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A rapid isolation method of extracellular proteins produced by Pseudomonad strains

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Manufacturing of protein/enzymes by biotechnological methods may contribute to develop new protein/enzyme preparations useful in various sectors of industry. Microbial enzymes released in growing medium and difficult to isolate. In this study, a rapid method has been developed for isolating extracellular proteins from cultured nutrient broth. After removing the bacterial cells from cultured broth through 0.4mm filter paper and dissolved proteins recovered by precipitation with 5% trichloroacetic acid (TCA). Sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the isolated crude enzymes from bacterial cell free culture filtrate showed the presence of different bands in the electrophoretic field.

Key words: *Cell free culture; Enzymes; Nutrient broth; R62 and R81; SDS-PAGE.*

Introduction

Enzyme and protein liberation from the cells is the first step of extraction. During microbial growth large numbers of enzymes or proteins are released in culture media from the microbial cells. Such biomolecules are difficult to isolate from the media. Microbial growth and development depends on the environment which is created in the media due to the presence of extracellular biomolecules. These biomolecules are difficult to isolate because they are immediately degraded by different factors (Murase et al., 2003). These enzymes may be recognized only by some indirect methods like enzyme assay, but still no reports have been reported for quantification. Many dissolved proteins have been observed in seawater and many other possible sources like bacterial membranes (Tanoue, 1995, 1996; Tanoue et al., 1995, Sharma and Sharma, 2015a). Pseudomonas fluorescens is a potential biological agent for controlling many plant diseases (Keel et al., 1992; Siddiqui and Shaukat, 2002; Sharma and Sharma, 2015b,c, 2016) it might be possible due to the production of protease in the external environment i.e. outside the cell (Sharma and Sharma, 2015a). Catalytic function of protease is to hydrolyze peptide bonds and break them into free amino acids. Extracellular serine protease from many fungi inhibits pathogen infection by degrading cuticle proteins (Clarkson and Charnley, 1996; Ahman et al., 2002; Meyer, 2003). Naturally they have been isolated from all the life forms especially from bacteria (Devi et al., 2008). Fluorescent pseudomonads reported for suppression of plant diseases (Whipps, 2001; Haas and Defago, 2005; Sarma et al., 2009; Mader et al., 2011; Sharma and Sharma, 2015b,c, 2016), it might be due to production of such extracellular biomolecules. Trichloroacetic acid (TCA) is useful to accumulate and concentrate the proteins/enzymes therefore, this study focused on the extracellular enzymes of pseudomonads which were released in the nutrient broth culture media.

Materials and methods

Fluorescent Pseudomonas jessenii strains R62 and P. synxantha strain R81 which were provided by rhizospheric biology lab, Department of Biological Sciences, GBPUAT, Pantnagar used as biological material. Plate assay for proteolysis activity was performed according to the method of Jayasree et al. (2009), bacterial isolates were streaked in protease specific medium containing (g/L); Casein - 3g, KNO_3 - 2g, NaCl - 2g, K_2HPO_4 - 2g, $MgSO_4$ - 0.05g, $CaCl_2$ - 0.02g, Yeast extract - 1g; agar - 20g. The clear zone was observed after 48h of incubation at 28°C. For total enzyme extraction, both the bacterial culture

grown in 300 ml of nutrient broth media for 24 h at 28^0C. The cultures were agitate with 200 ml of 67 mM phosphate buffer (pH 6.0) consisting of $Na_2HPO_4 \cdot 12H_2O$ (2.38 g/L) and KH_2PO_4 (8.17 g/L). After centrifugation the supernatant was filter sterilized through 0.4mm filter. 5% TCA was added to the filtrate and kept overnight at 4^0C. TCA-soluble components were removed by centrifugation at 3400 rpm for 30 min. The TCA insoluble fraction was washed with ethanol by centrifugation at 12,000 rpm for 20 min. The pellet was first resuspended in ethanol and further with diethyl ether and then subjected to centrifugation process. The dried pellet was redissolved in 20 ml of sample buffer for Sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS–PAGE) and applied for SDS–PAGE analysis (Laemmli, 1970) using protein marker.

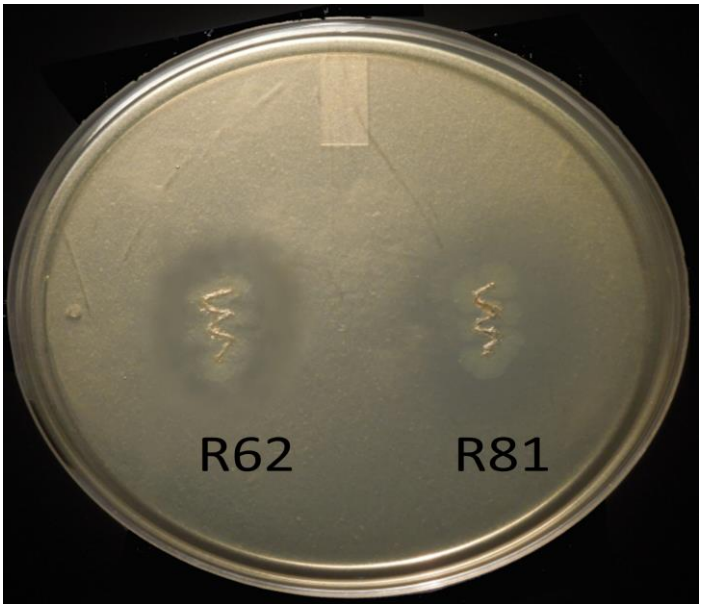

Figure 1. Proteolysis activity of R62 and R81

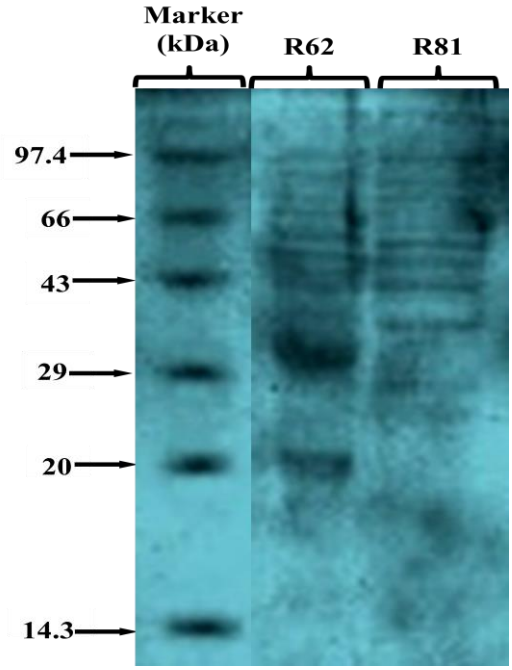

Figure 2. SDS-PAGE analysis of crude enzymes isolated from cell free culture filtrates of R62 and R81

Results and discussion

For proteolysis activity both bacterial strains were recorded positive (Figure 1). The molecular mass of different extracellular proteins which were released in nutrient broth media determined through SDS-PAGE analysis. The crude proteins

from cell free culture filtrate from both cultures showed the presence of different bands in the SDS-PAGE (Figure 2). These results suggested that the biomolecules which were release by the bacterial cells isolated by filter sterilization of culture through 0.4mm filter for removing bacterial cells, however similar observation was previously reported in greenhouse soil which was filter sterilized through No. 6 filter paper which is not valuable for removal of bacterial cells (Matsumoto et al., 2000; Murase et al., 2003) and bacterial culture through 0.4mm filter (Sharma and Sharma, 2015a). Extracellular proteins might be playing an important role in the microbial environment likewise as in nitrogen storage and for microbial growth etc. Such enzymes may also contribute to checking infection of hosts by degrading the host's protective barriers (Ahman et al., 2002; Huang et al., 2004). Many previous studies proposed that the microbial proteases are produced virulence factors against the pathogens (Ahman et al. 2002; Siddiqui et al. 2005; Tian et al. 2006). This finding further evaluated using different microbial samples and also identifying the various extracellular biomolecules for medicinal, pest resistance, stress resistance and many other perspectives.

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