Process Biochemistry 28 (1993) 23–27

Spectra of Ergot Alkaloids Produced by *Claviceps purpurea* 1029c in Solid-state Fermentation System: Influence of the Composition of Liquid Medium Used for Impregnating Sugar-cane Pith Bagasse

M.R. Trejo Hernández

Biotechnology Department, Autonomous Metropolitan University, Iztapalapa, AP 55-535, 09340 México City, México

B.K. Lonsane

Fermentation Technology and Bioengineering Discipline, Central Food Technological Research Institute, Mysore—570013, India

M. Raimbault & S. Roussos

Biotechnology Unit, ORSTOM Centre Montpellier, 911 Av. Agropolis, B.P. 5045, 34032 Montpellier Cedex 1, France

(Received 1 November, 1991; revised manuscript received and accepted 9 February, 1992)

A total of 16 different combinations of the liquid nutrient medium used for impregnating sugar-cane pith bagasse have been evaluated for the production of total alkaloids by Claviceps purpurea *1029c in a solid-state fermentation system, and for their effects on the spectra of the alkaloids produced. The data indicated large differences in the alkaloids' spectra. There is therefore the possibility of achieving tailor-made spectra of ergot alkaloids by changing the liquid nutrient media composition.*

INTRODUCTION

The demand for ergot alkaloids and their derivatives, an industrially important class of high-value biochemicals, has increased in recent years, as a result of a number of new uses, especially in the treatment of diseases.[1] The complexity of total chemical synthesis, which involves 15 steps and 140 possible intermediates,[2] rules out the synthesis of lysergic acid and other ergot alkaloids through the chemical route.[3] The traditional process exploited for many years in European countries involving growth on plants in the field, has many limitations such as a high dependence on weather, the loss of conidia during rain, damage to sclerotia during harvest and a high labour requirement.[1,4,6] Most of

Corresponding author: Dr B.K. Lonsane. Telephone: 37252.

these limitations are overcome to a large extent in the saprophytic production of ergot alkaloids through the fermentation route which, in addition, has advantages in the economy of production.[1,6,8] Consequently, considerable efforts have been made in the development of efficient fermentation processes.[7]

Submerged fermentation (SmF) techniques are currently used for the industrial-scale production of ergot alkaloids.[6] Other fermentation techniques investigated for improving productivity include semi-continuous fermentation with immobilized cells, biosynthesis with crude enzymes in cell-free systems and the use of protoplasts.[1,4,7] The simulation of the parasitic life-style in a fermentation system has been widely recognized as the method for obtaining high yields, and this has been partially achieved using immobilized cells or liquid surface fermentation.[1,4,6] The potential of mixed culture fermentation, solid-state fermentation (SSF) and stationary fermentation in plastic cushions has been the subject of speculation.[7]

Earlier comparative studies on the production of ergot alkaloids by *Claviceps fusiformis* ATCC 26019 in SmF and SSF processes revealed 3·9 times higher production of total alkaloids in the latter system.[9] The production was similar with *Claviceps purpurea* 1029c but the spectra of alkaloids produced were advantageous with the use of the SSF technique. For example, the ergonovine and ergotamine contents were 1·7 and 3·4 times higher in an SSF system.[9] In contrast, the lysergol content was reduced by 34·3 times in the SSF system.[9]

Studies on the solid substrates also indicated changes in the ergot alkaloid spectra in SSF systems,[10] including minor alterations in total alkaloid production by *C. purpurea* 1029c and more significant changes in the spectra of ergot alkaloids produced. For example, ergonovine accounted for 93% of the total alkaloids produced in wheat grain medium whereas lysergic acid derivatives and ergonovine comprised 66 and 32% respectively of total alkaloids in the rye grain medium.[10] In contrast, ergonovine, ergotamine and lysergic acid derivatives comprised 35, 35 and 27% respectively of the total alkaloids with the use of sugar-cane pith bagasse.[10]

The use of an inert solid support such as washed sugar-cane pith bagasse offers the facility of wide changes in the medium used for impregnating it in the SSF system. This paper describes the effects of medium composition on the spectra of the alkaloids produced in such a system.

MATERIALS AND METHODS

Microorganism and inoculum development

Claviceps purpurea 1029c was obtained from the Institute of Biochemistry and Molecular Biology, Technical University of Berlin, FRG, and maintained on potato dextrose agar (PDA) slants at 4 °C with subculturing every alternate month. In addition, it was preserved by lyophilization. The spore inoculum of the culture was prepared by the methodology of Sanglier,[11] using agar medium in Petri dishes of 13-cm i.d. After inoculation with freshly grown culture from a PDA slant, cultures were incubated at 26 °C for 5 days. The spores from the agar surface were harvested by using 50 ml of sterile distilled water containing a drop of Tween 80. All the experiments were conducted in triplicate and average values are reported, as the variations were less than ±5%.

Composition of liquid nutrient medium

The changes in the composition of the liquid nutrient medium used for impregnating washed sugar-cane pith bagasse are listed in Table 1. A mineral salt solution was added to each of the media to provide a final concentration of 0·03% KCl, 0·0025% $FeSO_4 \cdot 7H_2O$ and 0·01% $ZnSO_4 \cdot 7H_2O$ per litre of the liquid medium. The pH of the medium was adjusted to 5·2 by addition of 1 M NaOH. The media were sterilized at 121 °C for 20 min. Sucrose was sterilized separately at the same time and temperature, and mixed with the sterilized liquid nutrient medium at ambient temperature. The media were inoculated with the spore suspension to provide $0{\cdot}6 \times 10^7$ spores/ml of the final liquid medium.

Solid-state fermentation

Sugar-cane pith bagasse was used as an inert solid support in all cases. It was washed, sieved, autoclaved and dried by the processing methodology of Saucedo-Castañeda *et al.*[12] The processed sugar-cane pith bagasse was then impregnated with inoculated liquid medium at a ratio of 30:70 (w/v). The well-mixed moist solid medium in 60-g moist weight quantities was packed loosely into static column fermenters of 20-cm length × 4-cm diameter. These column fermenters were aerated at the rates specified in Table 1 using humidified air while the fermentation was carried on at 26 °C for 120 h. The fermenter assembly and its operational procedure have been described by Raimbault and Alazard.[13]

Table 1. Composition of Liquid Nutrient Media Used for Impregnating Sugar-cane Pith Bagasse.

Medium no.	*Carbon substrate*	*Composition (g/litre)*									*Aeration rate (litre/h per column)*
		$(NH_4)_2SO_4$, *urea*[a], *oxalate-*NH_3[c]	KH_2PO_4	$MgSO_4 \cdot 7\,H_2O$	$Ca(NO_3)_2$	*Citric acid*	*Tween 80*	*Valine*	*Proline*	*Tryptophan*	
1	RM	9·0[A] + 2·04[B]	0·5	0·625	2·5	10	5	2·92	2·87	1	2
2	S	9·0[A] + 2·04[B]	1	0·25	2·5	10	10	2·92	2·87	0·5	4
3	RM	11·8[C]	1	0·625	1	10	10	5·85	2·87	0·5	2
4	S	9·0[A] + 2·04[B]	0·5	0·625	2·5	5	10	5·85	5·75	0·5	2
5	S	11·8[C]	1	0·25	2·5	10	5	5·85	5·75	1	4
6	S	11·8[C]	0·5	0·625	1	10	10	2·92	5·75	1	4
7	RM	11·8[C]	0·5	0·25	2·5	5	10	5·85	2·87	1	4
8	RM	9·0[A] + 2·04[B]	0·5	0·25	1	10	5	5·85	5·75	0·5	4
9	RM	9·0[A] + 2·04[B]	1	0·25	1	5	10	2·92	5·75	1	2
10	S	9·0[A] + 2·04[B]	1	0·625	1	5	5	5·85	2·87	1	4
11	RM	11·8[C]	1	0·625	2·5	5	5	2·92	5·75	0·5	4
12	S	11·8[C]	0·5	0·25	1	5	5	2·92	2·87	0·5	2
13	RM	9·6[C] + 1·73[B]	0·5	0·625	–	–	–	–	–	0·5	4
14	RM	9·6[C] + 1·73[B]	1	0·625	–	–	–	–	–	1	4
15	S	9·6[C] + 1·73[B]	0·5	0·625	–	–	–	–	–	0·5	4
16	S	9·6[C] + 1·73[B]	1	0·625	–	–	–	–	–	1	4

The amount of sucrose or rye meal used was 200 g/litre in all cases.
RM. rye meal; S. sucrose.

Extraction of alkaloids

Ergot alkaloids from 25 g of moist fermented solids were extracted using 50 ml of solvent (1:1 mixture of acetone and 4% tartaric acid). The extraction was carried out at ambient temperature by allowing a contact time of 5 h. The extract was separated by centrifugation (5000 rev/min), concentrated under vacuum at 30 °C, and the alkaloids were solubilized in 3 ml of 4% tartaric acid solution.

Analytical methods

Total alkaloids were estimated spectrophotometrically using the methodology of Smith[14] and the Van Urk[15] reagent with ergonovine base used as standard. The spectra of the alkaloids were determined by high-pressure liquid chromatography.[10] CO_2 evolution during fermentation was determined by the methodology of Saucedo-Castañeda *et al.*[12]

RESULTS AND DISCUSSION

Growth and total alkaloid production

The fungus grew in 16 nutrient media studied, although the magnitude of growth varied (Table 2). The specific growth rates ranged from 0·01 to 0·06 in four media and from 0·10 to 0·17 μ/h in the remaining media. The data on the kinetics of CO_2 evolution during the entire course of fermentation (120 h) are given in Figure 1. The final pH of the medium was in the range of 4·86–8·56 and no

Table 2. Growth of *C. purpurea* 1029c and Total Alkaloid Production in Various Liquid Media Used for Impregnating Sugar-cane Pith Bagasse.

Medium no.	*Total* CO_2 *evolved (ml/g IDM)*	*Specific growth rate* (μ/h)	*pH*	*Total alkaloids produced* (μg/g *IDM*)
1	23·66	0·01	7·75	0
2	70·38	0·10	5·12	30·26
3	33·55	0·04	8·56	0
4	74·06	0·15	4·89	52·73
5	81·97	0·10	6·07	28·36
6	75·93	0·10	7·91	24·65
7	46·77	0·17	8·14	61·81
8	28·53	0·01	6·53	0
9	45·91	0·13	5·54	37·02
10	76·91	0·14	4·99	258·70
11	78·19	0·14	7·91	78·35
12	80·32	0·06	6·28	0
13	ND	ND	5·26	68·50
14	ND	ND	5·01	78·50
15	ND	ND	4·86	337·50
16	ND	ND	4·90	505·50

The values are for the fermentation period of 120 h.
ND. Not done.

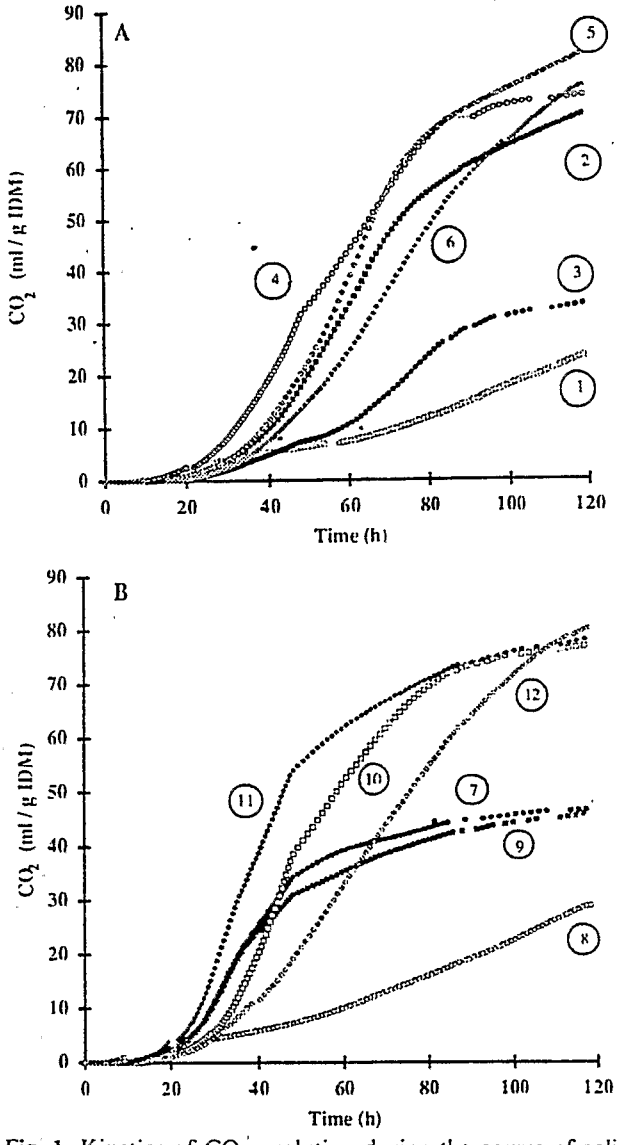

Fig. 1. Kinetics of CO_2 evolution during the course of solid-state fermentation involving the use of various liquid nutrient media for impregnating sugar-cane pith bagasse. (A) Media 1–6; (B) media 7–12.

correlation was found between the final pH and the growth of the culture or total alkaloid production (Table 2). The culture was not able to form ergot alkaloids in four media and no reason for this could be cited. The total alkaloid production in the remaining media (Table 2) was in the range of 24·65–505·50 μg/g initial dry matter (IDM). The total alkaloid production in the SSF system involving liquid nutrient media based on rye meal as carbon source was generally lower than in those based on sucrose. It is emphasized that sucrose is the best established carbon source for ergot alkaloid fermentation.[6,7] The highest production of total alkaloids was observed in medium 16, followed by media 15 and 10.

Table 3. Spectra of Alkaloids Formed in Various Liquid Media Used for Impregnating Sugar-cane Pith Bagasse.

Medium no.	*Alkaloid spectra (% of total alkaloids)*			
	Ergotamine	*Ergonovine*	*Lysergol*	*Derivatives of lysergic acid*
2	0	48·32	2·33	49·35
4	0·53	32·48	7·45	59·54
5	4·64	27·57	17·41	50·38
6	0	36·50	0	63·50
7	0	38·64	11·01	50·35
9	0	19·36	18·98	61·66
10	0·73	33·39	9·71	56·17
11	0	51·31	11·03	37·66
13	0	74·50	8·05	17·45
14	0	51·57	11·52	36·91
15	0	32·51	7·56	59·93
16	33·58	9·52	1·57	55·33

Alkaloid production was absent in media 1, 3, 8 and 12.

Alkaloid spectra

The data on the effect of the composition of the liquid nutrient media on the spectra of ergot alkaloids produced by *C. purpurea* 1029c in an SSF system showed some interesting trends (Table 3). For example, ergotamine was completely absent in eight media, whereas it was > 1% of the total alkaloids in media 4 and 10. In contrast, it accounted for 33·58 and 4·64% of the total alkaloids in media 16 and 5, respectively. Lysergol was not formed in medium 6 but it accounted for 2·33–18·98% of the total alkaloids in the other media.

Ergonovine and other derivatives of lysergic acid, however, were formed in higher quantities and together accounted for the major part of the alkaloids formed in most media (Table 3). The highest and lowest levels of ergonovine were 74·50 and 9·52% in media 13 and 16, respectively. These values for the other lysergic acid derivatives, in contrast, were 63·50 and 17·45% in media 6 and 13, respectively.

Implications of the data

These changes in the spectra of ergot alkaloids can be effected easily by changing the composition of liquid nutrient medium used for impregnating the sugar-cane pith bagasse. These changes can also be achieved without resorting to the use of amino acids, as is evident from the data for media 13–16, or by altering the concentration of tryptophan. It is of interest to note that tryptophan is well established as an inducer of ergot alkaloid biosynthesis.[5,7] Together with the solid substrate mediated changes

in alkaloid spectra,[10] the data open an entirely new possibility of achieving tailor-made spectra of ergot alkaloids at an economical cost. Moreover, the advantages of ergot alkaloid production in an SSF system are attractive.[9] On the whole, the production of ergot alkaloids by SSF processes appears to be potentially beneficial and economical as compared with the conventionally used SmF process.[9,16,17] The highly stable foam formation, sensitivity of *Claviceps* to chemical antifoam agents as well as mechanical stress, the requirement for high oxygen tension in the medium and the consequent need for a well-balanced system of aeration–agitation, the end-product inhibition of the enzymes involved in the biosynthesis of ergot alkaloids and the inhibition of these enzymes under the conditions of rapid growth are some of the serious and inherent problems encountered in the SmF processes for ergot alkaloids production.[1,5,6] These problems are either absent or of less consequence, or can be efficiently and successfully overcome in the SSF system.[9] More work on the topic, however, is needed before the commercial exploitation of the SSF system for the production of ergot alkaloids may be recommended.

ACKNOWLEDGEMENTS

M.R.T.H. is grateful to CONACyT (Mexico) and CEFI (France) for partial financial support for this work. B.K.L. thanks the Council of Scientific and Industrial Research, New Delhi, India, for deputation terms, and ORSTOM, Centre Montpellier, for excellent facilities and an associateship.

REFERENCES

1. Esser, K. & Düvell, A., Biotechnological exploitation of ergot fungus (*Claviceps purpurea*). *Process Biochem.*, **19** (1984) 142–9.
2. Kornfeld, E.C., Fornefeld, E.J., Kline, G.B., Mann, M.J., Morrison, D.E., Jones, R.G. & Woodward, R.B., The total synthesis of lysergic acid. *J. Am. Chem. Soc.* **78** (1955) 3087–114.
3. Kelleher, W.J., In *Advances in Applied Microbiology*, ed. D. Perlman, Academic Press, New York, 1969, pp. 211–45.
4. Řehàček, Z., New trends in ergot alkaloid biosynthesis. *Process Biochem.*, **18** (1983) 22–29, 33.
5. Řehàček, Z., Biotechnology of ergot alkaloids. *Trends in Biotechnol.*, **2** (1984) 166–72.
6. Robbers, J.E., In *Advances in Biotechnological Processes*, Vol. 3, ed. A. Mizrahi & A.L. Van Wezel, Alan R. Liss, New York, 1984, pp. 197–239.
7. Řehàček, Z. & Sajdl, P., *Ergot Alkaloids, Chemistry, Biological Effects, Biotechnology*, Academia, Prague, 1990.
8. Udvardy, E., Consideration of the development of an ergot alkaloid fermentation process. *Process Biochem.*, **15** (1980) 5–8.
9. Trejo-Hernández, M.R., Raimbault, M., Roussos, S. & Lonsane, B.K., Potential of solid state fermentation for production of ergot alkaloids. *Lett. Appl. Microbiol.*, in press.
10. Trejo-Hernández, M.R., Lonsane, B.K., Raimbault, M. & Roussos, S., Solid substrate mediated changes in ergot alkaloid spectra in solid state fermentation system. *Chem. Mikrobiol. Technol. Lebensm.*, in press.
11. Sanglier, J.J., Contribution à l'étude de la différenciation conidienne chez *Claviceps purpurea* (Fr.) Tul. Thèse Doct., Université de Clermont-Ferrand, Clermont-Ferrand, France, 1976.
12. Saucedo-Castañeda, G., Lonsane, B.K., Krishnaiah, M.M., Navarro, J.M., Roussos, S. & Raimbault, M., Maintenance of heat and water balances as a scale-up criteria for the production of ethanol by *Schwanniomyces castellii* in a solid state fermentation system. *Process Biochem.*, **27** (1992) 97–107.
13. Raimbault, M. & Alazard, D., Culture method to study fungal growth in solid fermentation. *Eur. J. Appl. Microbiol. Biotechnol.*, **9** (1980) 199–209.
14. Smith, M.I., Quantitative colorimetric reaction for the ergot alkaloids and its application in the chemical standardization of ergot preparations. *U.S. Public Health Rep.*, **45** (1930) 1466–81.
15. Van Urk, H.W., A new sensitive reaction for the ergot alkaloids, ergotamine, ergotoxine and ergotidine, and its adaptation to the examination and colorimetric determination of ergot preparations. *Pharm. Weekblad.*, **66** (1929) 473–81.
16. Lonsane, B.K., Ghildyal, N.P., Budiatman, S. & Ramakrishna, S.V., Engineering aspects of solid state fermentation. *Enzyme Microb. Technol.*, **7** (1985) 258–65.
17. Mitchell, D.A. & Lonsane, B.K., In *Solid Substrate Cultivation*, ed H.W. Doelle, D.A. Mitchell & C.E. Rolz, Elsevier Science Publishers, London, in press.