

ABSTRACT

The present study is concerned with the isolation and screening of thirty strains of *K. fragilis* for the biosynthesis of intracellular β -galactosidase in shake flask. Among all the isolates tested, *K. fragilis* IIB Y-12 gave maximum enzyme production i.e. 24.87 ± 0.271 $\mu\text{M/ml/min}$. Of all the media examined, maximum enzyme production was obtained with medium M3 consisting of (w/v), lactose (2.0 %), peptone (0.5 %), malt extract (0.3 %), and yeast extract (0.3 %). The parameters such as rate of enzyme synthesis (48 h), initial pH (6.5), volume of fermentation medium (50 ml), inoculum age (24 h) and inoculum size (2 %, v/v) were studied. The stimulatory effect of yeast extract or malt extract was also determined. While lactose and peptone were found to be the best carbon and nitrogen sources for maximum β -galactosidase production (45.24 ± 0.740 $\mu\text{M/ml/min}$) by the yeast.

The enzyme was subjected to partial purification by using different concentrations of ammonium sulphate. The maximum β -galactosidase protein was precipitated out (1.11-fold) at 60 % (w/v) saturation of ammonium sulphate. The highest enzymic activity was observed with 0.80 % (v/v) ONPG at 60°C after 15 min of incubation period. The maximum enzyme stability was sustained at -20 and 4°C temperatures, however, the enzyme inactivation occurred at 50°C.