PRODUCTION OF ERGOT ALKALOIDS BY FERMENTATION METHOD

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(*Received* 17 *March* 1967)

Capacity of three strains of the fungus, *Claviceps purpurea* to produce ergot alkaloids from synthetic media has been investigated. Of the three strains studied, *Claviceps purpurea* received from Prairie Regional Laboratory, Saskatoon, Canada gave high yields of ergot alkaloids. The effect of different carbohydrates, nitrogen sources and precursors on the production of ergot alkaloids has been studied. Highest yield (2·287 gm/l) of the total alkaloids has been obtained with maltose as a carbohydrate source, ammonium succinate as a nitrogen source and tryptophan (500 mg/l) as a precursor.

Ergot alkaloids are obtained commercially by extracting sclerotia of *Claviceps purpurea* parasitizing on rye (*Secale cereale Linn*) plants. The other important species of this fungus are *C. paspali*, *C. microcephale*, *C. miscanthi* and *C. syntherismae*. The alkaloids have also been isolated[1—4] from certain other fungi and plants.

The most important of these alkaloids are ergometrine and ergotamine. The former finds use in oxytocic effect and the latter in migraine, adrenolytic, hallucinogenic effect etc. Studies on the effect of various factors on yields of ergot alkaloids have been made by several workers [5—11] by artificial cultivation of this fungus on rye. Such methods of production of ergot alkaloids are however uneconomical from commercial standpoint. Production of these alkaloids by fermentation method has interested many scientists[1, 2—18 and 20—25], however, it is still a virgin field of research. This paper describes some of the recent work carried out in this laboratory on the production of ergot alkaloids by fermentation method.

MATERIALS AND METHODS

Strains—The following strains of *Claviceps purpurea* were studied.

(*a*) *C. purpurea* R. 29 obtained from Regional Research Laboratory, Jammu, India.

(*b*) *C. purpurea* obtained from National Chemical Laboratory, Poona, India.

(*c*) *C. purpurea* PRL 1980 obtained from Prairie Regional Laboratory, Saskatoon, Canada.

The strains (*a*) and (*c*) were maintained on potato-dextrose-agar medium at *pH* 5·6. The strain (*b*) was maintained on the medium consisting of dextrose, bacto-peptone, potassium dihydrogen phosphate, magnesium sulphate, ferrous sulphate and agar at *pH* 6·0.

Synthetic media—The various media used for production of ergot alkaloids are given in Appendix I.

Conditions of fermentation—The fermentation was carried out by stationary surface culture method.

Estimation of ergot alkaloids—The total ergot alkaloids were estimated using Vanurk's[19] reagent. The intensity of blue colour developed was measured colorimetrically. The mycelium and culture filtrate were separated by straining. The total ergot alkaloid content was estimated in the culture filtrate after drying at 40°C under vacuum and in the mycelium by drying it at room temperature (32°±2°C).

APPENDIX I

MEDIA USED FOR PRODUCTION OF ERGOT ALKALOIDS

Serial No.	Ingredients of media per litre
1.	Maltose 50 gm Glucose 10 gm Yeast extract 5 gm Asparagine 0·3 gm Peptone Difco 6·0 gm Magnesium sulphate 1·0 gm Potassium phosphate (monobasic) 2·0 gm Biotin 2·5 γg
2.	Mannitol 50·0 gm Glutamic acid 10·0 gm Potassium phosphate (dibasic) 1·0 gm Magnesium sulphate 0·3 gm
3.	Glucose 50·0 gm Yeast 10·0 gm
4.	Mannitol 50 gm Sucrose 20 gm Potassium phosphate (monobasic) 1 gm Magnesium sulphate 0·3gm Manganese sulphate 0·1 gm Ferrous sulphate 0·004 gm Succinic acid 5·4 gm
5.	Maltose 50 gm Sucrose 10 gm Asparagine-1 gm Peptone 6 gm Magnesium sulphte 1 gm Potassium phosphate (monobasic) 2 gm
6.	Mannitol 50 gm Succinic acid 6·8 gm Potassium phosphate (monobasic) 1·0 gm Magnesium sulphate 0·3 gm Ferrous sulphate 13 mgm Zinc sulphate 4 mgm
7.	Mannitol 50 gm Ammonium succinate 8 gm Potassium phosphate (monobasic) 1·0 gm Magnesium sulphate 0·3 gm Ferrous sulphate 5·0 mgm Calcium chloride 5·95 mgm Zinc sulphate 4·40 mgm Manganese sulphate 2·75 mgm Sodium chloride 2·55 mgm Ammonium molybdate 1·82 mgm Biotin 5 γg
8.	Medium 2+tryptophan
9.	Medium 2+indoleacetic acid
10.	Medium 7+tryptophan

Yield of mycelium—The yield of mycelium was calculated by drying a part of it to constant weight.

RESULTS AND DISCUSSIONS

The optimum conditions for growth of strains studied were found to be *pH* 5·6 for strains (*a*) and (*c*) and *pH* 6·0 for strain (*b*), temperature 24°C to 28°C and growth period 35 days. The strain (*a*) grown on the different media (Appendix I) gave satisfactory growth in almost all the media tried but pigmentation or alkaloid formation did not take place. The strain (*b*) was grown on medium (7) only. It gave positive results with Vanurk's reagent but the yields of ergot alkaloids were insignificant. The strain (*c*) was also grown on medium No. (7) and was found suitable for the production of ergot alkaloids. In further studies on production of ergot alkaloids only the strain (*c*) was used. The capacity of this strain to produce ergot alkaloids was studied using medium No. (7) (containing mannitol as the carbon source) along with tryptophan or phenylalanine as precursors. The yields of mycelium and total alkaloids formed under these conditions are given in Table 1. Higher yields of alkaloids were obtained when tryptophan was added as a precursor in the medium.

The effect of varying the carbon sources on the yield of alkaloids was studied by taking various carbohydrates. The results are given in Table 2. The highest yield of ergot alkaloids (323 mg/l) was obtained when maltose was used as a carbohydrate source. The mixture of galactose and glucose (19 : 1) which was found as the best by Taber & Vining[21] gave a yield of 207 mg/l. The lowest yield was obtained with galactose alone (7 mg/l). Mannitol

TABLE 1

YIELD OF MYCELIUM AND ERGOT ALKALOIDS

Claviceps purpurea PRL 1980

Medium	Yield of mycelium and culture filtrate in 1 L of original medium		Yield of alkaloids in			Total alkaloids in 1 L of fermented medium
	Wt. of dry mycelium	Vol. of culture filtrate	Mycelium	Culture filtrate	Total alkaloids in 1L of original medium	
	gm.	ml.	mg/gm.	mg/100 ml.	mg.	mg.
Medium 7+ Tryptophan	18·0	720	0·822	1·381	24·739	34·36
Medium 7+ Phenylalanine	20·32	726	0·493	0·945	16·878	23·25

TABLE 2

EFFECT OF DIFFERENT CARBOHYDRATES ON YIELDS OF ERGOT ALKALOIDS

Claviceps purpurea PRL 1980

Carbohydrate	Yield of mycelium and culture filtrate in 1L of original medium		Yield of alkaloids in			Yield of total alkaloids in one litre of fermented medium
	Wt. of dry mycelium	Vol. of culture filtrate	Mycelium	Culture filtrate	Total alkaloids in one litre of original medium	
	gm.	ml.	mg/gm.	mg/100ml.	mg.	mg.
Mannitol	18·73	788	0·274	..	..	..
Galactose	2·03	850	0·247	0·644	5·975	7·03
Glucose	14·31	780	2·343	1·836	46·85	60·03
Maltose	18·82	820	3·617	24·413	264·64	322·7
Galactose* + Glucose	16·85	820	3·494	13·549	169·972	207·2
Sucrose	16·38	800	0·370	0·689	11·67	14·59
Fructose	21·00	808	0·315	1·014	14·808	18·33

*in ratio of 19 : 1

which has been shown to be advantageous by other workers[12,20] was found unsatisfactory for this strain. High yields with maltose have not been obtained in similar studies [15, 21].

The study of varying the nitrogen sources was carried out using maltose as a carbon source in each case. The results are presented in Table 3. Highest yield of 2·287 gm. of total alkaloids per litre of the fermented medium has been obtained when ammonium succinate is supplemented with tryptophan. Good results were obtained when yeast extract or glutamic acid were used as nitrogen sources. No growth of the mycelium took place when urea or tryptophan alone was used. Taber & Vining[21] has reported that tryptophan may not be a desirable supplement for increasing the yield of "natural" alkaloids. It does influence the course of indole metabolism and produces clavine alkaloids such as agroclavine, penniclavine etc. Good results have been obtained by Johansson[22] using whey as a nitrogen source.

Studies on some of the precursors other than tryptophan viz., phenylalanine, alanine and trigonellin were carried out. These precursors were used alongwith maltose as carbon source and ammonium succinate as nitrogen source. No increase in the yields of the alkaloids was obtained with any of these precursors.

TABLE 3

EFFECT OF NITROGEN SOURCES ON YIELD OF ERGOT ALKALOIDS AND MYCELIUM

Claviceps purpurea PRL 1980

Nitrogen sources	Yield of mycelium and culture filtrate from 1L of original medium		Yields of alkaloids in			Total in 1L of fermented medium
	Wt. of dry mycelium	Vol. of culture filtrate	Mycelium	Culture filtrate	Total Alkaloids in 1L of original medium	
	gm.	ml.	mg/gm.	mg/100 ml.	mg.	mg.
Ammonium succinate	16·0	800	4·95	48·29	465·52	582·0
Ammonium succinate + tryptophan	14·5	800	9·65	211·20	1829·52	2287·0
Yeast extract	15·5	800	4·26	87·68	767·47	959·0
Glutamic acid	11·0	850	3·3	89·37	795·95	936·0
Urea	..	No Growth	..	..	..	..
Tryptophan alone	..	No Growth	..	..	..	..

The organism *C. purpurea* PRL 1980 appears to grow well under stationary surface culture, as preliminary experiments with the organism in shake cultures did not yield any growth.

The yield of 1 gm/l has been reported by Aracmone[24] *et al* and Mary[25] *et al* using the organism *C. paspali* in each case. The yields of ergot alkaloids by *C. purpurea* strain PRL 1980 obtained by us are very encouraging and highest than hitherto reported. The effect of various other factors such as vitamins, trace element, organic acids, phosphate concentration and other fermentation conditions are under study in order to get maximum production of ergot alkaloids. The isolation and identification of individual alkaloids is also in hand.

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