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SOLID SUBSTRATE MEDIATED CHANGES IN ERGOT ALKALOID SPECTRA IN SOLID STATE FERMENTATION SYSTEM.

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Summary: Use of different solid substrates resulted in minor alterations in total alkaloid production by *Claviceps purpurea* 1029c in solid state fermentation system but the changes in the spectra of ergot alkaloids were of significantly higher magnitudes. Ergonovine accounted for 93% of the total alkaloid production in wheat grain medium while lysergic acid derivatives and ergonovine comprised of 66% and 32% of total alkaloids in rye grain medium. In contrast, ergonovine, ergotamine, and lysergic acid derivatives were 35, 35, and 25% respectively of the total alkaloids with the use of sugar cane pith bagasse impregnated with sucrose. No information on these aspects was available earlier.

Durch das Substrat verursachte Änderungen im Spektrum der in einem Festphasenfermentationssystem produzierten Mutterkornalkaloide.

Zusammenfassung: Der Einsatz verschiedener fester Substrate in einem Festphasenfermentationssystem für *Claviceps purpurea* 1029c verursachte lediglich geringe Änderungen in der Gesamtausbeute an produzierten Alkaloiden. Die Veränderungen im Verteilungsspektrum der einzelnen Mutterkornalkaloide waren im Gegensatz dazu signifikant. In einem Weizenkornmedium machte Ergonovin 93% des gesamten Alkaloidertrages aus, in einem Roggenkornmedium dagegen erhält man 66% Lysergінsäurederivate und nur 32% Ergonovin. In einem mit Saccharose getränkten Mark von ausgepreßtem Zuckerrohr machten Ergonovin und Ergotamin je 35% sowie Lysergінsäurederivate 25% der gesamten Alkaloidproduktion aus. Über diese Aspekte im Zusammenhang mit der Produktion von Mutterkornalkaloiden gibt es bisher keine Literaturhinweise.

INTRODUCTION

Relative concentration and type of ergot alkaloid complex, formed during saprophytic production through fermentation route, are controlled by the genetically fixed characteristics of the microbial culture [1]. These can be influenced within certain limits by cultural conditions or externally applied specific chemicals such as amino acids [1,2]. Among these avenues, the use of amino acids is highly cost-intensive, while the genetic improvements dictate concentrated and long term efforts. Due to the vital importance of the fermentation process in meeting ever-increasing demand of ergot alkaloids

[3], considerable efforts have been successfully put up to genetically improve the cultures by using conventional and novel approaches [4-6]. Advantageous changes in spectra of ergot alkaloids produced by *Claviceps purpurea* have been observed recently with the substitution of submerged fermentation (SmF) technique by solid state fermentation (SSF) system [7]. During further work on process standardization, solid substrate mediated changes in spectra of ergot alkaloids became apparent. Hence, detailed studies were undertaken and the data are reported in present communication. No information on these aspects was available earlier.

MATERIALS AND METHODS

Microorganisms

Claviceps purpurea 1029c was obtained from Institute of Biochemistry and Molecular Biology, Technical University of Berlin, Germany and maintained on PDA slants by subculturing every alternate month, in addition to the preservation by lyophilization. The spore inoculum of the culture was prepared as per methodology of Sangler [8] at 26 °C for 5 days. The inoculum size used was $0.6 \cdot 10^7$ spores·ml^{-1} liquid medium. All the experiments were conducted in triplicate.

Solid state fermentation technique

Wheat or rye grains were cut into 3 pieces and moistened with distilled water to 50% moisture level for autoclaving at 121 °C for 15 min. The lumps formed were broken to separate the grain particles for drying at 60 °C for about 24 h. The grains thus processed can be stored upto 6 months without any contamination. The methodology for processing of sugar cane pith bagasse involved washing, sieving, autoclaving, and drying as per the details reported elsewhere [9].

Liquid nutrient medium which was used for moistening the processed grains or impregnating processed sugar cane pith bagasse contained (g·l^{-1}): ammonium oxalate 9.6, urea 1.73, KH_2PO_4 0.625, $MgSO_4 \cdot 7\,H_2O$ 0.025, $ZnSO_4 \cdot 7\,H_2O$ 0.01, and NH_4OH to raise the pH to 5.2.

The medium was sterilized at 121 °C for 20 min and inoculated with spores. Wheat or rye grains (40 g) were mixed with 60 ml of the inoculated liquid medium to get 60% moisture in the moist solid medium. In case of sugar cane pith bagasse, separately sterilized (121 °C for 15 min) solution of sucrose was added to inoculated liquid medium to achieve 30% sugar concentration and then 70 ml of the inoculated medium was used to impregnate 30 g dry processed sugar cane pith bagasse. In all these cases, 60 g moist medium was charged in static column fermenter of size 20 cm length x 4 cm diameter. The columns were aerated at a rate of 4 l humidified air·h^{-1} per column and the fermentation was carried out at 26 °C for 10 days. The details of fermenter operations were as per Raimbault and Alazard [10].

Extraction of alkaloids

The fermented solids in 25 g moist weight quantity were homogenized for extracting the alkaloids in 50 ml solvent (1:1 mixture of acetone and 4% tartaric acid solution) at room temperature and 5 h contact time with intermittent shaking. The spent solids were removed by centrifugation (5,000 rpm) and the extract was concentrated under vacuum at 30 °C before solubilizing the alkaloids in 3 ml of 4% tartaric acid solution.

Analytical aspects

Total alkaloids present in dissolved form in 4% tartaric acid solution were estimated by spectrophotometric method using van Urk reagent [11] against the standard solution of ergonovine base. The alkaloid spectra were determined by high pressure liquid chromatograph (PU 4100, Philips, London) using Nucleosil RP_{18} of 5 μm particle size (Macherey-Nagel, Germany) as adsorbent in a 125 mm x 4 mm packed column. For this purpose, the pH of the extract was raised to about 9.5 with 10% NH_4OH solution before solubilizing the alkaloids in chloroform. The alkaloids in chloroform were concentrated under vacuum and dissolved in small quantity (1 ml) of methanol. A mixture of acetonitrile and 0.02% solution of ammonium carbonate (40:60) was used for elution. The integrations were performed using PU 6031 electronic integrator (Philips, London).

Sucrose concentration in the media was estimated as per the method of Dubois *et al.* [12] while starch was estimated by hydrolizing it and assaying the glucose formed by the method of Miller [13]. The biomass estimation was by glucosamine method [14].

RESULTS AND DISCUSSION

Growth and total alkaloid production

Data on the growth of *Claviceps purpurea*

Table 1: Effect of different solid substrates on production of ergot alkaloids by *Claviceps purpurea* 1029c in solid state fermentation system.

Attribute	Solid substrate		
	Wheat grain	Rye grain	Sugar cane pith bagasse + sucrose
Specific growth rate μ (h^{-1})	0.21	0.16	0.11
Substrate utilization (%)	46.38	52.85	51.12
Biomass formation (g per g substrate consumed)	0.22	0.25	0.21
Total alkaloid production (mg·100 g^{-1} IDM)	35.21	37.73	40.87
Alkaloid productivity (mg·g^{-1} dry biomass)	3.52	2.85	3.81
Ergotamine (% of total alkaloids)	0.47	0.08	35.53
Ergonovine (% of total alkaloids)	93.91	32.21	35.51
Lysergol (% of total alkaloids)	5.18	1.14	1.51
Lysergic acid derivatives (% of total alkaloids)	0.44	66.57	27.45

1029c in SSF system involving the individual use of the solid substrates indicated that the biomass formation at the end of 10 days fermentation was nearly same in all the cases (Table 1). However, the specific growth rates were quite different. The %-substrate utilization was in the range of 46.38-52.85, thereby indicating that the substrate was not a limiting factor in influencing the specific growth rates. The total alkaloid production per 100 g IDM or g dry biomass formed was also nearly same and ranged between 35.21-40.87 mg·100 g^{-1} IDM in case of all the three substrates (Table 1).

Alkaloid spectra

In contrast to the nearly equal production of total alkaloids by *Claviceps purpurea* 1029c on three different solid substrates in SSF system, the spectra of alkaloids produced on these substrates differed significantly (Table 1). For example, ergonovine was the major alkaloid formed and accounted for 93.91% of the total alkaloids in the wheat grain medium. The concentrations of ergotamine and lysergic acid derivatives, in this case, were >0.5% while that of lysergol was 5.18% of the total alkaloids formed. In contrast, lysergic acid derivatives and ergonovine together accounted for 98.78% of total alkaloids formed in rye grain medium, their ratio being 1:0.48 (Table 1). Lysergol accounted for 1.14% of total alkaloids while ergotamine was present in traces in the rye grain medium. A totally different pattern of the alkaloid spectra was observed with the use of sugar cane pith bagasse impregnated with sucrose. Ergotamine, ergonovine, and lysergic acid derivatives together accounted for 98.49% of the total alkaloids, their ratio being approximately 1:1:0.77 (Table 1). In this case, lysergol formation was only 1.51% of total alkaloids.

The spectra of ergot alkaloids are known to be influenced by amino acids present in the medium [3, 15]. It is probable that the changes in spectra observed in the present studies are due to differences in the composition of solid substrates studied. The amino acids are highly cost-intensive and their use in medium to influence the spectra of ergot alkaloids thus will lead to increased cost of production. The use of SSF system offers cheaper alternative in this context. However, the composition of the solid substrates used in the present study is known to vary widely in different varieties of same cereals/grains. A simple solution for this problem is to evaluate different races/varieties of same cereals/grains and to select appropriate type for use in fermentation.

It is emphasized that ergonovine and other lysergic acid derivatives are commercially important due to their use in pharmaceutical industries [15]. Moreover, lysergic acid serves as a starting material for the chemical synthesis of some of the medicinally used ergolines such as ergonovine and its derivatives [15]. On the other hand, clavine alkaloids such as lysergol are less important from commercial point of view as compared to lysergic acid types [15]. But it is desirable to use strains which allow minimum production of secondary alkaloids from the view points of economy and ease in downstream processing [1]. In the light of these above facts, the solid substrate mediated changes in the alkaloid spectra produced by *Claviceps purpurea* 1029c are not only interesting from academic angles but also from commercial point of view.

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