ABSTRACT

Production of ergot alkaloids was achieved in culture liquid (extracellular) and mycelial (intracellular) filtrate extracts of *Penicillium commune* and *Penicillium* sp. IIB in M5 fermentation medium using surface culture fermentation technique. Various species of genus *Penicillium* were screened for their ability to produce ergot alkaloids from their culture liquid (extracellular) and mycelial (intracellular) filtrate extracts of five different fermentation media. Among all the species tested, *Penicillium commune* (CLFE=1.359±0.002 mg/ml; MFE=0.958±0.001 mg/ml) and *Penicillium* sp. IIB (CLFE=1.154±0.002 mg/ml; MFE=0.635±0.001 mg/ml) produced maximum ergot alkaloids after 21 days of incubation at initial pH 5.2 and at 25°C in M5 fermentation medium. Optimization of culture conditions such as effect of different substrates (carbon and nitrogen sources), culture medium ingredients (tryptophan, asparagine, succinic acid, KH_2PO_4, NH_4Cl, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$ and $ZnSO_4$), and various process parameters (pH, incubation temperature, incubation time and size of inoculum) were optimized using one factor at a time technique (OFAT). Maximum yield of ergot alkaloids in extracellular filtrate extracts of *Penicillium* commune (4.38 mg/ml) and *Penicillium* sp. IIB (5.51 mg/ml) was achieved at optimum levels of sucrose (35%), yeast extract (30%), KH_2PO_4 (2%), tryptophan (2%), asparagines (2% to 2.5%), succinic acid (2%), NH_4Cl (1.5% to 2%), $MgSO_4.7H_2O$ (1.5%) , $FeSO_4.7H_2O$ (1.0%), $ZnSO_4$ (1%, 1.5%) at pH 5.0 and 25°C after 21 days of incubation. Statistical designs of response surface methodology (RSM) such as Plackett-Burman Design (PBD) and Box-Behnken Design (BBD) were used for screening and optimization of different factors for ergot alkaloids production for the enhanced production of ergot alkaloids by *Penicillium commune* and *Penicillium* sp. IIB. Among various factors, sucrose, yeast extract and $FeSO_4.7H_2O$ were selected due to their significant positive effects on ergot alkaloids yield. Maximum response for ergot alkaloids production was observed from experimental run 6 and 13 of *Penicillium commune* and *Penicillium* sp. IIB with a maximum yield of 14.64 mg/ml

and 35.60 mg/ml respectively, using Box-Behnken Design (BBD) as compared to their predicted values. The high correlation between the predicted and observed values indicated the validity of RSM.

Strains of *Penicillium commune* and *Penicillium* sp. IIB were subjected to various mutagens to improve the yield of ergot alkaloids. Wild strains were treated with UV irradiations and EMS (ethyl methane sulfonate) for different time intervals (1-150 min). It was found that mutant strains such as PCUV-4 and PCEMS-3 showed maximum ergot alkaloids yield as compared to wild strain of *Penicillium commune* in pre-optimized culture conditions. During the scale up process, it was found that PCUV-4 mutant strain of *Penicillium commune* showed 12.32 mg/ml yield which was 3-fold higher than the wild strain. This strain was designated as the best positive mutant for the enhanced production of ergot alkaloids. The further analysis of ergot alkaloids from filtrate extracts was performed through analytical techniques such as TLC and HPLC. It was found that large *Rf* values for the extracellular and intracellular ergot alkaloids of *Penicillium commune* and *Penicillium* sp. IIB were obtained in mobile phase A1 and H2. PCCLFE9, PCMFE9 of *Penicillium commune* and PCLFE9, PMFE9 of *Penicillium* sp. IIB exhibited maximum colored spots and large *Rf* values revealing pinkish purple and blue colors indicating the presence of ergotamine, agroclavine and ergocriptine alkaloids. The chromatographic separation of ergot alkaloids present in filtrates was achieved on HPLC using chloroform:isopropanol (80:20 v/v) as mobile phase. Maximum retention time (3.83 min) was recorded from the mycelial (intracellular) filtrate (PCMFE12 sample) of *Penicillium commune* which indicated the presence of ergocriptine. Highest concentration of ergot alkaloids (ergotamine) i.e. 20.12 mg/ml was investigated from the culture liquid (extracellular) filtrate of sample PCLFE9 of *Penicillium* sp. IIB. The ergot alkaloids yield from wild and mutant strain of *Penicillium commune* and *Penicillium* sp. IIB appeared as the potential source for up-scaling the process at required levels for the commercial production of ergot alkaloids for pharmaceutical purposes. Protocol improvement in this regard is strongly

recommended for uplifting the ergot alkaloids production to fulfill the indigenous demands.