Abstract

L-lysine is one of the essential amino acids which is in great demand therefore, efforts are being focused to enhance the microbial production of L-lysine. Present study was also aimed at enhancing the production of L-lysine through bioprocess and metabolic engineering. Six hundred and ninety strains (IIB1 to IIB690) were isolated from 110 soil samples collected from different areas of Punjab and Azad Kashmir. Only nine bacterial isolates were capable of producing L-lysine during submerged fermentation, with two isolates namely IIB187 and IIB646 showing maximum L-lysine production i.e. 0.25 g/L and 2.31 g/L, respectively. These strains i.e. IIB187 and IIB646 were identified as *Bacillus megaterium* and *Corynebacterium glutamicum*, respectively. Fifteen different fermentation media were screened for L-lysine production by the two selected isolates in shake flasks. Both the *B. megaterium* produced maximum L-lysine in medium FM13. After preliminary optimization, *B. megaterium* (IIB187) produced maximum (0.59 g/L) L-lysine and *C. glutamicum* (IIB646) produced maximum of 6.3 g/L of L-lysine at 30°C after 72 hours of incubation. Physical and chemical mutagens were used to create random mutagenesis for enhancing the yield of L-lysine in the selected strain of *C. glutamicum* (IIB646). From all the treated survivors, ten homoserine auxotroph mutants were obtained, which showed higher L-lysine production as compared to wild strain. Out of these ten mutants, $IIB646^{EMS18}$ produced maximum of 7.3 g/L L-lysine while all other mutants showed less than 6.5 g/L L-lysine. To develop a metabolically engineered strain, overexpression of pyruvate carboxylase gene in L-lysine producing strain of *Coynebacterium glutamicum* (IIB646) was achieved by using recombinant pET 21a (+) vector. Engineered strain of *Corynebacterium glutamicum* (IIB646) produced maximum 12.2 g/L of L-lysine in FM13 medium. Wild and mutant strains produced maximum L-lysine after 96 hrs of incubation period, while engineered strain showed maximum L-lysine production after 72 hrs of incubation period at an optimum incubation temperature of 30°C. Different pH values (6.0 to 8.5) of fermentation medium were studied and maximum growth and L-lysine production of these three strains were observed at pH value of 7.0. L-lysine production was also carried out at different agitation rates ranging from 50 to 250 rpm in shaking incubator and maximum L-lysine production was found at an agitation rate of 200 rpm. Maximum amount of L-lysine was produced by wild,

mutant and engineered strain when 22 hrs old inoculum at a concentration of 8% was used for fermentation. Different carbon sources were screened and maximum L-lysine production was observed in the presence of 8% glucose in the culture medium. Similarly, different nitrogen sources were also screened for the production of L-lysine and all the strains showed higher L-lysine production (7.4g/L by wild, 9.5 g/L by mutant and 15.3 g/L by engineered strain) in the medium supplemented with 2.5% ammonium sulphate. Wild, mutant and engineered strains produced maximum amounts of L-lysine in the presence of 1.5% calcium carbonate in the culture medium. Thiamin HCl and biotin were also found to be responsible for enhancing L-lysine production by *C. glutamicum*. During the optimization of casamino acids, wild strain produced maximum 9.5 g/L L-lysine, mutant 11.6 g/L and engineered strain of *C. glutamicum* (IIB646) produced maximum of 17.5 g/L L-lysine in the presence of 2.5 g/L of casamino acids in the fermentation medium. In fermenter studies, optimum rate of aeration, agitation and medium pH were found to be 1.5 vvm, 250 rpm and pH 7.0, respectively.