealed that in all treatments the mycelia of fusant protoplasts yielded more protein in comparison to dikaryon mycelia.

The data on the effect of carbon sources revealed that glucose is the best carbon source for the growth and yield of protein by dikaryon and protoplast-fusant mycelia of *P. sajor-caju*. Both the strains are able to utilize fructose , sucrose, lactose, starch, arabinose, mannitol and sodium acetate.

The data on the effect of nitrogen sources on the growth and yield of protein by the dikaryon and protoplast-fusant mycelia of *P. sajor-caju* revealed that potassium nitrate was the best source of nitrogen. It is being followed by ammonium sulphate, asparagine and others. The data further reveal that there is a wide variation in the responses exhibited by both the strains in utilizing different sources of nitrogen.

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