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Potential of solid state fermentation for production of ergot alkaloids

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Production of total ergot alkaloids by *Claviceps fusiformis* in solid state fermentation was 3·9 times higher compared to that in submerged fermentation. Production was equal in the case of *Claviceps purpurea* but the spectra of alkaloids were advantageous with the use of solid state fermentation. The data establish potential of solid state fermentation which was not explored earlier for production of ergot alkaloids.

A significant increase in demand of ergot alkaloids have been witnessed in recent years (Esser & Düvell 1984). Consequently, their saprophytic production by submerged fermentation (SmF) has gained critical importance as conventional parasitic production has many limitations (Rehácek 1984). Considerable R and D efforts have been put up in recent years to improve the productivity of the fermentation process. These include: (a) thorough optimization of technology; (b) genetic improvements of strains; (c) semicontinuous fermentation with immobilized cells; (d) synthesis with crude enzymes in cell-free systems; and (e) use of protoplasts of the cultures (Rehácek & Sajdl 1990; Esser & Düvell 1984). Beneficial effects of simulation of a parasitically living cell in the fermentation system have been recognized and partially achieved by using immobilized mycelial cells or liquid surface fermentation, in addition to speculation of the potential of mixed culture fermentation, solid state fermentation (SSF) and stationary fermentation in plastic cushions (Rehácek & Sajdl 1990; Kobel & Sanglier 1986). Present communication reports the potential of SSF technique, which is known to simulate natural growth of micro-organisms (Lonsane *et al.* 1985) but was never evaluated earlier for production of ergot alkloids.

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Materials and Methods

Claviceps purpurea 1029c was obtained from the Institute of Biochemistry and Molecular Biology, Technical University of Berlin, Germany, while *C. microcephala* MT5 was isolated from infected *Mollinia coerulea* (kindly supplied by Professor Lacoste, Laboratoire de Cryptogamie, Muséum d'Histoire Naturelle, Paris). Three other *Claviceps* were procured from ATCC, MD, USA. The cultures of PDA slants were maintained and subcultured every alternate month. Spore inoculum was prepared on agar medium as per the methodology of Sanglier (1977) in the case of *C. purpurea* 1029c while liquid medium in a shake flask (Kobel & Sanglier 1986) was used for all other cultures. In both the cases, the inoculum was grown at 26°C for 5 d. The inoculum size for *C. purpurea* 1029c was $0{\cdot}6 \times 10^7$ spores/ml liquid medium present in the system while it was 20% in the case of inoculum grown in liquid medium.

FERMENTATION TECHNIQUES

The SmF medium contained (g/l): sucrose, 210; ammonium oxalate, 9·6; urea, 1·73; KH_2PO_4, 0·625; $MgSO_4 . 7H_2O$, 0·625; KCl, 0·3; $FeSO_4 . 7H_2O$, 0·025; $ZnSO_4 . 7H_2O$, 0·01; and NH_4OH to raise the pH to 5·2. The solutions of sucrose and other minerals were sterilized separately at 121°C for 20 min and mixed before inoculation. The flasks (50 ml medium in 250 ml Erlenmeyer flasks) were agitated on a shaker (220 rev/min) at 26°C for 10 d. For the SSF process, the above liquid medium (70 ml) was inoculated and mixed with 30 g sugar cane pith bagasse, obtained as per the processing methodology described by Saucedo-Castañeda *et al.* (1992). Sixty g of medium was charged in a static column fermenter and aerated at a rate of 4 l/h/column. The other cultural conditions were as described above while the fermenter operations were as reported by Raimbault & Alazard (1980).

DOWNSTREAM PROCESSING AND ANALYTICAL ASPECTS

The medium in the case of the SmF process was centrifuged to separate mycelial cells and the clear broth was subjected to spectrophotometric estimation of total alkaloids using van'Urk reagent (Banks *et al.* 1974) against ergonovine base as standard. The mycelial cells were washed and homogenized for extracting the alkaloids in 50 ml solvent (1 : 1 mixture of acetone and 4% tartaric acid) with 5 h contact time. The solids were removed by centrifugation and the extract was concentrated under vacuum at 30°C before solubilizing the alkaloids in 3 ml of 4% tartaric acid for estimation of total alkaloids as described above. The methodology for extraction of total alkaloids from fermented moist solids obtained in SSF process was similar to that from mycelial cells except for the use of 25 g moist solids instead of mycelial cells.

For determination of alkaloid spectra using a high pressure liquid chromatogram (PU 4100, Philips, London), pH of the extract was raised to 9·5 with 10% NH_4OH solution. The alkaloids were solubilized in chloroform and concentrated under vacuum for extraction with methanol. The adsorbent used was Nucleosyl RP_{18} for 5 μm particle size (Macherey-Nagel, Germany) in a 125 mm × 4 mm packed column while a mixture 0·02% solution of ammonium carbonate and acetonitrile (60 : 40) was used for elution. The integrations were performed with a PU 6031 electronic integrator (Philips, London) Sucrose concentration in the media was estimated as per the method of Dubois *et al.* (1956) while the mycelial cells were dried for determining biomass in the SmF process. The biomass estimation in the SSF process was by the glucosamine method (Elson & Morgan 1933).

Results and Discussion

A total of five cultures belonging to four species of *Claviceps* were studied. *Claviceps purpurea* ATCC 20102, *C. paspali* ATCC 13892 and *C. microcephala* MT5 produced traces of alkaloids in the SmF process and were not studied further. *Claviceps purpurea* 1029c and *C. fusiformis* ATCC 26019 were good alkaloid producers. Among these, *C. fusiformis* ATCC 26019 produced 3·9 times higher total alkaloids in SSF as compared to the SmF process (Table 1). The ability of SSF to give many times higher product concentration has been documented for a number of microbial metabolites (Lonsane *et al.* 1985) and the present data add one more metabolite to the list. The total alkaloid production was, however, nearly equal in both the fermentation techniques in the case of *C. purpurea* 1029c. But the results indicate significant changes in spectra of alkaloids with the use of the SSF system as compared to the SmF process. For example, ergonovine content of the total alkaloids was increased by about 1·7 and 8·0 times in case of *C. purpurea* and *C. fusiformis*, respectively in the SSF system. Similarly, the increase in ergotamine was 3·3 and 2·9 times for these cultures in the SSF process. In contrast, lysergol content was reduced by 34·3 times in *C. purpurea* as against the minor change in the case of *C. fusiformis*. Lysergic acid derivatives were higher by 1·7 times in *C. purpurea* but were lower by 1·9 times in *C. fusiformis* with the use of the SSF technique. In terms of market cost, lysergol is much cheaper than ergonovine and lysergic acid derivatives while ergonovine is the most costly among all the alkaloids assayed in the present studies, thereby indicating the economic importance of these advantageous changes in the spectra of alkaloids with the use of the SSF system. Sucrose consumption and productivities were found to be higher in the

Table 1. Comparative production of ergot alkaloids by two different cultures in submerged and solid state fermentations

Attribute	Unit	Submerged culture		Solid state culture	
		A	B	A	B
Sucrose consumption	g/l	101·0	64·9	116·7	70·5
$Y_{s/x}$	g/g dry biomass	3·5	1·8	6·2	1·3
$Y_{p/s}$	mg/g sucrose	1·7	2·4	1·5	8·8
Productivity	mg/g dry biomas	6·1	4·4	9·1	11·2
Total alkaloid production	mg/l	175·1	160·1	171·5	623·2
Ergonovine	% of total alkaloids	21·3	5·5	35·5	44·1
Lysergol	% of total alkaloids	51·5	2·5	1·5	3·1
Ergotamine	% of total alkaloids	10·6	2·6	35·5	7·5
Lysergic acid derivatives	% of total alkaloids	16·6	88·1	27·5	45·8

A, *Claviceps purpurea* 1029c; B, *Claviceps fusiformis* ATCC 26019.
Initial sucrose, 200 g/l in SmF; 0·56 g of sucrose/g of dry matter in SSF.

SSF system for both the cultures (Table 1). The SSF system promoted higher $Y_{s/x}$ and $Y_{p/s}$ for *C. purpurea* and *C. fusiformis*, respectively. In contrast, the $Y_{p/s}$ and $Y_{s/x}$ values were marginally lower for these cultures, respectively, in the SSF system.

A critical evaluation of the limitations of the SmF process for ergot alkaloids production and the well recognized advantages of the SSF technique indicate that the latter is more suited for alkaloid production. For example, a stable foam is formed in the SmF process for alkaloids production due to the presence of surface-active components and cereal proteins in the medium as well as release of foam-promoting substances from natural autolysis of fungi during a long fermentation period (Rehácek 1984). Moreover, the use of antifoams was shown to result in considerable loss in alkaloid yield (Rehácek 1984) due to sensitivity of *Claviceps* to antifoam agents (Esser & Düvell 1984). These problems are completely eliminated in the SSF process due to absence of foam formation (Lonsane *et al.* 1985). Sensitivity of *Claviceps* cultures to mechanical stress and the essential requirement of high oxygen tension in the medium collectively demand a well-balanced system of aeration–agitation in the SmF process (Rehácek 1984). The use of a static SSF process and the usual practise of aeration of the fermenting solids at higher rate for metabolic heat removal by evaporative cooling (Saucedo-Castañeda *et al.* 1992) can overcome these problems efficiently. The end-product inhibition of the enzymes involved in biosynthesis of ergot alkaloids and inhibition of these enzymes under conditions of rapid growth are well documented (Robbers 1984; Rehácek 1984). The ability of the SSF system to significantly overcome end-product inhibition (Ramesh & Lonsane 1991) and the characteristic slower microbial growth in SSF processes (Lonsane *et al.* 1985) are noteworthy in these respects.

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