

Original Research Article

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Extracellular Cold Active Alkaline Proteolytic Enzyme Produced By *Pseudomonas gessardii* Strain N-86 Isolated from Antarctica

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ABSTRACT

To produce cold active protease, investigation was conducted using bacteria isolated from the core sample of Nella Lake, East Antarctica. Isolates were screened for protease using plate assay method followed by submerged fermentation at cold temperature. Potential isolate was identified as *Pseudomonas gessardii* strain N-86 by molecular technique. Further, investigations were performed to enhance the protease production capabilities by submerged fermentation technique using strain N-86 with various physiological (temperature and pH) and chemical (Carbon, nitrogen, minerals, amino-acids, and various substrates) parameters. Production optimization revealed the maximum protease production by using skim milk (2% w/v), pH 9.5 and 10% v/v of inoculum size at 15°C. Additional potential inducers for protease production were sucrose (1% w/v), L-Glutamine (0.01% w/v) and MgCl₂ (0.1% w/v). Final productions with optimized conditions gave 5.42-fold increase in protease production on 72 h of incubation. The present study successfully produced cold-active protease which may have novel properties to degrade protein based wastes which can contribute in cleaning of environment at cold regions without harming the climate and also can be used in various detergent, textile, food and beverage industries effectively. The present study is the first report on *Pseudomonas gessardii* isolated from Nella Lake, Antarctica producing cold active protease.

Keywords

Cold adapted, East Antarctica, Larsemann Hills, Nella Lake, Protease, Psychrotolerant, Sediment core

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Introduction

Proteases are the hydrolytic enzymes that act up on different kinds of proteins lead to formation of smaller peptides and amino acids. On the basis of action it is of exo- or

endo-peptidase and it is of four types on the basis of functional groups viz. serine-, cystein-, aspartic- and metallo-protease. The protease types depend on the composition of the production medium; therefore, one microorganism can produce various types of

proteases (Maharana *et al.*, 2016). Proteases can be found in almost all kingdom viz. Animalia, Plantae, Fungi, Bacteria, Archaea and even in viruses. However, microbial origin proteases are preferred due to diversified uses and compatibility. Microbial proteases have immense applications as compared to other hydrolytic enzymes in various spheres of science and industries. Alkaline proteases are one of the most important groups of enzymes, used in various industrial products and processes as detergents, pharmaceuticals, leather, meat tenderizers, protein hydrolyzates, food products and even in the waste processing (Sen *et al.*, 2009).

Cold active enzymes are in high demand in various food and beverages, detergent and textile industries and to fill this huge requirement, it is necessary to investigate on cold active microorganisms capable of producing cold enzymes (Maharana, 2016). Enzymes from psychrotolerants are more adaptable than mesophiles and psychrophiles due to their wider range of temperature stability, and which can be used for bio-remediation and wastewater treatments at cold regions (Maharana and Ray, 2015a).

Huang *et al.*, (2016) isolated *Pseudomonas gessardii* strain LZ-E from wastewater discharge site of a petrochemical company of Lanzhou city which showed degradation capabilities of naphthalene and hexavalent chromium. The production of enzymes depends on many physical and chemical variables which control the production by regulating the growth of isolates used in the processes and optimization is necessary for the hyper production of any enzymes before doing large-scale production (Maharana and Ray, 2015b).

Pseudomonas spp. is well known for production of lipase enzymes which may be

of cold or mesophilic (Gao *et al.*, 2000; Rashid *et al.*, 2001; Maharana and Ray, 2014c; Maharana and Ray, 2015a). Some reports are available showing *Pseudomonas* spp. having potentiality to produce proteases at low temperatures (Hamamoto *et al.*, 1994; Vazquez *et al.*, 2002; Baghel *et al.*, 2005; Reddy *et al.*, 2009; Maharana and Ray, 2013; Singh *et al.*, 2014, 2015; Caldera *et al.*, 2016; Matsui *et al.*, 2017). Besides, cold active proteases were well produced by psychrotolerant fungi (Singh *et al.*, 2012b; Maharana and Ray, 2014a; Maharana and Ray, 2014b).

Lake Nella (76°22' S, 69°24' E), an ultra-oligotrophic lake is located at an altitude of 15 m a.s.l. in the Broknes peninsula, Larsemann Hills region, East Antarctica. The lake holds fresh to slightly saline waters and has high possibilities of getting cold microorganisms which in turn have many potential applications to various sectors of science. As microbiological study is concerned, Nella Lake is not yet much explored except studies from Singh *et al.*, (2017) and Maharana and Singh, (2018 a,b,c) where cold active enzymes like lipase and amylase were explored from bacterial and fungal isolates. There was no study performed on cold active proteases in that habitat; therefore, one approach was taken in the present study to produce cold active extracellular protease from microbes isolated from sediment cores of Nella Lake using a psychrotolerant bacteria *i.e.* *Pseudomonas gessardii* strain N-86.

Materials and Methods

Chemicals, reagents and instruments

The chemicals and reagents were of analytical grade (Hi-Media laboratories, Mumbai, India; Bangalore Genei, India; Sigma Aldrich Chemicals Private Limited, Bengaluru,

Karnataka, India). Instruments used were microscope (Epi-fluorescence research microscope, BX51 Olympus, Japan), -20°C freezer (Biomedical freezer, MDF-U537D, Sanyo), low temperature static incubator (MIR 253 & MIR-554-PE, Sanyo, Japan), refrigerated incubator shaker (IS-971RF, Jeio tech, Korea), pH analyzer (Orion 930, Thermo Electron, USA), refrigerated table top centrifuge (5810, Eppendorf, Germany), refrigerated circulatory water bath (F25-MC, Julabo, Germany) and UV-Vis spectrophotometer (Specord S205, Analytik Jena, AG Germany) *etc.*

Sampling and isolation of bacteria

Microorganisms investigated in this present study were isolated from the sediment core samples of Lake Nella in the Broknes peninsula, Larsemann Hills region, East Antarctica. Core samples were serially diluted by 10-fold dilution technique and spread over various media viz. Antarctic Biological Medium (ABM), ABM/10 and Nutrient Agar (NA), NA/10 for isolation of bacteria.

Plates were incubated for 1-2 weeks at 1, 5, 15 and 22°C and after visible, cultures were streaked on the same agar plate for further purification microscopically. Purified isolate was stored at -20°C with 20% glycerol (v/v) and 1/10th dilution of nutrient broth. This culture was revived from glycerol stock by spreading on the same isolation agar medium and multiple copies were also made and stored at 4°C for further use.

Screening for cold active protease

Isolates were investigated qualitatively by spot inoculation method on skim milk agar (Hi-media) by incubating respective plates at 4, 15 and 22°C. Hydrolytic zone diameters were calculated by subtracting the whole diameter to that of bacterial colony diameter.

Potent enzyme producers were selected on the basis of maximal zone of clearances.

Production strategies for cold active protease

Those isolates again investigated for quantitative screening to select best isolate for cold active extracellular protease production. For this potential isolates were inoculated in nutrient broth (Hi-media) and incubated for 2 days at 15°C with 150 rpm. From that about 5% (v/v) inoculum was taken in protease production medium (g.L⁻¹: NaCl, 2; CaCl₂, 0.1; MgSO₄, 0.4; K₂HPO₄, 0.3; KH₂PO₄, 0.3; (NH₄)₂SO₄, 0.5; casein, 10; pH, 7) and incubated at 15°C with 150 rpm for 96 h.

Crude enzyme (supernatant) was collected by centrifugation of production medium at 12,000 rpm at 4°C for 20 min. For production purposes freshly prepared inoculum of isolates were used. Two steps selection procedure were adopted to select one best bacterial isolate for further study. The potent isolate was outsourced for 16S rDNA gene sequencing for identification in molecular level.

Protease assay

The quantitative assay of the protease activity was performed using a modified method of Park *et al.*, (2014). One ml of crude enzyme was incubated with 0.65% casein solution (prepared with 0.05 M phosphate buffer, pH 7.0) for 10 min at 15°C in water bath. Reaction was stopped by adding 5 ml of 110 mM Tri-chloro acetic acid (TCA) and incubated for 30 min.

It was then centrifuged at 12,000 rpm at 4°C for 5 min. and about 5 ml sodium carbonate (500 mM) followed by 1 ml Folin reagent was added. Respective enzyme blanks were also made by eliminating the incubation period.

The absorbance at 660 nm was measured, and the amount of tyrosine released from casein was calculated using a standard curve for *L*-tyrosine. One unit of enzyme activity was defined as the amount of enzyme that released 1 µg of tyrosine per minute under assay conditions.

Production optimization for proteolytic degradation

Production optimization for protease was done using submerged fermentation technique by “one factor at-a-time” (OFAAT) method. Factors investigated were various substrates, incubation temperature, initial medium pH, substrate concentrations, nitrogen and carbon sources, amino acids, minerals, inoculum size, and incubation period. In each experiment, the optimized factor was integrated with the control medium in triplicates. Besides, one control in every production was also considered in each step devoid of supplied factors for comparative selection among the parameters. After optimization final protease production was done using optimized conditions.

Statistical analysis

The data recorded during the investigation were subjected to significance test by t-test and analysis of variance (ANOVA). Statistical significance was set at $p < 0.05$, and for high significance set at $p < 0.001$. Results were denoted as mean \pm standard error (SE) of triplicate experiments.

Results and Discussion

Screening and selection for cold active protease producers

A total of 178 bacteria were isolated at various temperatures. All isolates were investigated for protease production

qualitatively by plate assay method at various temperatures i.e. 4, 15 and 22°C. Almost 90 isolates (50.56%) showed positive for protease at 4°C while 96 isolates (53.93%) showed positive at both 15 and 22°C. Some of isolates showing positive for protease on skim milk agar plates are depicted in Figure 1.

Among them potential fifteen (15) isolates were selected on basis of zone of clearances for further quantification using casein as substrate at 15°C and incubated for 96 h. Figure 2 denotes protease production by those 15 potential isolates.

For final selection best four (4) isolates were chosen and investigated using two substrates i.e. skim milk and casein, which were investigated day-wise (Fig. 3). It reveals maximum production was achieved by strain N-86 on 2nd day of incubation at 15°C. Therefore, from onwards N-86 was taken as potent isolate for protease production. The N-86 was outsourced and identified as *Pseudomonas gessardii* strain N-86 (GenBank accession no. KY783362), which is a Gram negative and rod shaped bacterium. ANOVA reveals that the differences in protease activity due to various bacteria *w.r.t.* two substrates are highly significant ($p < 0.001$).

Production optimization of protease using *Pseudomonas gessardii* strain N-86

Effects of incubation temperature

Various temperatures viz. 5, 10, 15, 25, 30, 35 and 45°C were investigated for protease production using strain N-86. *Pseudomonas gessardii* strain N-86 showed maximum protease production by incubation temperature of 15°C and after this there is a gradual decrease in production (Fig. 4). It showed a possibility of getting an enzyme that can be active at a lower temperature.

The effect of incubation temperature on protease production is significant as per ANOVA test at $p < 0.01$. The t-stat also shows a significant difference between their population mean at 0.01 probability level.

Effects of initial pH

Initial pH of the production medium was set at various pH ranging from 4.5-11.5, while production of protease was more at alkaline range than acidic and optimum pH was 9.5 (Fig. 5). There is a positive and high significant correlation found among the initial pH of the medium and protease activity ($r = 0.914$) at $p < 0.001$ which denotes with an increase in pH of the medium, protease production increases. Significance is again confirmed by the t-test even at population level at $p < 0.001$. ANOVA test reveals that there is a high significant difference between the effects of initial pH of the medium on protease production at 0.001 probability level.

Effects of substrate concentrations

It is confirmed from Figure 3 that, the maximum protease activity was found by using skim milk as substrate. Therefore, various concentration of skim milk was further investigated at concentration of 0, 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5% (w/v). Figure 6 reveals maximum protease production at a skim milk concentration of 2.0% (w/v).

There is a positive and significant correlation found among the initial pH of the medium and protease activity ($r = 0.936$) at $p < 0.01$ which denotes with an increase in concentration of skim milk, protease production increases. Significance is again confirmed by the t-test even at population level at $p < 0.05$. ANOVA test reveals that there is a significant difference between the effects of substrate concentrations on protease production at 0.05 probability level.

Effects of nitrogen sources

Various organic nitrogen sources like Yeast Extract, Beef Extract, Peptone and in-organic nitrogen sources like NaNO_3 , KNO_3 and $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ at a concentration of 0.5 and 1.0% (w/v) were taken for investigation. But, there is no significant production found by using these nitrogen sources as compared with the previous control medium. This might be due to the presence of nitrogen source in the form of skim milk in the medium, which was self-sufficient for the productions of protease using strain N-86.

Effects of carbon sources

Various carbon sources like Xylose, Fructose, Lactose, Maltose, Glucose and Sucrose at a concentration of 1.0% (w/v) were taken for investigation with a control medium having previous optimized medium. Among all the carbon sources Sucrose (1% w/v) was regarded as the potential carbon source which induce strain N-86 for more production of protease at 15°C (Fig. 7). ANOVA reveals a high significant differences among the carbon sources used at $p < 0.001$.

Effects of amino-acids

Various amino acids as indicated in Figure 8 at a concentration of 0.01% (w/v) were taken for investigation with a control medium having previous optimized medium, which devoid of any amino acids. Among 20 amino acids *L*-Serine, *L*-Phenylalanine, *L*-Methionine, *L*-Histidine, *L*-Glutamine and *L*-Ascorbic acid were regarded as inducers for protease production by strain N-86.

However, maximum production of protease was observed using *L*-Glutamine. ANOVA reveals a high significant differences among the amino acids for production of protease at $p < 0.001$.

Effects of minerals

Among the eleven (11) mineral chlorides as depicted in Figure 9, only three have inductive effect for the protease production i.e. KCl, MgCl₂ and CaCl₂. MgCl₂ showed maximum protease activity w.r.t. control. ANOVA reveals a high significant differences among the mineral chlorides for protease production at $p < 0.001$.

Effects of inoculum size

Inoculum sizes of 0.1, 0.5, 1.0, 2.5, 5.0, 10 and 20% (v/v) were taken for investigation. Maximum production was found at 10% v/v of strain N-86 using skim milk as substrate (Fig. 10). The t-test reveals that there is a significant variation among the population means of protease activity with respect to varied inoculum sizes at $p < 0.05$.

Effects of incubation period

After all parameters investigated final protease production was done using optimized conditions for 168 h (Fig. 11). Maximum protease production of 1.414 U/ml was achieved on 72 h of incubation at 15°C, which was 5.42 fold increases in protease production as compared with the un-optimized medium condition. The effect of incubation period for protease production is significant as per ANOVA test at $p < 0.01$. The t-stat shows a significant difference between their population mean at 0.01 probability level.

Discussion

Present study investigated on protease production in cold temperature conditions. Therefore, cold temperature bacterial isolates were isolated from sediment samples of Nella Lake and screened primarily on skim milk agar plates and then the potential isolates were selected on the basis of the zone of

clearances followed by quantitative assay using submerged fermentation technique. The potential isolate was identified as *Pseudomonas gessardii* strain N-86 having GenBank accession no. KY783362. It is a Gram -ve bacterial and psychrotolerant in nature. There are very few reports on *Pseudomonas gessardii* strains. Among them many researches were focused on lipase production (Ramani *et al.*, 2010; Ramani and Sekaran, 2012; Veerapagu *et al.*, 2013; von Neubeck *et al.*, 2017). A research was found on *Pseudomonas gessardii* DSM 17152^T isolated from bovine raw milk which showed proteolysis at 4°C on plate assay, while it showed negative at 25°C (von Neubeck *et al.*, 2017) which is contradicting with our results having wide range of temperature tolerance capacity of studied *Pseudomonas gessardii* strains N-86. The same pattern of getting protease positive at both 5°C and 25°C was observed by Caldera *et al.*, (2016). Besides, there are some reports available on cold active protease production by *Pseudomonas* spp. but it was limited to primary screening only (Hamamoto *et al.*, 1994; Baghel *et al.*, 2005; Maharana and Ray, 2013). Zeng *et al.*, (2003) reported on a psychrophilic *Pseudomonas* strain DY-A isolated from deep sea produces cold active serine alkaline proteases at 10°C. A psychrophilic strain *Pseudomonas* TAC II 18 was isolated from frozen vegetables abandoned for 40 years at the fire destroyed Antarctic station Port Martin in Terre Adelie, showed maximum protease production at 4°C than 25°C (Chessa *et al.*, 2000). Shivaji *et al.*, (2011) reported on *Pseudomonas gessardii* CIP 105469^T isolated from sediment sample of fresh water lake situated at Schirmacher Oasis, Queen Maud Land, Antarctica, which showed negative for protease production at 4°C.

Effects of various physical and chemical parameters were considered for optimize protease production by the strain N-86.

Incubation temperature is not only necessary for the growth of a micro-organism but it also induces the microbes to produce respective hydrolytic enzymes for the utilization of substrates efficiently for survival (Maharana and Singh, 2018a). Low temperature regarded as an extreme environment and present study was focused on isolation of cold active protease. Therefore, protease production was investigated from 5°C to 45°C. As depicted in Figure 4, optimum temperature is 15°C and the production is more at lower temperature than the higher ranges; therefore, it can be regarded as cold active protease having maximal activity towards low temperature which may have potential application to various industrial sectors. In contrary, psychrotrophic *Pseudomonas* spp. were isolated from Blue-green Algal Mats of Schirmacher Oasis, Antarctica having optimum temperature as 30°C for protease production (Singh and Ramana, 1998).

From Figure 5 one can conclude that the protease production is more in alkaline condition (optimum is at 9.5 pH) than acidic ranges which denotes alkalinity nature of the strain N-86 and its protease. This cold tolerant alkaline protease can be used in various food and detergent industries. While in contrary, Singh and Ramana, (1998) isolated *Pseudomonas* spp. from Schirmacher Oasis, Antarctica, which showed pH 7.0 as optimum for protease production. The effect of substrate concentrations is a crucial parameter for enzyme production which is depicted in Figure 6. An appropriate amount of substrate in a production medium not only will be utilized by the concerned microorganisms for their growth but also it can help to enhance the enzyme production effectively (Maharana and Singh, 2018c). A concentration of 2% (w/v) of skim milk was regarded as optimum for protease production by the strain N-86. After that protease production decreases due to increase in viscosity of the production

medium which in turn reduces the effective circulation of nutrients and oxygen (Dhume *et al.*, 2019).

Additional carbon and mineral sources and some amount of amino acids may enhance the enzyme production capacity of a bacterial isolate by giving additional nutrients to the microorganisms for growth which in-turn enhance the enzyme production in same proportion (Maharana and Singh, 2018c). Singh and Ramana, (1998) isolated psychrotrophic *Pseudomonas* spp. from Antarctica, which was assessed for hydrolytic enzymes including proteases. They reported best carbon sources for protease production at 15°C were Lactose and Maltose while in contrary showing optimum with 1% (w/v) Sucrose (Fig. 7). Carbon sources are not having the direct stimulating effects on enzyme production besides, giving an additional nutrients for the microorganisms for growth which successively increase the enzyme production in same ratio (Maharana and Singh, 2018a). Among 20 amino acids, maximum production of protease is found in production medium having 0.01% (w/v) *L*-Glutamine (Fig. 8). Reduction in enzyme activity by the other amino acids was presumably due to non-utilization or inhibitory effects of the amino acids on the growth of strain N-86 and, as the growth is retarded, so is the activity (Maharana and Ray, 2015b). Mineral salts are required for microbial growth and other activities. MgCl₂ shows maximum protease activity w.r.t. control as depicted in Figure 9. Chlorides of Cobalt and Mercury drastically minimized protease production by strain N-86.

Initial inoculum concentration in a production medium plays an essential role in enzyme production by controlling the growth of a microbe. Small inoculum size controls and shortens the initial lag phase whereas larger inoculum size increases the duration of lag

phase (Singh *et al.*, 2012a). Maximum protease production was showed using 10% (v/v) of inoculum of strain N-86 (Fig. 10) and a sharp fall was observed at 20% of the same. After that a final production was conducted using all the optimized parameters for final production which showed maximum protease production was found at 15°C using *Pseudomonas gessardii* strain N-86 on 72 h. At initial stage of production, a gradual

increase in the protease production was experienced which was due to the abundant nutrients and no growth inhibitors. But after 72 h there was a slight fall of protease production which might be due to the depletion of nutrients, accumulation of toxic end products and the change in pH of the medium. Table 1 depicts the comparisons between the initial production medium with optimized medium conditions.

Table.1 Comparison between initial un-optimized medium conditions w.r.t. final optimized conditions for production of cold active protease

| Parameters & Chemical composition | Optimized conditions (Strain N-86) | Un-optimized conditions |
|---------------------------------------|------------------------------------|-------------------------|
| Substrate, skim milk (w/v) | 2% | 1% |
| NaCl (w/v) | 0.2% | 0.2% |
| CaCl ₂ (w/v) | 0.01% | 0.01% |
| MgSO ₄ (w/v) | 0.04% | 0.04% |
| K ₂ HPO ₄ (w/v) | 0.03% | 0.03% |
| KH ₂ PO ₄ (w/v) | 0.03% | 0.03% |
| NH ₄ SO ₄ (w/v) | 0.05% | 0.05% |
| pH | 9.5 | 7.5 |
| Inoculum size (v/v) | 10% | 5% |
| Nitrogen source (1% w/v) | - | - |
| Carbon source (1% w/v) | Sucrose | - |
| Minerals (0.1% w/v) | MgCl ₂ | - |
| Amino-acid (0.01% w/v) | L-Glutamine | - |
| Incubation temperature (°C) | 15 | 15 |
| Incubation period (h) | 72 | 48 |
| Protease (U/ml) | 1.414 | 0.261 |
| FOLD INCREASE | | 5.42 |

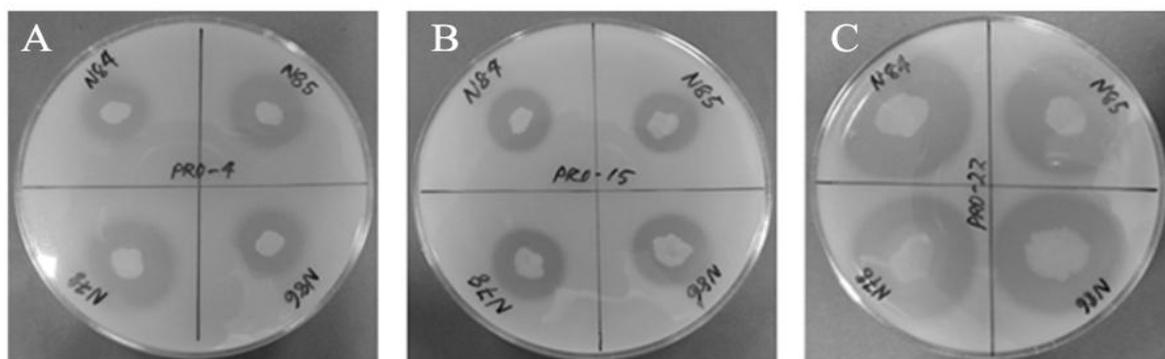


Fig.1 Qualitative plate assay of some Nella lake bacterial isolates for protease using skim milk agar (A) 4°C, (B) 15°C and (C) 22 °C

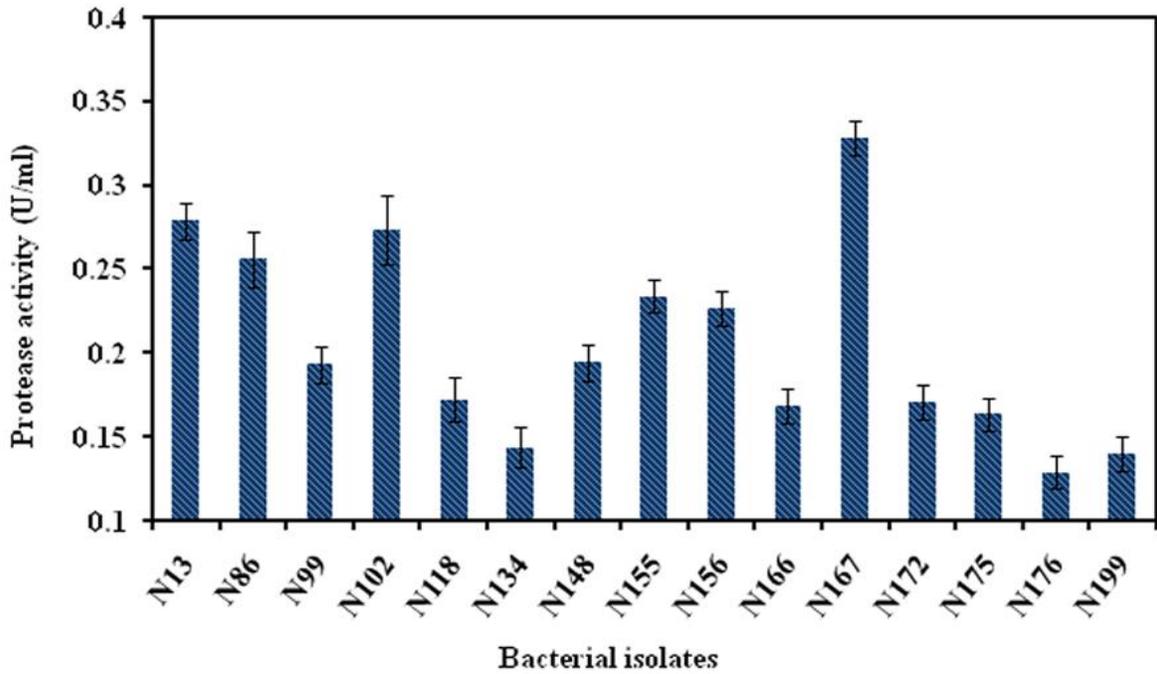


Fig.2 Cold active protease production using 15 potential bacterial isolates at 15°C using casein as substrate incubated for 48 h.

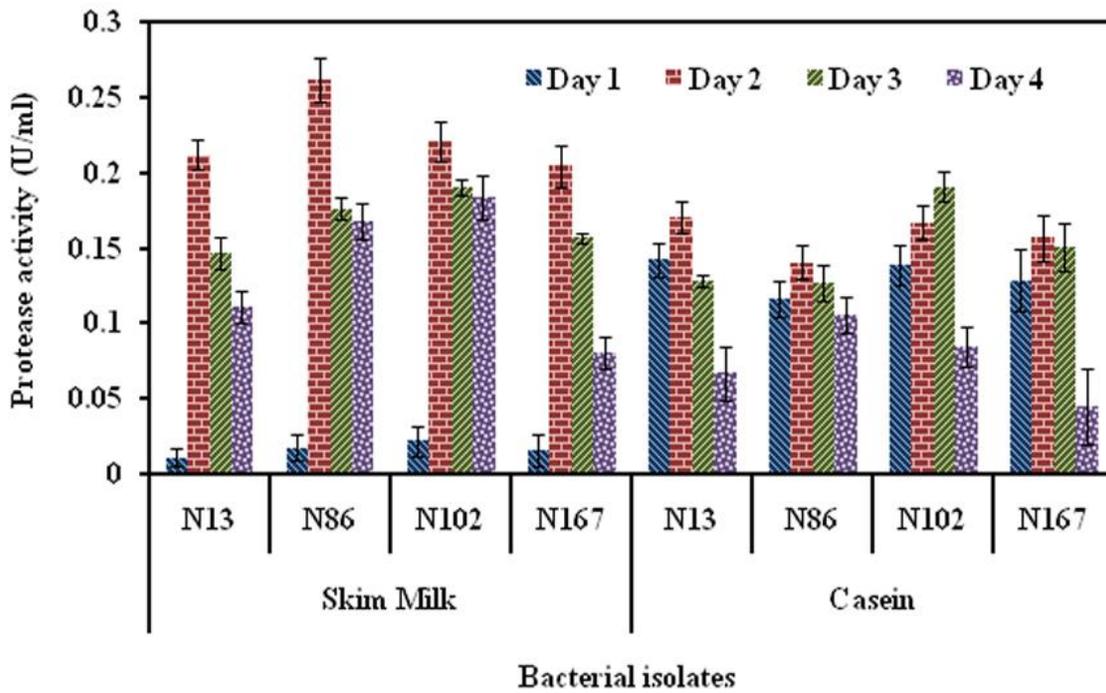


Fig.3 Day-wise cold active protease production using best 4 bacterial isolates using various substrates at 15°C

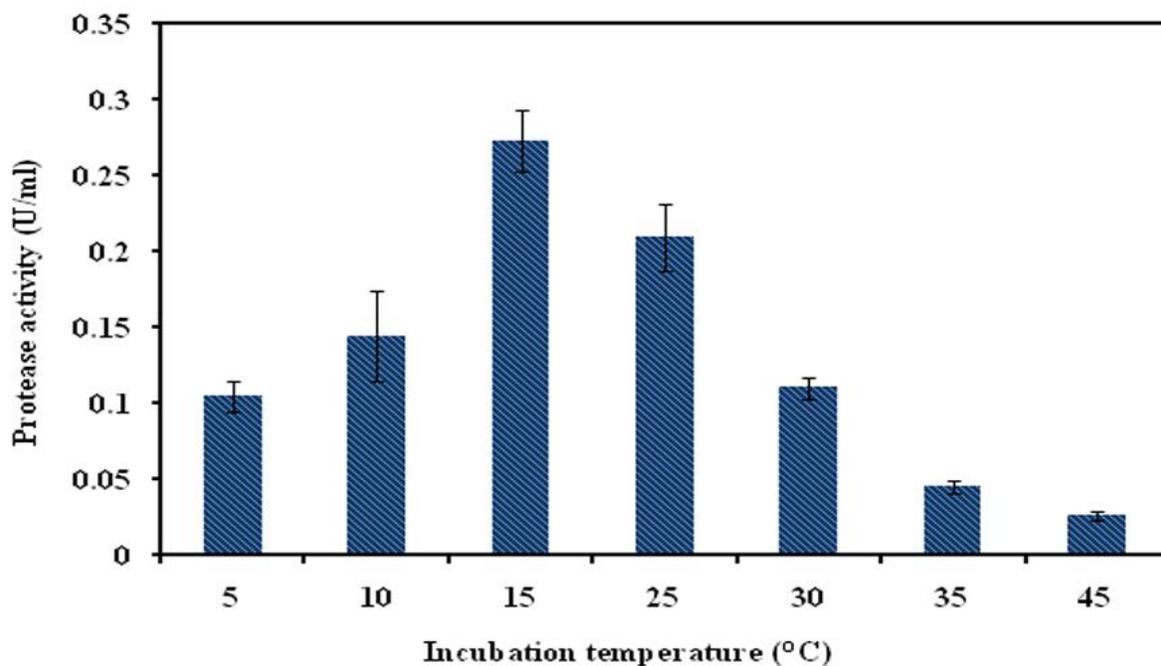


Fig.4 Effects of various incubation temperatures on cold active protease production by strain N-86. The results are the means of 3 independent experiments and the bars correspond to standard errors

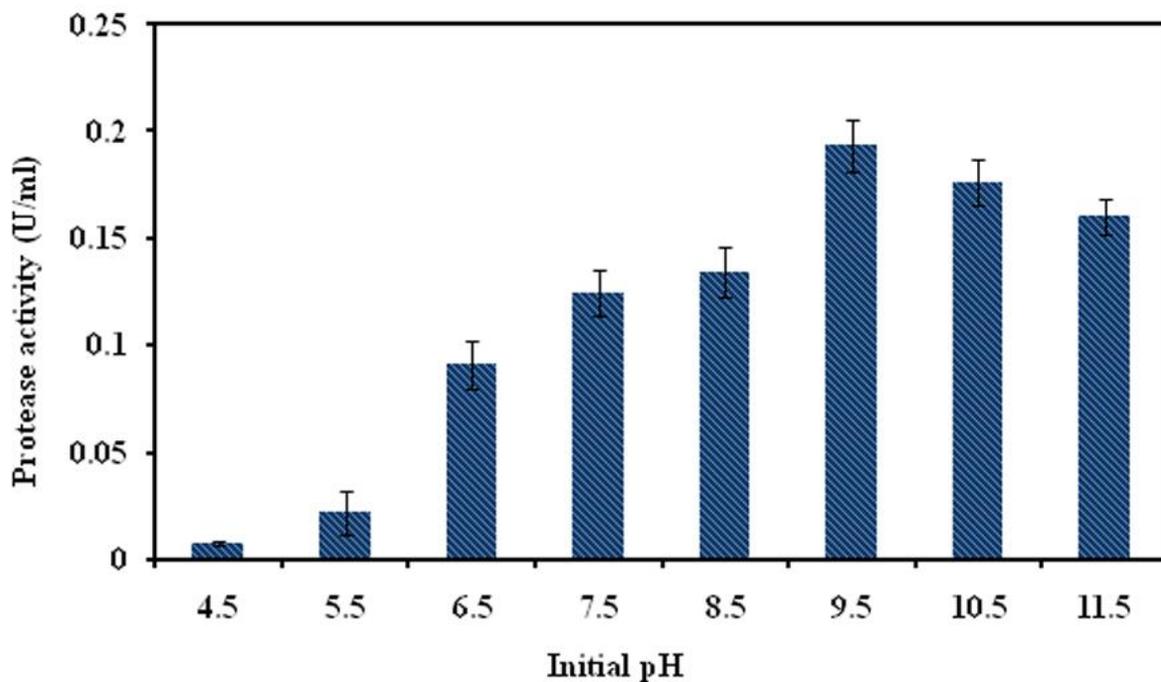


Fig.5 Effects of initial pH of the medium on cold active protease production by strain N-86 at 15°C. The results are the means of 3 independent experiments and the bars correspond to standard errors

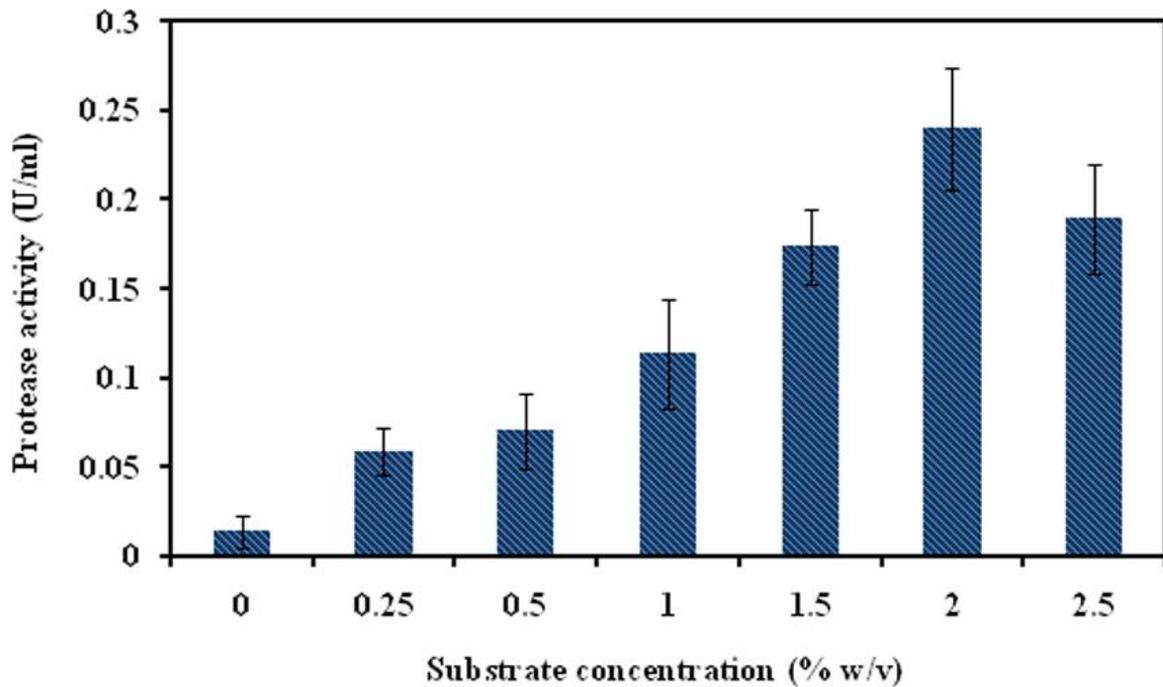


Fig.6 Effects of substrate concentrations on cold active protease production by strain N-86 at 15°C. The results are the means of 3 independent experiments and the bars correspond to standard errors

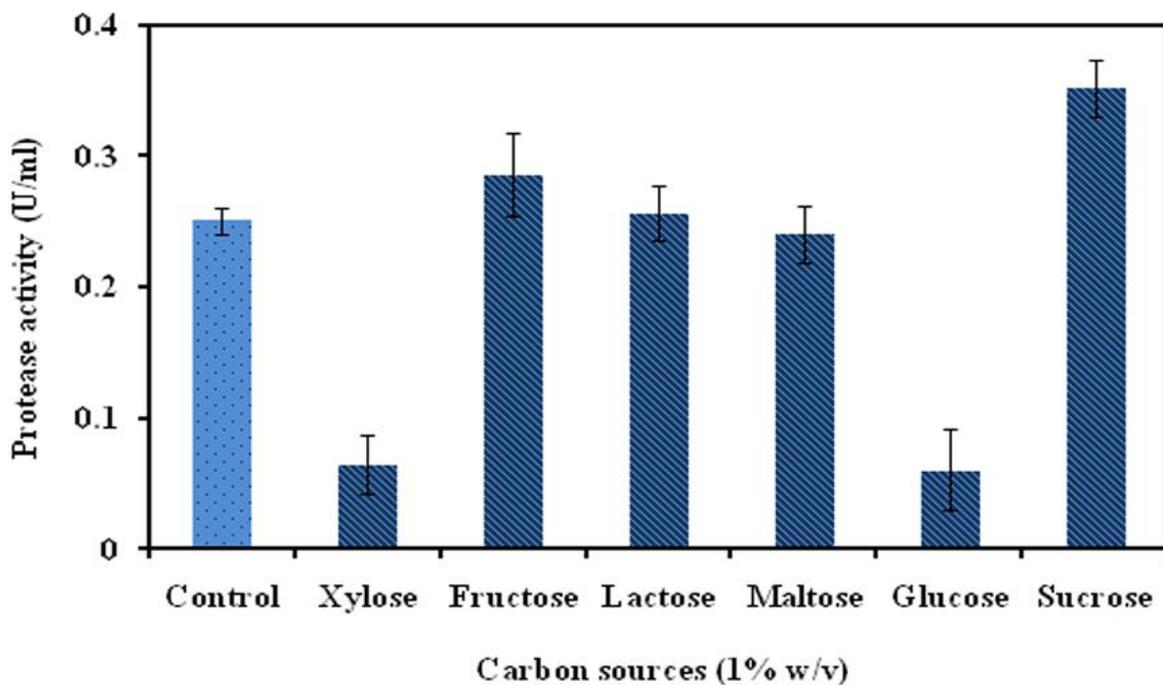


Fig.7 Effects of carbon sources on cold active protease production by strain N-86 at 15°C. Control medium is devoid of any additional carbon sources. The results are the means of 3 independent experiments and the bars correspond to standard errors

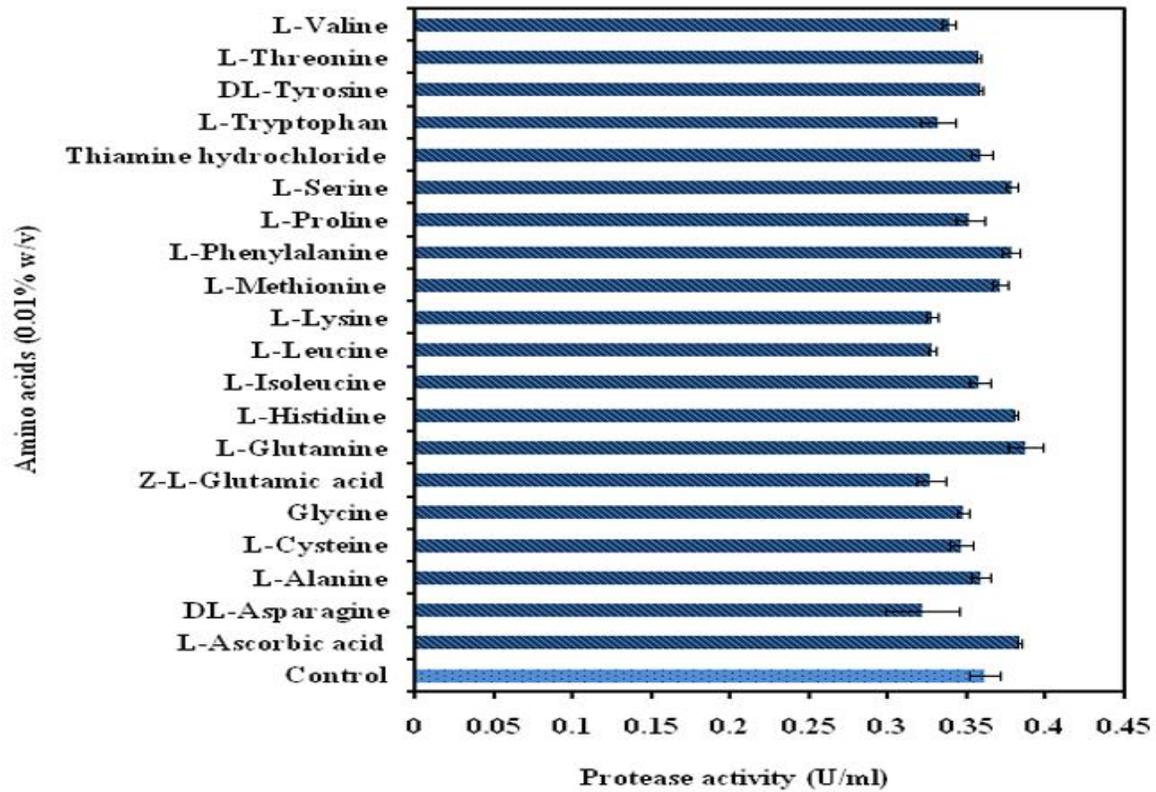


Fig.8 Effects of amino-acids on cold active protease production by strain N-86 at 15°C. Control medium is devoid of any additional amino acids. The results are the means of 3 independent experiments and the bars correspond to standard errors

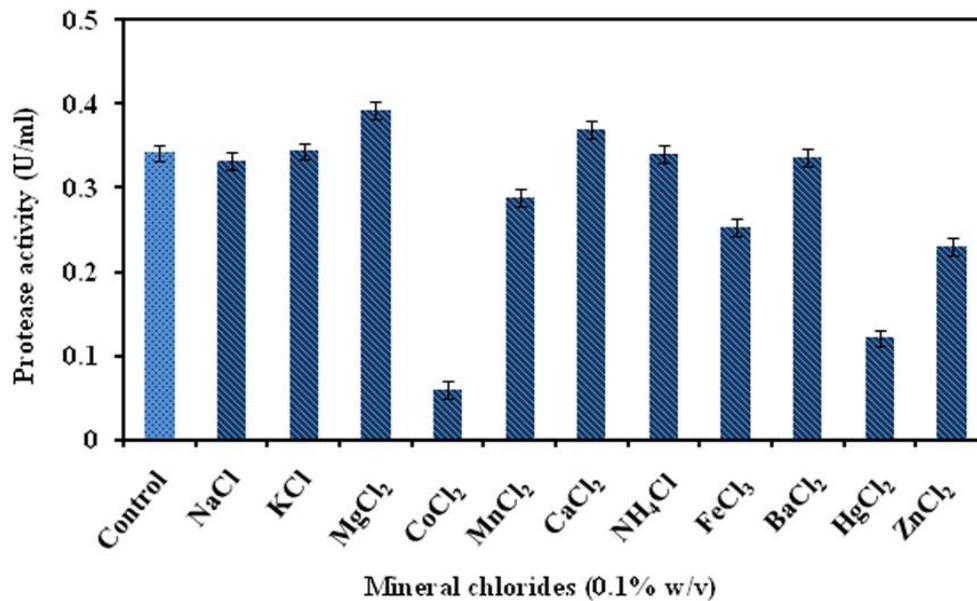


Fig.9 Effects of mineral chlorides on cold active protease production by strain N-86 at 15°C. Control medium is devoid of any additional minerals. The results are the means of 3 independent experiments and the bars correspond to standard errors

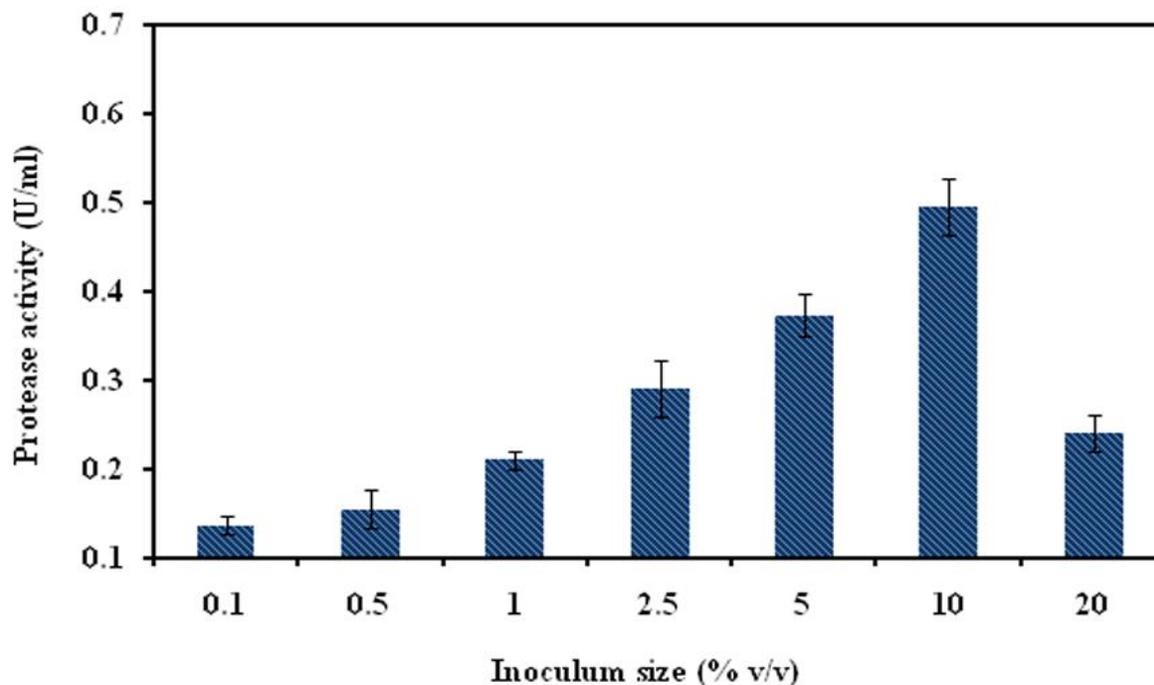


Fig.10 Effects of inoculum sizes on cold active protease production by strain N-86 at 15°C. The results are the means of 3 independent experiments and the bars correspond to standard errors

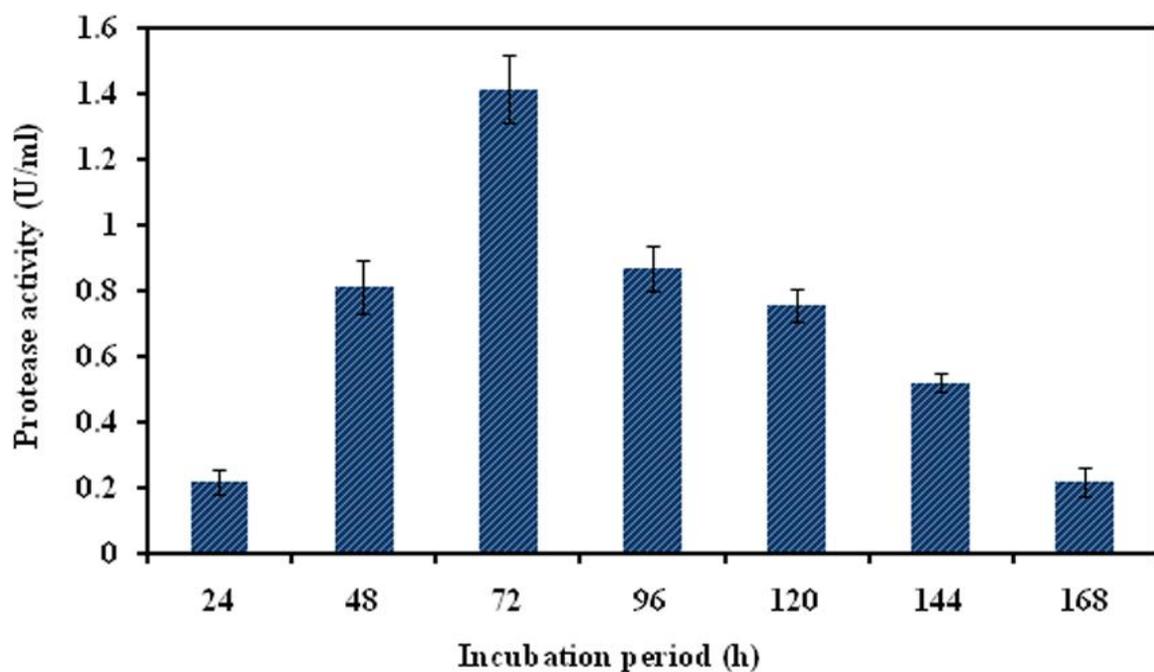


Fig.11 Effects of incubation period on cold active protease production by strain N-86 at 15°C. The results are the means of 3 independent experiments and the bars correspond to standard errors

In summary, the present study successfully optimized the production medium enhancing the protease production effectively from un-optimized conditions, which confirmed from the fold increase in protease by 5.42 using *Pseudomonas gessardii* strain N-86.

Besides, to the best of our knowledge, our study is the first report on *Pseudomonas gessardii* isolated from Nella Lake, Antarctica producing cold active protease. Therefore, protease produced from above cold tolerant microorganisms can be used in various fields of science like detergent formulation for cold temperature washing of clothes and protein contaminants removal from waste water in cold and temperate regions due to their alkaline and cold tolerance capabilities.

Presently studied cold active protease may provide new insights into the potential biotechnological applications of the isolate in Antarctic environments.

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References

Baghel, V. S., R. D. Tripathi, P. W. Ramteke, K. Gopal, S. Dwivedi, R. K. Jain, U. N. Rai and Singh, S. N. 2005. Psychrotrophic proteolytic bacteria from cold environment of Gangotri glacier, Western Himalaya, India. *Enzyme Microb. Technol.* 36, 654-659.

- Caldera, L., L. V. Franzetti, E. V. Coillie, P. D. Vos, P. Stragier, J. D. Block and Heyndrickx, M. 2016. Identification, enzymatic spoilage characterization and proteolytic activity quantification of *Pseudomonas* spp. isolated from different foods. *Food Microbial.* 54, 142-153.
- Chessa, J. P., I. Petrescu, M. Bentahir, J. V. Beeumen and Gerday, C. 2000. Purification, physico-chemical characterization and sequence of a heat labile alkaline metalloprotease isolated from a psychrophilic *Pseudomonas* species. *Biochimica. et. Biophysica. Acta.* 1479(1-2): 265-274.
- Dhume, G. M., A. K. Maharana, M. Tsuji, A. K. Srivastava and Singh, S. M. 2019. Cold-tolerant endoglucanase producing ability of *Mrakia robertii* A2-3 isolated from cryoconites, Hamtha glacier, Himalaya. *J. Basic Microbial.* 59(7): 667-679.
- Gao, X-G., S-G. Cao and Zhang, K-C. 2000. Production, properties and application to non-aqueous enzymatic catalysis of lipase from a newly isolated *Pseudomonas* strain. *Enzyme Microb. Tech.* 27, 74-82.
- Hamamoto, T., M. Kaneda, K. Horikoshi and Kudo, T. 1994. Characterization of protease from a psychrotroph, *Pseudomonas fluorescens* 114. *Appl. Environ. Microbiol.* 60(10): 3878-3880.
- Huang, H., K. Wu, A. Khan, Y. Jiang, Z. Ling, P. Liu, Y. Chen, X. Tao and Li, X. 2016. A novel *Pseudomonas gessardii* strain LZ-E simultaneously degrades naphthalene and reduces hexavalent chromium. *Bioresour. Technol.* 207, 370-378.
- Maharana A. K., 2016. Extracellular cold active endoglucanase and pigment producing psychrotolerant *Penicillium Pinophilum*. *Int. J. Pharm. Pharm. Sci.* 8(10): 164-169.
- Maharana A.K., I. Sinha, P. C. Behera and Ray, P. 2016. Extracellular alkaline protease producing halo-alkalitolerant bacteria isolated from marine coasts of Odisha. *Int. J. Pharm. Pharm. Sci.* 8(5): 379-386.
- Maharana, A. and Ray, P. 2015a. A novel cold-

- active lipase from psychrotolerant *Pseudomonas* sp. AKM-L5 showed organic solvent resistant and suitable for detergent formulation. *J. Mol. Catal. B: Enzym.* 120, 173-178.
- Maharana, A. K. and Ray, P. 2013. Isolation and screening of cold active extracellular enzymes producing psychrotrophic bacteria from soil of Jammu City. *Biosci. Biotechnol. Res. Asia.* 10(1): 267-273.
- Maharana, A. K. and Ray, P. 2014a. Low temperature degradation of various substrates by psychrotolerant *Fusarium* spp. isolated from soil of Jammu city. *J. Adv. Microbiol.* 1(1): 52- 56.
- Maharana, A. K. and Ray, P. 2014b. Screening of psychrotrophic micro-fungi for cold active extracellular enzymes isolated from Jammu city, India. *J. Pure Appl. Microbiol.* 8(3): 2369-2375.
- Maharana, A. K. and Singh, S. M. 2018a. A cold and organic solvent tolerant lipase produced by Antarctic strain *Rhodotorula* sp. Y- 23. *J. Basic Microbiol.* 58(4): 331-342.
- Maharana, A. K., and Ray, P. 2014c. Application of Plackett-Burman Design for improved cold temperature production of lipase by psychrotolerant *Pseudomonas* sp. AKM-L5. *Int. J. Curr. Microbiol. Appl. Sci.* 3(4): 269-282.
- Maharana, A. K., and Ray, P. 2015b. Optimization and characterization of cold active endoglucanase produced by *Aspergillus terreus* strain AKM-F3 grown on sugarcane bagasse. *Turk. J. Biol.* 39, 175-185.
- Maharana, A. K., and Singh, S. M. 2018b. Cold active amylases producing psychrotolerants isolated from Nella Lake, Antarctica. *Biosci. Biotechnol. Res. Asia* 15(1): 5-16.
- Maharana, A. K., and Singh, S. M. 2018c. Cold active lipases produced by *Cryptococcus* sp. Y-32 and *Rhodococcus erythropolis* N149 isolated from Nella Lake, Antarctica. *Int. J. Curr. Microbiol. App. Sci* 7(3): 1910-1926.
- Matsui, M., A. Kawamata, M. Kosugi, S. Imura, and Kurosawa, N. 2017. Diversity of proteolytic microbes isolated from Antarctic freshwater lakes and characteristics of their cold-active proteases. *Polar Sci.* 13, 82-90.
- Park, H. J., Y. M. Lee, S. Kim, A. R. Wi, S. J. Han, H. W. Kim, I. C. Kim, J. H. Yim and Kim, D. 2014. Identification of proteolytic bacteria from the Arctic Chukchi Sea expedition cruise and characterization of cold-active proteases. *J. Microbiol.* 52, 825-833.
- Ramani, K., and Sekaran, G. 2012. Production of lipase from *Pseudomonas gessardii* using blood tissue lipid and thereof for the hydrolysis of blood cholesterol and triglycerides and lysis of red blood cells. *Bioproc. Biosyst. Eng.* 35(6): 885-896.
- Ramani, K., E. Chockalingam, and Sekaran, G. 2010. Production of a novel extracellular acidic lipase from *Pseudomonas gessardii* using slaughterhouse waste as a substrate. *J. Ind. Microbiol. Biot.* 37(5): 531-535.
- Rashid, N., S. Yuji, E. Satoshi, A. Haruyuki and Tadayuki, I. 2001. Low temperature lipase from psychrotrophic *Pseudomonas* sp. Strain KB700A. *Appl. Environ. Microbiol.* 67, 4064-4069.
- Reddy, P. V. V., S. S. S. N., Rao, M. S., Pratibha, B., Sailaja, B., Kavya, R. R., Manorama, S. M., Singh, T. N. R. Srinivas and Shivaji, S. 2009. Bacterial diversity and bio-prospecting for cold-active enzymes from culturable bacteria associated with sediment from a melt water stream of Midtre Lov' enbreen glacier, an Arctic glacier. *Res. Microbiol.* 160(8): 538-546.
- Sen, S., B. Mandal and Dasu, V. V. 2009. Alkaline Protease: a tool to clean environment. In: AIChE Spring National Meeting.
- Shivaji, S., K. Kumari, K. H. Kishore, P. K. Pindi, P. S. Rao, T. N. R. Srinivas, R. Asthana, and Ravindra, R. 2011. Vertical distribution of bacteria in a lake sediment from Antarctica by culture-independent and culture-dependent approaches. *Res. Microbiol.* 162(2): 191-203.

- Singh, A. K., A. K. Maharana, H. Masih, Y. Kumar and Mishra, S. K. 2012a. Production, optimization and purification of bacterial cellulase by solid-state bio-processing of agro biomass. *Res. J. Pharm. Biol. Chem. Sci.* 3(2): 977-989.
- Singh, L. and Ramana, K. V. 1998. Isolation and characterization of psychrotrophic Antarctic bacteria from blue-green algal mats and their hydrolytic enzymes. Fourteenth Indian Expedition to Antarctica, Scientific Report, Department of Ocean Development, Technical Publication No. 12, pp 199-206.
- Singh, P., S. M. Singh and Dhakephalkar, P. 2014. Diversity, cold active enzymes and adaptation strategies of bacteria inhabiting glacier cryoconite holes of High Arctic. *Extremophiles*.18(2): 229-242.
- Singh, P., S. M. Singh and Roy, U. 2016. Taxonomic characterization and the bio-potential of bacteria isolated from glacier ice cores in the High Arctic. *J. Basic Microbiol.* 56(3): 275-285.
- Singh, S. M., A. K. Maharana and Naik, S. 2017. Microbial communities of Nella Lake, Larsemann Hills, East Antarctica. In: Anton Van de Putte (Ed.), Book of Abstracts, XIIth SCAR Biology Symposium. Leuven, Belgium, Scientific Committee on Antarctic Research (SCAR), Cambridge, United Kingdom. Pp. 512.
- Singh, S. M., S. K. Singh, L. S. Yadav, P. N. Singh and Ravindra, R. 2012b. Filamentous soil fungi from Ny-Ålesund, Spitsbergen, and screening for extracellular enzymes. *Arctic*. 45-55.
- Vazquez, S. C., and Mac Cormack, W. P. 2002. Effect of isolation temperature on the characteristics of extracellular proteases produced by Antarctic bacteria. *Polar Res.* 21(1): 63-72.
- Veerapagu, M., A. S. Narayanan, K. Ponmurugan, and Jeya, K. R. 2013. Screening selection identification production and optimization of bacterial lipase from oil spilled soil. *Asian J. Pharm. Clin. Res.* 6(3): 62-67.
- von Neubeck, M., C. Huptas, C. Glück, M. Krewinkel, M. Stoeckel, T. Stressler, L. Fischer, J. Hinrichs, S. Scherer and Wenning, M. 2017. *Pseudomonas lactis* sp. nov. and *Pseudomonas paralactis* sp. nov., isolated from bovine raw milk. *Int. J. Syst. Evol. Microbiol.* 67(6): 1656-1664.
- Zeng, R., R. Zhang, J. Zhao and Lin, N. 2003. Cold-active serine alkaline protease from the psychrophilic bacterium *Pseudomonas* strain DY-A: enzyme purification and characterization. *Extremophiles*. 7(4): 335-337.

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