Abstract

The main objective of this study was the optimization of medium for the maximum production of avermectin B1b from *Streptomyces avermitilis* DSM 41445. Different media were used for the production of avermectin B1b. However the maximum production of avermectin B1b (17 mg/l) was obtained by using SM2 growth medium containing soluble corn starch, yeast extract, KCl, $CaCO_3$ and $MgSO_4$ which was detected qualitatively by using TLC and quantitatively by HPLC. Maximum production was observed with initial medium pH of 7, 10% inoculum size with incubation temperature of 31°C for 10 days of fermentation period. In the next step the production of avermectin B1b from strain of *Streptomyces avermitilis* 41445 was enhanced by mutagenesis using Ultraviolet irradiation, ethidium bromide (EB) and ethyl methanesulfonate as mutagens. Selection of avermectin B1b hyper producing mutant produced from these three different methods was made on the basis of HPLC results. Mutants obtained after 45 minutes irradiation of ultraviolet rays on the spores of *Streptomyces avermitilis* 41445 was found to be the best mutant for the enhanced production of avermectin B1b component (254.14 mg/L). Other avermectin B1b hyper producing mutants obtained from EMS (1 µL/mL) and EB (30 µL/mL) treatment gave 202.63 mg/L and 199.30 mg/L of B1b respectively. The hereditary stability analysis of UV 45(m) 3 mutant showed that the production of avermectin B1b remained constant and there were no reverse mutation occurred after 15 generations. *Streptomyces avermitilis* belonging to *Actinomycetes* are specialized for the production of avermectin being used as anthelmintic and insecticidal agent. They are mostly present in the soil and their isolation from the soil is very crucial so s to obtain the medically important avermectin. In the present study 10 bacterial isolates lacking antimicrobial activities were isolated from soil samples collected from different areas of Lahore. Three distinctive localities of Lahore were opted for the assortment of soil to isolate *Streptomyces avermitilis*. About fifty isolates of *Streptomyces* species were attained through selective prescreening procedures. All of these isolates were studied for the production of secondary metabolite, the avermectin. Different test like soluble pigment colour and melanin formation were used for identification. Biochemical characterization of isolates closely resembling the control was done. The 10 selected isolates were identified as avermectin producing strain by fermentation and were characterized on ISP2 medium for aerial and reverse side mycelia colour, soluble pigment colour and melanin formation in comparison with

Streptomyces avermitilis DSM 41445. The best avermectin (10.15mg/L) producing isolate S1-C was when subjected for culture characteristics analysis in different media along with biochemical characterization showed similar result as were obtained for *S.avermitilis* DSM 41445. From the [redacted]s it was concluded that agricultural lands around PCSIR Campus Lahore were the rich source of industrially important *Streptomyces* especially the *S.avermitilis*. Avermectin is an environment friendly bio-insecticide. Optimization of the culture conditions for avermectin B1b production was not carried out before using Artificial Neural Network (ANN) method. The present work is therefore conducted to optimize some important factors including yeast extract, $MgSO_4.7H_2O$ and temperature for the avermectin B1b production using ANN methodology from *Streptomyces avermitilis* DSM 41445. The optimum levels for the yeast extract, $MgSO_4.7H_2O$ and temperature were 16.0 (g/L), 5.0 (g/L) and 32 °C respectively. Maximum effect was observed by yeast extract. Avermectin B1b yield was increased up to 150% after optimization. ANN was found a powerful technique for the optimization and prediction of avermectin B1b production from *Streptomyces avermitilis* DSM 41445. Present study was conducted to optimize avermectin B1b production from *S.avermitilis* 41445 UV45(m)3 using artificial neural network and Response surface methodology. Three variables NaCl, KCl and pH were used for optimization. Coefficient of determination and adjusted coefficient of determination have very poor values for RSM. Values predicted by RSM for experiments were also much different from the observed avermectin production. Comparatively predicted avermectin levels by ANN were very close to observed values with much higher R^2 and adjusted R^2. Optimum levels of NaCl, KCl and pH predicted by ANN were 1.0g/L, 0.5g/L and 7.46 respectively. Sensitivity analysis predicted highest effect being shown by pH followed by NaCl and KCl. About 37.89 folds increase in avermectin B1b production was observed at optimum levels of three variables envisage by ANN. Optimum levels, ranking order of variables and the predicted avermectin on the optimum levels by the RSM was much different from ANN values. Results revealed that ANN is better optimization tool for given strain than RSM.

Use of avermectin B1b as anthelmintic and insecticidal agent has increased to protect the soil and for enhanced crop production. Enhanced production of avermectin B1b was obtained from mutant strain of *Streptomyces avermitilis* 41445. Modeling of mutant strain *Streptomyces avermitilis* 41445 UV 45(m) 3 growth and avermectin B1b production is therefore required for optimization during fermentation process. Kinetics of intracellular avermectin B1b production

ies in shake flask culture during submerged fermentation. Mathematical models based upon Logistic and Piret Equations have been used to investigate the kinetics of avermectin B1b production and substrate utilization from *Streptomyces avermitilis* 41445 UV 45(m)3. Effect of various carbon sources (glucose, maltose, lactose, potato starch, soluble corn starch, and wheat starch), pH (6.0, 6.5, 7.0, and 7.5), agitation speed (150, 200, and 250 rpm) on microbial growth and product formation were evaluated. Maximum avermectin B1b production (420.02 ±0.01 mg/L) and cell biomass (31.74 ±0.05 g/L) was obtained in media having potato starch as carbon substrate, medium pH of 7.5 with agitation speed of 250 rpm. Maximum specific growth rate (μ_{max}), growth associated avermectin B1b production coefficient (α) and non-growth associated avermectin B1b production coefficient (β) obtained were $0.16h^{-1}$, 0 mg $cell^{-1}$ h^{-1} and 3.5 mg $cell^{-1}$ h^{-1} respectively. From the above results we can conclude that avermectin B1b production was non-growth associated process.