International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Pectinase Production by *Delftia Acidovorans* Isolated From Fruit Waste under Submerged Fermentation

Vibha Bhardwaj[1], Neelam Garg[2]

[1, 2] Department of Microbiology, Kurukshetra University, Kurukshetra, Haryana, India

Abstract: ***Delftia acidovorans strain IAC/BECa-023 was isolated from waste of fruit processing unit and selected as maximum pectinase producing bacterium under alkaline conditions. The morphological, cultural and biochemical characteristics of Delftia were studied and it was identified on the basis of nucleotide homology and phylogenetic analysis. Maximal quantities of pectinase were produced in the alkaline range of the production medium at 25 ºC to 37 ºC under shaking conditions at 150 rpm in 24 hr of incubation period. Best carbon source was found to be starch supplemented with pectin and Potassium Nitrate as nitrogen.***

Keywords: Delftia acidovorans , pectinase, microorganisms, optimization, submerged fermentation.

1. Introduction

Pectin is an important component of middle lamella and primary cell wall of higher plants. It is a high molecular weight acid polysaccharide of α (1-4) linked D-galacturonic acid residues (Torres-Fanela et al, 2003). Pectinases constitute a unique group of enzymes which catalyze the degradation of pectic polymers present in the plant cell walls (Fogarty & Kelly 1982). Pectinases are produced by many organisms such as bacteria (Horikoshi 1972; Karbassi & Vaughn 1980), fungi (Aguilar & Huitron 1990) and yeasts (Gainvors & Belarbi 1993). In the industrial sector, acidic pectinases are used in the extraction and clarification of fruit juices (Rombouts & Pilnik 1986), whereas alkaline pectinases find immense use in the degumming of ramie fibers (Cao et al. 1992), retting of flax (Sharma 1987), plant protoplast formation and treatment of effluents discharged from fruit processing units (Tanabe et al.1987). Although the major source of acidic pectinases is fungi, alkaline pectinases are produced from alkalophilic bacteria, mainly *Bacillus* spp. However, selection of a particular organism remains a tedious task and the choice gets tougher when commercially competent enzyme yields are to be achieved. The present investigation was carried out to isolate and identify a potential pectinase producing bacterium and to optimize the conditions for pectinase production.

2. Materials and Methods

Chemicals and Reagents-Pectin, D-galacturonic acid monohydrates were obtained from Sigma Chemicals Co. All the other chemicals and reagents used for the study were of analytical /microbiological grade of Hi Media Chemicals Pvt Ltd.

2.1 Isolation and identification

Samples of soil, waste of fruit processing units and vegetables were procured locally and used for isolation pectinase producing bacteria using pectin agar medium at pH 7.2 .The serially diluted samples were screened for pectinase producing microorganisms. Out of 109 isolates, NV59 isolate produced maximum zone of clearance after incubation at 37ºC for 48 hours on addition of 1% cetyl trimethyl ammonium bromide (CTAB) and was selected for further study. The NV59 was characterized by performing various morphological, cultural and biochemical test based on standard methods and identified by Xcleris Lab Ltd Ahmadabad based on nucleotide homology and phylogenetic analysis.

2.2 Production of Pectinase

Isolate NV59 was cultivated in production medium (Reda 2008) under submerged fermentation at pH-7 and incubated at 37°C for one day on a rotary shaker (NSW- 256) at 150 rpm for production of enzyme.

2.3 Optimization of cultural conditions for pectinase production

Cultural conditions viz. pH, temperature, incubation time, agitation, inoculum size and age, sources of carbon and nitrogen and their concentration were optimized for pectinase production using one variable at a time. The inoculated broth after incubation was centrifuged at 10,000g for 10mins at 4°C and the clear supernatant was used as crude enzyme for enzyme assay.

2.4 Assay of pectinase activity

Pectinase activity was assayed by the method of Miller (1959) and absorbance was measured at 540 nm. One unit of enzyme was defined as the amount of enzyme which catalyses the formation of 1µmol of galacturonic acid/min.

2.5 Effect of pH, Temperature and Incubation Time

To optimize the pH for enzyme production, the pH of the production medium was set at 4, 5, 6, 7, 8, 9, 10, 11 and 12. Most favorable temperature was studied by incubating the production medium at different temperatures such as 20°C, 25°C, 30°C, 35°C, 37°C, 40°C, 45°C and 50°C. Effect of incubation time was studied by incubating the inoculated

production medium for different time intervals 6 h, 12 h, 18 h, 24 h, 48 h , 72 h, 96 h, 120 h,144 h, 168 h, 192 h and 216 h.

2.6 Effect of agitation

Optimum shaking conditions were determined by agitating the inoculated production medium in an incubator shaker at 120,130, 140,150 and 160rpm.

2.7 Effect of Inoculum Age and Inoculum Size

Inoculum age was optimized by inoculating the production medium with inoculum of varying age viz.0 h, 6 h, 12 h, 18 h, 24 h, 30 h, 36 h and 48 h old. To study the effect of inoculum size, 0.5ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5ml and 3.0ml of inoculum was used to inoculate the production medium.

2.8 Effect of Carbon source and its concentration

Various carbon sources citrus pectin, dextrose, fructose, galactose, lactose, maltose, sucrose, mannose, trehalose , starch, cellulose and xylose were used in the production medium at a concentration of 1% w/v as carbon source. Production medium containing pectin as the only carbon source was used as control. Concentration of best carbon source was optimized for maximal enzyme production by using different concentrations 0.25%, 0.50%, 1.00%, 1.50%, 2.00%, 2.50%, 3.00% and 3.50% w/v.

2.9 Effect of Nitrogen source and its concentration

The effect of various nitrogen sources viz. peptone, ammonium sulphate, gelatin, ammonium chloride, urea, ammonium nitrate, ammonium dihydrogen phosphate, potassium nitrate, sodium nitrate on the production of enzyme was studied by supplementing 0.2% w/v of these to the production medium. The production medium without any nitrogen source was used as control. To study the effect of concentration of best nitrogen source for maximal enzyme production, it was used at different concentrations 0.05%, 0.10%, 0.20%, 0.30%, 0.40% and 0.50% w/v in the production media.

3. Result and Discussion

In the recent years, the potential of using microorganisms as biotechnological sources of industrially relevant food processing enzymes has stimulated renewed interest in the exploration of extracellular enzymatic activity. Furthermore, most commercial pectinases are from microbial sources. Pectinases are increasing in commercial importance. In the present study, isolation and screening of pectinase producing microorganisms were carried out and production conditions for enhanced pectinase production were optimized. NV59, a bacterium isolated from the soil of a fruit-processing unit exhibited a largest zone of clearance .One bacterial colony showing maximum zone of clearance on pectin agar plates. On morphological examination NV59 was found to be Gram negative, nonsporing, motile, aerobic rod shaped bacterium. On pectin agar medium colonies were smooth, shiny, opaque, raised and irregular .The biochemical characteristics are given in Table 1.

Table 1: Biochemical Characterization of Isolate NV59

Biochemical test	*Result*	*Carbohydrate fermentation*	*Result*
Indole	-	Glucose	+
Voges	-	Mannitol	+
Citrate	+	Xylose	-
Lysine	+	Inositol	-
Ornithine	-	Sorbitol	-
Arginine	+	Rhamnose	-
Nitrate	+	Sucrose	+
Malonate	+	Lactose	+
Urease	-	Arabinose	+
Phenyl	-	Adonitol	-
H_2S	+	Raffinose	-
ONPG	-	Salicin	+
Catalase	+	Trehalose	+

The isolate NV59 was identified as ***Delftia acidovorans strain IAC/BECa-023 (***GenBank Accession Number: **JX155411.1**) based on nucleotide homology and phylogenetic analysis by Xcleris Lab Ltd Ahmadabad.

3.1 Production of pectinase

Delftia acidovorans was cultivated in pectinase production medium (Reda 2008) for optimization under submerged fermentation. The pectinase production by ***Delftia acidovorans*** was tested by adjusting the pH of medium between pH 4.0-12.0.Maximal pectinase production was observed at pH 11(Figure 1). The incubation temperature was also found to influence the pectinase production. The optimum temperature was found to be 25°C and further increase in temperature decreases the pectinase production (figure 2).Optimal incubation period for pectinase production was found to be 24 hours(figure 3).Agitation increases pectinase production upto150 rpm(figure 4). The Maximal enzyme production was observed at 24 hours inoculum age (figure 5) and 0.5% inoculums size (figure 6). Among the various carbon sources tested starch was found to be the best source when its 1% concentration was supplemented to the production medium containing pectin. The pectinase production was suppressed greatly when was grown either on maltose, fructose and galactose as C-source (figure 7,8).Of the various nitrogen sources used maximal pectinase production was observed when supplemented with potassium nitrate 0.2% (figure 9,10)

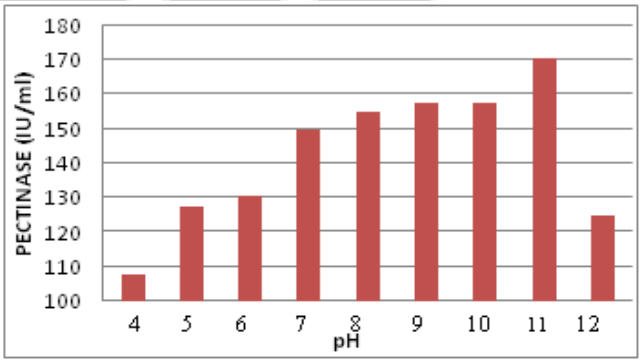

Figure 1: Effect of pH on pectinase production under submerged fermentation

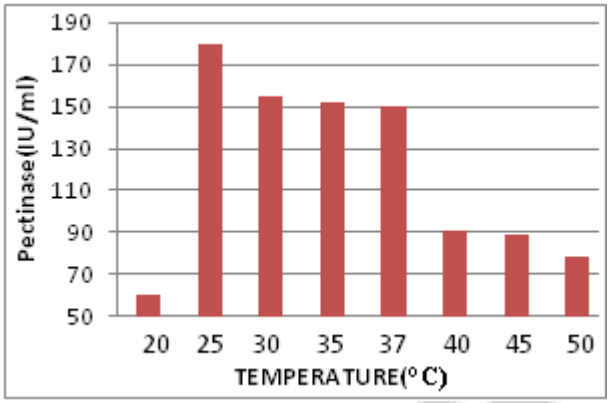

Figure 2: Effect of temperature on pectinase production under submerged fermentation

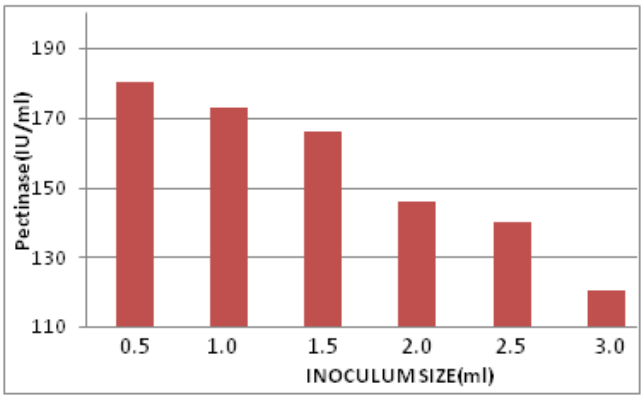

Figure 6: Effect of inoculum size on pectinase production under submerged fermentation

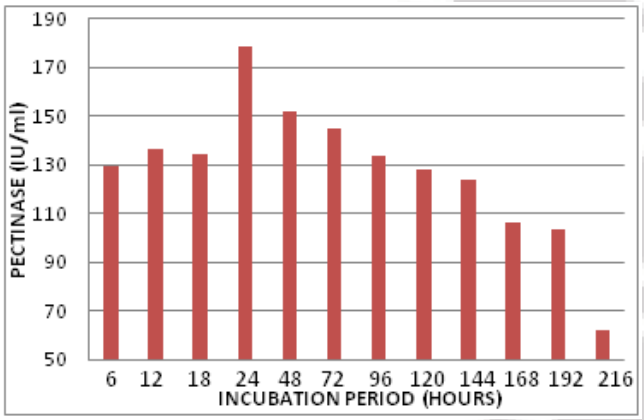

Figure 3: Effect of Incubation period on pectinase production under submerged fermentation

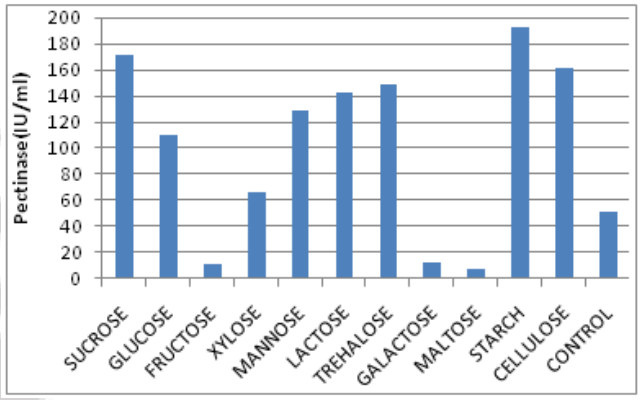

Figure 7: Effect of carbon sources on pectinase production under submerged fermentation

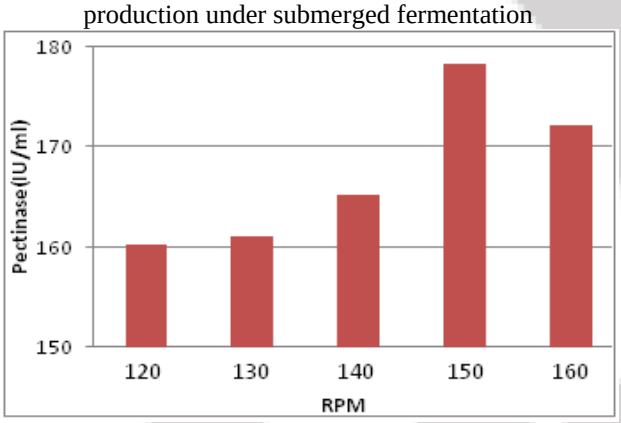

Figure 4: Effect of agitation on pectinase production under submerged fermentation

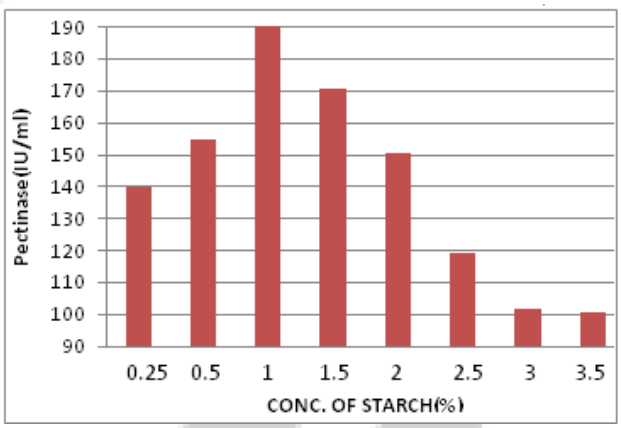

Figure 8: Effect of starch conc. on pectinase production under submerged fermentation

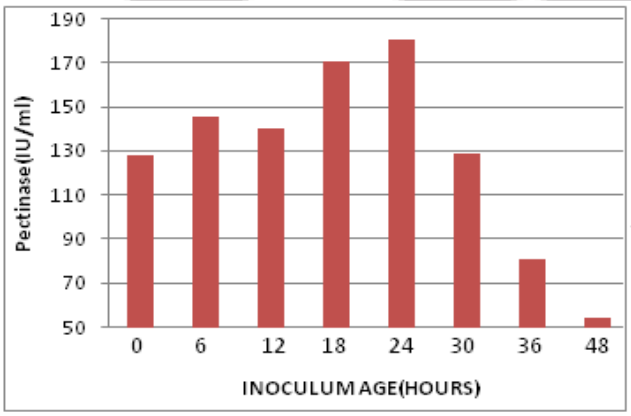

Figure 5: Effect of inoculum age on pectinase production under submerged fermentation

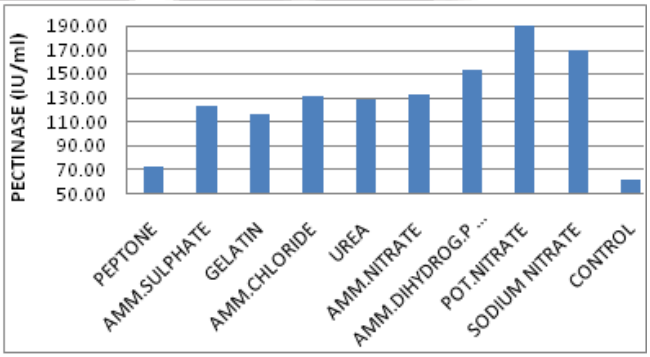

Figure 9: Effect nitrogen sources on pectinase production under submerged fermentation

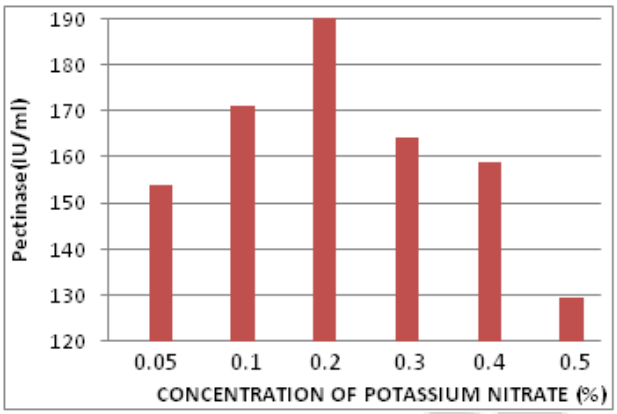

Figure 10: Effect of potassium nitrate conc. on pectinase production under submerged fermentation

4. Conclusion

This is probably the first report of pectinase production by ***Delftia acidovorans strain IAC/BECa-023*.** The organism *Delftia acidovorans* has been isolated, characterized and identified. The culture conditions were optimized for pectinase production. *Delftia acidovorans* has shown best results at pH 11 alkaline range and temperature of 25°C to 37 °C in 24 h of incubation period at 150 rpm under submerged fermentation.

References

[1] Acuna-Arguelles, M.E., Gutierrez-Rajas, M., Viniegra-Gonzalez, G. & Favela-Toress, E. 1995 Production and properties of three pectinolytic activities produced by *A. niger* in submerged and solid state fermentation. Applied Microbiology and Biotechnology 43, 808-814.

[2] Aguillar, G. & Huitron, C. 1990 Constitutive exopectinase produced by *Aspergillus* sp. CH-Y-1043 on different carbon sources. Biotechnology Letters 12, 655-660.

[3] Borin, M.D.F., Said, S. & Fonseca, M.J.V. 1996 Purification and biochemical characterization of an extracellular endopolygalacturonase from *Penicillium frequentans*. Journal of Agriculture and Food Chemistry 44, 1616-1620.

[4] Cao, J., Zheng, L., & Chen, S. 1992 Screening of pectinase producer from alkalophilic bacteria and study on its potential application in degumming of rammie. Enzyme and Microbial Technology 14,1013-1016.

[5] Dave, B.A. & Vaughn, R.H. 1971 Purification and properties of a polygalaturonic acid trans eliminase produced by *Bacillus pumilus*. Journal of Bacteriology 108, 166-174.

[6] Fanseca, M.J.V. & Said, S. 1995 Sequential production of pectinase by *Penicillium frequentans*. World Journal of Microbiology and Biotechnology 11, 174-177.

[7] Fogarty, M.V. & Kelly, C.T. 1983 In Microbial Enzymes and Biotechnology. ed. Fogarty, M.W. pp. 131-182. London & New York: Elsevier Applied Science Publishers. ISBN 1-85166486-6

[8] Gainvors, A. & Belarbi, A. 1995 Detection methods for polygalacturonase producing strains of *Saccharomyces cerevisae*. Yeast 10, 1311-1319.

[9] Garibaldi, A. & Bateman, D.F. 1971 Pectic enzymes produced by *Erwinia chrysanthemi* and their effect on plant tissue. Physiological Plant Pathology 1, 25-40.

[10] Horikoshi, K. 1972 Production of alkaline enzymes by alkalophilic microorganisms Part III. Alkaline pectinase of *Bacillus* No P-4-N. Agricultural and Biological Chemistry 36, 285-293.

[11] Karbassi, A. & Vaughn, R.H. 1980 Purification and properties of polygalacturonic acid trans-eliminase from *Bacillus stearothermophilus*. Canadian Journal of Microbiology 26, 377-384.

[12] Kashyap D.R., Chandra S. K. A., and Tewari R., (2000). Production, purification and characterization of pectinase from a *Bacillus* sp. DT7. World J. Microbiol. Biotech.16,277−282.

[13] Keller V., Jen J. J., and Brunner R. J., (2006). Purification of commercialpectinase by hydrophobic chromatography. J. Food. Sci. 47,2076−2077.

[14] Kelly, C.T. & Fogarty, W.M. 1978 Production and properties of polygalacturonate lyase by an alkalophilic microorganism Bacillus sp. RK9. Canadian Journal of Microbiology 29, 1164-1172.

[15] Koponen J. M., Buchert J., Kaisa S. P., and Torronen R. A., (2008). Effect of pectinolytic juice production on the extractability and fate of bilberry and black currant anthocyanins. Euro. Food. Res. Tech. 222,485−494.

[16] Laemmli, U.K. 1970 Cleavage of structural proteins during the assembly of head bacteriophage T4. Nature 22, 680-685.

[17] Martinez, M.J., Alconda, M.T., Guillrn, F., Vazquez, C. & Reyes, F. 1991 Pectic activity from Fusarium oxysporium f. sp. melonis:purification and characterization of an exopolygalacturonase. FEMS Microbiology Letters 81, 145-150.

[18] Merril, C.R., Goldman, D. & Ibert, M.H. 1981 Ultrasensitive stain for proteins in polyacrylamide gel shows regional variations in cerebrospinal fluid proteins. Science 211, 1437-1438.

[19] Miller, L.G. 1959 Use of dinitrosalicylic acid reagent for the determination of reducing sugars. Analytical Chemistry 31, 426-428.

[20] Moran, F. & Starr, M.P. 1969 Metabolic regulation of polygalacturonic acid trans eliminase in Erwinia. European Journal of Biochemistry 11, 291-295.

[21] Nagel, C.W. & Vaughn, R.H. 1961 The characteristic of a polygalacturonase produced by Bacillus polymyxa. Archives of Biochemistry and Biophysics 93, 344-352.

[22] Nagel, C.W. & Hasegawa, S. 1968 The isolation and characterization of an 1,4 (4,4 dihydrogalacturonosyl) galacturonate hydrolase. Journal of Food Science 33, 378-382.

[23] Nasumo, S. & Starr, M.P. 1967 Polygalacturonic acid tran eliminase of Xanthomonas compestris. Biochemical Journal 104, 178-184.

[24] Panda T., Nair S. R., and Kumar P. M., (2004). Regulation of synthesis of pectinolytic enzymes of *Aspergillus niger.* Enz. Microbial. Technol. 34,466−473.

[25] Pitt, O. 1988 Pectic lyase from Rhoma medicabinis var. pinodella. Methods in Enzymology 161, 350-365.

[26] Reda,A.,Bayoumi .2008.Production of bacterial pectinases from Agroindustrial wastes under solid state

fermentation conditions.Journal of Applied Sciences Research,4(12):1708-1721.
[27] Rombouts, F.M. & Pilnik, W. 1986 Pectinases and other cell-wall degrading enzymes of industrial importance. Symbiosis 2, 79-89.
[28] Siessere, V. & Said, S. 1989 Pectic enzyme production in solid state fermentation using citrus pulp pellets by Talaromyces flavus, Tubercularia vulgaris and Penicillium charlessi. Biotechnology Letters 11, 343-344.
[29] Sharma, H.S.S. 1987 Enzymatic degradation of residual non cellulosic polysaccharides present on dew retted flax fibers. Applied Microbiology and Biotechnology 26, 358-362.
[30] Starr, M.P. & Nasumo, S. 1967 Proteolytic activity of phytopathogenic Xanthomonads. Journal of General Microbiology 46, 425-433.
[31] Tanabe, H., Yoshihara, K., Tamura, K., Kobayashi, Y., Akamatsu, I., Niyomwan, N., & Footrakul, P. 1987 Pretreatment of pectic waste water from orange canning process by an alkalophilic Bacillus sp. Journal of Fermentation Technology 65, 243-246.
[32] Torres-Favela E., Aguilar C., Esquivel-Contreras C.J., and Gustavo G.V., (2003). Pectinase. In Enzyme Technology. Asiatech Publisher Inc. Delhi., 273−296.

Author Profile

Vibha Bhardwaj, a research scholar in department of microbiology of Kurukshetra University Haryana India. She is the member of Indian Women Scientist Association, National Environmentalists Association, Sustainable Development and Environment Protection. She has published papers in reputed journal and also chapters in books. She got **Mahila Gaurav Puraskar {Women's pride Award} from Government of Haryana India in 2010**. She has presented papers in National and International conferences and got prizes. She had worked in Forensic Department, Hospitals, and Pharmaceuticals.

Dr Neelam is Associate Professor in department of microbiology Kurukshetra University Kurukshetra Haryana India. She is MSc. and PhD in microbiology and has teaching experience of 27 years at postgraduate level. She has to her credit a number of research papers published on International and National journals of repute and in national and international proceedings. She has published one book as editor with international publishers. She has been sanctioned a University Grant Commission (India) major project on Chitin deacetylase production in 2012.