

ABSTRACT

This is thought that oxidative stress may cause the pathogenesis of chronic fatigue syndrome (CFS). In the current study the oxidative stress was measured in pregnant women with chronic fatigue syndrome and compared with those without CFS. For this purpose five different assays (ABTS: 2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid, FRAP: ferric reducing antioxidant power, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, SOD: superoxide dismutase and metal chelating activity assays) were used. There was a significant ($P<0.05$) lower plasma level of antioxidant capacity in pregnant women with chronic fatigue syndrome (3.12 ± 0.80 mmol/L, 10.57 ± 4.01 mmol/L) compared with those without CFS (4.69 ± 0.73 mmol/L, 21.01 ± 5.39 mmol/L) using ABTS and FRAP assay respectively. The plasma level of radical scavenging capacity was significantly ($P<0.05$) lower in pregnant women with chronic fatigue syndrome ($60.75 \pm 3.91\%$, $33.75 \pm 6.70\%$) compared with healthy volunteers ($77.90 \pm 3.58\%$, $43.76 \pm 8.80\%$) using DPPH and SOD assay respectively. There was a significant ($P<0.05$) lower plasma level of bound iron in pregnant women with CFS ($30.14 \pm 8.11\%$) compared with pregnant women without CFS ($37.69 \pm 10.28\%$) using metal chelating activity assay. It is apparent that oxidative stress might be involved in developing CFS, and significant lower levels of TEAC value, FRAP value, DPPH anion scavenging capacity, superoxide anion scavenging capacity and bound iron in pregnant women with CFS indicate that these women and their infants are at higher risks as compared to pregnant women without CFS and their infants.

Summary

The present study was focused on the isolation of protease producing bacterial isolates from local environment. Twenty four soil samples were collected from different sites, heat shocked and forty bacterial isolates were screened. For further study only six isolates were selected. On the basis of 16S rRNA sequencing it was found that GCU-BSu-5.1, GCU-BSu-22.1 and GCU-BSu-24.1 isolates showed 99% nucleotide sequence homology with *Bacillus subtilis*, GCU-BSu-9 isolate showed 98% nucleotide sequence homology with *Bacillus subtilis*, GCU-BSu-8.1 showed 99% nucleotide sequence homology with *Bacillus licheniformis* whereas GCU-BSu-14b showed 99% nucleotide sequence homology with *Bacillus amyloliquefaciens*.

Optimization of cultural conditions was also performed for maximum production of alkaline protease. M-2 medium containing 2% soybean meal, 1% skim milk, 0.3% NaCl, 0.1% CaCl₂ and 1% Na₂CO₃ was selected as all the isolates gave maximum yield of alkaline protease in this medium. It was found that all bacterial isolates GCU-BSu-5.1, GCU-BSu-8.1, GCU-BSu-9, GCU-BSu-14b, GCU-BSu-22.1 and GCU-BSu-24.1 showed maximum enzyme production of 152U/ml, 144U/ml, 165U/ml, 138U/ml, 172U/ml and 150U/ml at 37⁰C respectively. Effect of pH was evaluated for the production of alkaline protease from selected isolates. The *Bacillus* species GCU-BSu-5.1 was showed maximum activity 148U/ml at pH 10. In case of GCU-BSu-8.1, GCU-BSu-9, GCU-BSu-22.1 and GCU-BSu-24.1 bacterial isolates showed maximum enzyme productivity 140U/ml, 152U/ml, 157U/ml and 170U/ml at pH 10. Whereas the optimum pH for protease productivity of bacterial isolates GCU-BSu-14b was 138U/ml at pH 8.

On the alkaline protease production the effect of inoculum size was also studied and it was found that the inoculum size of 10% was giving the highest yield of alkaline protease and *Bacillus* species GCU-BSu-5.1, GCU-BSu-8.1, GCU-BSu-9, GCU-BSu-14b, GCU-BSu-22.1 and GCU-BSu-24.1 showed maximum enzyme production 154U/ml, 170U/ml, 220U/ml, 220U/ml, 220U/ml and 245U/ml at 10% inoculum size. Time course for the production of alkaline protease in the fermenter was also studied and it was found that the enzyme activity in all bacterial isolates GCU-BSu-5.1, GCU-BSu-8.1, GCU-BSu-9, GCU-BSu-22.1 and GCU-BSu-24.1 reached maximum 150U/ml,

144U/ml, 158U/ml, 172U/ml and 158U/ml after 48 hours of incubation whereas GCU-BSu-14b gave maximum enzyme production of 153U/ml after 54 hours of incubation.

Effect of aeration was also studied as all the bacterial isolates are aerobes. It was found that *Bacillus* spp. GCU-BSu-5.1, GCU-BSu-9, GCU-BSu-22.1 and GCU-BSu-24.1 showed maximum production of enzyme 165U/ml, 154U/ml, 170U/ml and 158U/ml in 25ml of fermentation medium. Whereas *Bacillus* spp. GCU-BSu-8.1, GCU-BSu-14b showed maximum production of enzyme 165U/ml, 150U/ml in 50ml of fermentation medium.

Characterization of alkaline protease produced by bacterial isolates was performed. The effect of fermentation period was studied and it was found that alkaline protease from GCU-BSu-5.1, GCU-BSu-8.1, GCU-BSu-9, GCU-BSu-14b, GCU-BSu-22.1 and GCU-BSu-24.1 was stable at 50°C. It was found that enzyme activity of all bacterial isolates GCU-BSu-5.1, GCU-BSu-8.1, GCU-BSu-9, GCU-BSu-14b, GCU-BSu-22.1 and GCU-BSu-24.1 was reached maximum at alkaline pH of 11.5. Effect of thermal stability was also studied and it was found that in *Bacillus* species GCU-BSu-5.1, GCU-BSu-8.1, GCU-BSu-9, GCU-BSu-14b, GCU-BSu-22.1 and GCU-BSu-24.1 no enzyme activity was observed at 70°C when enzyme was treated with and without CaCl₂. At 60°C there was no proteolytic activity was found in control (without CaCl₂) but significant enzyme activity was determined in the presence of CaCl₂.