

Abstract

Rennin like acid proteases produced by nonpathogenic fungi belonging to Mucorales are also known as milk clotting enzymes. This study is basically concerned to isolation and screening of milk clotting enzyme producing fungal isolates from the soil samples of different areas of Punjab Pakistan (Sheikhupura, Thokar Niaz Baig, Faisalabad). Out of all the strains two strains were able to show milk clotting activity i.e., MCS1 and MCS2 having Milk Clotting Activity (MCA) values of 571.43 ± 3.6 and 234.21 ± 2.1 Soxhlet Units per milliliter (SU/ml) respectively. Then crude enzyme was produced using solid state fermentation by using wheat bran as a substrate, mineral solution as moisturizer, spore suspension of Milk Clotting Strain 1 (MCS1) strain and distilled water. Crude enzyme from MCS1 was selected for optimization of different parameters such as substrate, mineral solution, substrate concentration, incubation period, incubation temperature and pH optimization under solid state fermentation. Then enzyme under optimized conditions i.e., 10 g wheat bran as substrate, M-9 as mineral solution, incubation period of 96 hours at 30° C at pH 6.0, was then partially purified and applied to make cheese after optimizing certain factors such as incubation period, incubation temperature and milk source to produce maximum amount of cheese i.e., 4 g/10 ml yield was obtained by using goat milk incubated for 4 minutes at 35°C. According to present study it is possible to propose partially purified enzyme from MCS1 strain as an alternate to rennet, but further optimization, purification and cheese production tests are required.