



ABSTRACT

Acetoin is a natural compound and widely used in various industries such as food, dairy, cosmetics, paint and pharmaceutical industry. In present study, a new strain coded as SFS-13 was isolated from agricultural soil and identified as *B. subtilis* SFS-13. The acetoin production and glucose consuming efficiency by wild type SFS-13 were 8.62 ± 0.04 g/l and $25.33 \pm 0.02\%$, respectively. After chemical mutagenesis of SFS-13 with ethyl methyl sulphate, 99 mutant survivors were picked through shake flask fermentation selection procedure. The strategy of random mutagenesis improved acetoin producing capability and GCE of wild type strain by 163.34 and 18.08%, respectively. The culture conditions and medium optimization were performed by varying one factor at a time. The maximum acetoin synthesis was attained by performing fermentation at 37°C , pH-6, 160 rpm for 24 h and consuming 10% (w/v) glucose, yeast extract (12 g/l) and peptone (8 g/l). As a result acetoin production, dry cell mass and GCE were increased to 38.03 g/l, 13.24 g/l and 38.03%, respectively, representing an increase of 67.53%, 36.35% and 27.15% in corresponding values. The optimized immobilization conditions were sodium alginate 3% (w/v), 0.3 M CaCl_2 , hardening time 40 min, inoculum 2.64% (w/v) wet weight of *B. subtilis* SFS-13-75 cells, beads size 3 mm and number of beads 250. The maximum acetoin titer, acetoin yield and glucose consuming efficiency by immobilized cells of mutant survivor were 45.67 ± 0.07 g/l, 0.457 ± 0.01 g/g glucose and $45.62 \pm 0.14\%$, respectively. Moreover, to our knowledge, it is the first report on detailed process optimization of immobilization technique using cells of mutant survivor for maximum acetoin production.