

Biosynthesis of ergot alkaloids exposed to 2,4-dinitro phenyl hydrazine hydrochloride

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Abstract : Biosynthesis of ergot alkaloids by *Claviceps purpurea* H-18 was carried out using 20% sucrose solution at 26°C and pH 5.4 in 9 days of optimum incubation period along with incorporation of 2,4-dinitro phenyl hydrazine hydrochloride. The results show that addition of 2,4-dinitro phenyl hydrazine hydrochloride to the production medium slightly stimulates the fermentation process and thereby slightly enhances the yield of ergot alkaloids in comparison to control fermentor flask.

(Keywords : Ergot alkaloids fermentation, 2,4-dinitro phenyl hydrazine hydrochloride and *Claviceps purpurea* H-18).

Introduction

Ergot or ergot fungi refers to a group of fungi of the genus *Claviceps*. The most prominent member of this group is *Claviceps purpurea*. This fungus grows on rye and related plants, and produces alkaloids that can cause ergotism in humans and other mammals who consume grains contaminated with its fruiting structure.

The efficacy of some substances on the growth and activity of microbes are quite miraculous. During the recent past commendable development towards ergot alkaloids fermentation exposed to some active organic molecules has been studied by many workers¹⁻¹¹. In view of the importance and good physiological activities of the organic molecules, the authors have undertaken to access and analyse the production of ergot alkaloids by *Claviceps purpurea* H-18 exposed to 2,4-dinitro phenyl hydrazine hydrochloride

Experimental

The medium used for production of ergot alkaloids was composed of the following: Sucrose: 200.00g; citric acid: 20.00g; Yeast Extract: 0.15g; KH_2PO_4 : 0.55g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.55g; KCl: 0.15g; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 0.010g ; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$: 0.008g; pH : 5.4 Adjusted by NH_4OH Distilled water to make up 1000 ml.

Now from the above composition 100 ml of the production medium was taken into 250 ml conical fermenter flask. The flasks were plugged with non absorbent cotton wool plugs and sterilized in an autoclave maintained at 15 lbs steam pressure for 30 minutes. For production of ergot alkaloids by submerged fermentation fungal strain of *Claviceps purpurea* H-18 has been employed.

Strain : *Claviceps purpurea* H-8 has been used in the present study as a source of enzymes for ergot alkaloids fermentation.

Assay method : Evaluation of total ergot alkaloids formed was made colorimetrically described by Pacific *et al.*¹² using Van Urk's reagent¹³ modified by Smith¹⁴ based on the formation of a blue colour with the addition of Van Urk's reagents.

Concentration of the, 2,4-dinitro phenyl hydrazine hydrochloride used :

M/10 solution of 2,4-dinitro phenyl hydrazine hydrochloride has been prepared and 1.0 ml to 10.00 ml of this solution were incorporated

Table - 1
Biosynthesis of ergot alkaloids exposed to 2,4-dinitro phenyl hydrazine hydrochloride

Concentration of mutagen used	*Yield of ergot alkaloids in mg/100ml			% Difference in the yield of ergot alkaloids after 9 days	
	8 days	9 days	10 days		
Control	595	888	801	—	
1.0 x 10 ⁻³ M**	581	880***	790	(-)	0.90090
2.0 x 10 ⁻³ M	571	879	772	(-)	1.01351
3.0 x 10 ⁻³ M	560	865	663	(-)	2.59009
4.0 x 10 ⁻³ M	529	818	632	(-)	7.88288
5.0 x 10 ⁻³ M	511	799	715	(-)	10.02252
6.0 x 10 ⁻³ M	500	750	643	(-)	15.54054
7.0 x 10 ⁻³ M	495	710	620	(-)	20.04504
8.0 x 10 ⁻³ M	469	698	608	(-)	21.39639
9.0 x 10 ⁻³ M	440	615	590	(-)	30.74324
10.0 x 10 ⁻³ M	436	606	588	(-)	31.75675

* Mean of three observations, ** Optimum concentration of dimethyl alloxan

*** Optimum yield of ergot alkaloids, (+)ve values indicate % increase in the yield of ergot alkaloids
 Experimental deviation (±) 1.5% to 3.5%

to the production medium (1st to 10th set respectively). The control flasks contained no 2,4-dinitro phenyl hydrazine hydrochloride.

Results and Discussion

The data recorded in the table-1 shows that 2,4-dinitrophenyl hydrazine hydrochloride has very much detrimental action on the fungus *Claviceps purpurea H-18* and fermentative production of ergot-alkaloids. The maximum yield of ergot-alkaloids, i.e. 880 mg/litre has been observed at initial concentration 1.0 x 10⁻³ M which was less in comparison to that of control flask, i.e. 888 mg/litre in 9 days of incubation period. The detrimental action of this mutagen may also be considered like *p*-nitrophenyl hydrazine⁴⁸. As 2, 4-dinitrophenyl hydrazine hydrochloride bears one more nitro group it is comparatively more toxic and hence retards the fermentative production of ergot alkaloids. It has been observed and established that the compounds having nitro group(s) in their structure has been found

poisonous^{86,92,93,42} to the microbes and microbial process in many fermentation processes.

Recently Singh et al⁹⁴⁻⁹⁵ reported N-sulphonyl urethane as an effective mutagen for *L. casei* NCIM-2125. Faizi et al⁹⁶ has also studied some chemical mutagens on fermentation processes and has reported only benzyl carbamate has significant influence while 2, 4-dinitrophenyl hydrazine, acetone phenyl hydrazone and acetaldehyde phenyl hydrazone insignificant for fermentative process, especially for citric acid fermentation. On the other hand Singh et al.⁹⁷ studied efficacy of p-toluenesulphonyl azide and p-toluene sulphonyl hydrazine on microbial synthesis of bioalcohol and found that p-toluene sulphonyl azide retards the yield of bioalcohol while p-toluene sulphonyl hydrazine enhances the yield significantly. Narayan and Sharma⁹⁸ found nitrosoguanidine and ethylene imine mutagens useful and significant for higher yields of alcohol. Singh⁹⁹ in her investigation has reported benzoyl hydrazine and salicyloyl hydrazine as a specific mutagen for fermentative production of lactic acid.

References

1. S.P. Singh, B. Kumar and R. Kant, *Nat. Acad. Sci. Letters* **15**, 367 (1992).
2. S. P. Singh, Md. Shamim, K.P. Kamal and K.B. Lal, *Indian J. agric. Chem.* **30**, 73 (1997).
3. S.P. Singh, Md. Shamim, R.K. Singh, A. K. Singh, M. K. Nirala and K. P. Kamal *Asian J. Chem.* **8**, 165(1996).
4. F. R. Faizi *J. Chemtracks* **5**, 73 (2003)
5. F.R. Faizi K. Ahmad, A. Suraiya and S. P. Singh, *J. Chemtracks* **8**, 69 (2006).
6. H. Kumar, S. M. Abdullah and H. N. Singh *J. Chemtracks* **9**, 173 (2007).
7. Reeta Rani *Ph.D. Thesis Chemistry M.U. Bodh Gaya* pp. 140-168 (1991).
8. Lalan Kumar *Ph. D. Thesis Chemistry M.U. Bodh-Gaya* pp. 175-199 (1991).
9. K.P. Tiwari, and A. Pandey, *Zbl. Bakt. II Abt.* **136**, 70 (1981).
10. R. Singh, *Ph. D. Thesis Chemistry M.U. Bodh-Gaya* pp. 163-164 (1990).
11. S.P. Singh, A.P. Sinha, M. Prasad, L. Kumar and S. K. Yadav, *Biojournal* **4**, 241 (1992).
12. L. P. Pacifici, and Schwarting, A. E. Kelleher, W. J. : *loydia*, **25**, 37 (1962).
13. H. W. VanUrsk, *pharm. weekbl.* **66**, 473 (1929).
14. M.I. Smith, : U. S. Public Health Rep. **45**, 1466 (1930).
15. R. Singh : Ph. D. Thesis Chemistry Magadh University, Bodh-Gaya, pp. 112-148 (1990)
16. K. P. Tiwari and S. P. Singh : *Nati. Acad. Sci. Letters*, **1**, 146-148 (1978).
17. N. Rathor, : Ph. D. Thesis Chemistry, Magadh University, Bodh-Gaya, pp. 136-137 (1988).
18. N. Zahid, and R. M. Baxter : Paper presented at A. Ph. A. Acad. of Pharma Sci. Miami, May, (1968).
19. A. M. Conney, : *Pharmacol. Rev.* **19**, 317 (1967).
20. S. P. Singh, V. Kumar and S. S. Chandra, *Indian J. agric. Chem.* **34**, 80, (2001)
21. S.P. Singh, A. Singh, V. Kumar and B. Singh. *Vijnana Parishad Anushandhan Patrika* **48**, 215 (2005)
22. F. R. Faizi, M. A. Khan, Md. Irfan, P. K. Chaurasia and S. P. Singh *J. Chemtracks*, **5**, 37, (2003).
23. S.P. Singh, C. K. Singh, D. Narayan, and R. Kumar : *J. Chemtracks* **5**, 51 (2003)
24. D. Narayan and R. N. Sharma : *J. Chemtracks* **6**, 41 (2004).

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