ISSN: 2230-9926

*Available online at http://www.journalijdr.com*

IJDR

International Journal of DEVELOPMENT RESEARCH

*International Journal of Development Research*
*Vol. 5, Issue, 03, pp. 3766-3767, March, 2015*

## *Full Length Research Article*

# ISOLATION OF PROTEIN FROM CELL FREE CULTURE FILTRATE: A NOBLE APPROACH

***Ishwar Prakash Sharma and Sharma, A. K.**

Department of Biological Sciences, College of Basic Sciences and Humanities, G. B. Pant University of Ag. & Tech., Pantnagar, 263145, U. S. Nagar, India

**ARTICLE INFO**

***Article History:***
Received 30th December, 2014
Received in revised form
08th January, 2015
Accepted 11th February, 2015
Published online 31st March, 2015

***Key words:***
Cell free culture,
Protease,
R62 and R81,
SDS-PAGE.

**ABSTRACT**

Bacteria are well known for their ability to liberate proteins and enzymes into their environment. Pseudomonad sp. being important micro-organisms produces wide range of extra-cellular enzymes including proteases. The pseudomonad strains R62 and R81 has been used in this study for electrophoresis analysis of extracellular protease enzymes which were present in the cell free culture of nutrient broth (NB) and protease specific broth (PB) media. The SDS-PAGE analysis of the crude protein from cell free culture filtrate from both the media when analyzed for proteases, showed the presence of different bands in the electrophoretic field.



## INTRODUCTION

The first step in the extraction of protein is liberation of protein from the cells. Large numbers of proteins were release in the culture media during the microbial growth. Protease is also a type of enzyme which liberate outside the cell, their catalytic function is to hydrolyze peptide bonds and break them into free amino acids. Commercially, they are usage in detergents, leather, food and pharmaceutical industries (Bhaskar *et al*., 2007 and Jellouli *et al*., 2009). Naturally they have been isolated from all the life forms. They are of three type's *i.e*. acid protease mostly produced by fungi and best performed at pH 2.0-5.0; neutral proteases produced by plants and function at pH 7.0 and alkaline proteases have optimum activity at pH 8.0 and they are mostly produced by microorganisms (Devi *et al*., 2008). Fluorescent pseudomonads have been reported previously for plant growth promotion and suppression of crop diseases (Whipps, 2001; Haas and Defago, 2005). The selected pseudomonads strains (R62 and R81) which were used in this study have been reported positive for phosphate solubilization, indole-3-acetic acid, siderophores and 2,4-diacetyl phloroglucinol (DAPG) production, which makes them potential plant growth promoting rhizobacteria (PGPR) as well as for disease suppression (Sarma *et al*., 2009; Mader *et al*., 2011). It was suggested that, these bacteria might be responsible for the production of some extracellular proteins which is responsible for the suppression of plant pathogens.

***Corresponding author: Ishwar Prakash Sharma***
*Department of Biological Sciences, College of Basic Sciences and Humanities, G. B. Pant University of Ag. & Tech., Pantnagar, 263145, U. S. Nagar, India*

## MATERIALS AND METHODS

In this study, fluorescent *Pseudomonas jessenii* strains R62 and *P. synxantha* strain R81 were used. For total protein extraction, both the bacterial culture grown in the 300 ml of nutrient broth (NB) for 24 h and in 300 ml of protease broth (PB) containing (g/L) glucose - 5.0, peptone - 7.5, $MgSO_4.7H_2O$ - 5.0, $KH_2PO_4$ - 5.0 and $FeSO_4.7H_2O$ - 0.1, pH-7.0 medium for 72 h. The cultures were mixed 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).The supernatant was centrifuged and filter sterilized through 0.4mm filter. After that 5% TCA was added to the bacterial culture filtrate and kept at $4^0$C for overnight. Next morning the solution was centrifuged in 50-ml centrifuge tubes at 3400 rpm for 30 min to remove the TCA-soluble component. The TCA insoluble fraction was washed with ethanol into a 1.5-ml microtube and centrifuged at 12,000 rpm (11,000g) 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 SDS–PAGE and applied for SDS–PAGE analysis (Laemmli, 1970).

## RESULTS

The molecular mass of protease enzyme was determined through SDS-PAGE analysis. The crude protein from cell free culture filtrate of both the media (NB and PB media) when analyzed for protease, showed the presence of different bands in the SDS-PAGE (Fig 1) within the range of different protease isomers, it might be suggested that the bands of different isomers of proteases were present in the bacterial cell free supernatant.

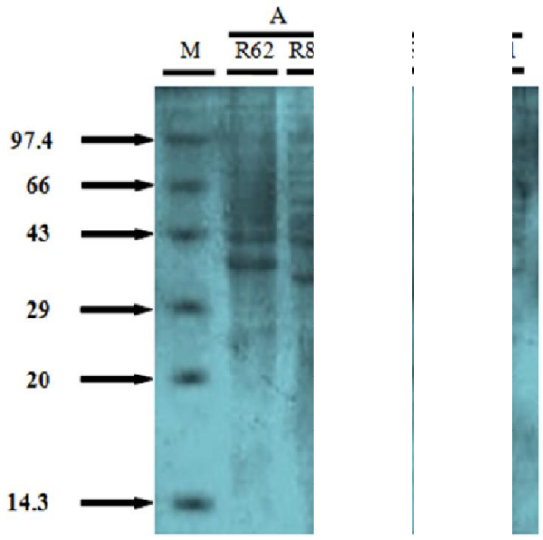


**Figure 1. SDS-PAGE analysis of cell free supernatant of R62 and R81 in the different medium. M = Protein marker (kDa), A = Supernatant from protease broth (PB), B = Supernatant from nutrient broth (NB) medium**

## DISCUSSION

The molecular weight of proteases range from 18-90 kDa (Sidney and Lester, 1972). On the basis of zymogram study, the molecular weight of proteases was found to be approximately 38 kDa (Devi *et al*., 2008), 41 kDa (Tian *et al*., 2007), 46 kDa (Muthulakshmi *et al*., 2011) and 60 kDa (Dubey *et al*., 2010). Extracellular proteases are important for the hydrolysis of proteins in cell-free environments and enable the cell to absorb and utilize hydrolytic products (Ayaz, 2012). Different isoforms of proteases i.e. serine protease (temperature labile), cysteine protease, alkaline protease, metalloprotease (temperature stable) and neutral protease have been previously identified from the cell free supernatants of *Aeromonas hydrophila* strain B51 (Leung and Stevenson, 1988; Park *et al*., 2003).

**Acknowledgment:** Authors are thankful to the Rhizosphere Biology Lab, Department of Biological Sciences, CBSH, Pantnagar for providing lab facilities for the research work.

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