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Isolation and characterization of *Pseudomonas stutzari* strain K-1 protein

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Abstract

Pseudomonas stutzari is one of the important nitrogen fixing agents, based on its physiological properties, and denitrification relevance to overall nitrogen recycling. Considering the significance of these microorganisms playing a symbiotic role serving for improving crops production; this study was aimed to explore the temperature optimization for *Pseudomonas stutzari* strain K-1 proteins` isolation and characterization. The effect of the suitable temperature conditions ranging from 20°C to 60°C for this strain was found through genome expression in the form of protein production and 42°C temperature was found to be best for optimum organism growth. Several *Pseudomonas* genus has been applied to cereal seeds and few directly applied to soil as bio-control agents and preventing the growth or establishment of crop pathogens.

Keywords: Denitrification, Protein estimation, *Pseudomonas stutzari*, SDS-PAGE, Soil bio-control.

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1. Introduction

Pseudomonas stutzari is very important among ten major groups of *Pseudomonas* (gram negative single cell bacterium), which is recognized as *rRNA* homology groups [1].Mostly members of *P.Stutzari* were isolated from the soil and aquatic habitats and colonies of this group have their specific and distinguish shape, wrinkled shape reddish brown, hard and dry [2-3]. Phenotypically *Pseudomonas spp*, grows on minimal, chemically defined media with ammonium ions or nitrate and a single organic compound, sole source of carbon and energy [4]. The species most closely related to *P. stutzari* is *P. balearica* on phylogenetic bases when compared phenotypically and grows over 42°C [5]. Denitrification is a stable trait of *Pseudomonas stutzari* which is a heterotrophic bacterium and has been considered as model organism.

Pseudomonas stutzari plays imperative role in plant development by controlling the growth hormones like Auxin, Cytokines, BNF and IAA which plays a key part in plant growth [6-7]. Certain members of this group have been applied to cereals seeds or applied directly to soil as bio-control agent and thus preventing growth or founding of crop pathogens. *Pseudomonas spp.* promote the plant growth by improving the growth restricting conditions directly by producing antagonistic substances like antibiotics, siderophores and variety of enzymes. Salicylic acid is the compound with siderophores activity has been found under iron limitations in *Pseudomonas spp.* [8]. From taxonomic and physiological perspective, pyoverdines are important pigments and their production is enhanced under conditions of iron starvation [9]. Some strains of *P. stutzari* are important in synthesizing siderophores and have been described due to their higher absorption potential and resistance to metals such as aluminum, chromium, cobalt, germanium, lead, manganese, nickel, plutonium, selenium, silver, thallium, uranium, vanadium, and zinc [10].

Pseudomonas stutzari are involved in environmentally important metabolic activities, like metal recycling and degradation of compounds. In view of importance of this organism, the present research work was designed to study the effect of temperature on growth and proteins profiling in the *Pseudomonas stutzari*, which having the ability of BNF hormones and siderophores production [11]. This study is very important in the field of plant production as *Pseudomonas stutzari* strain K-1 and an important tool to be used to enhance plant production. Temperature optimization for the growth of *Pseudomonas stutzari* strain K-1 and isolation and characterization of protein under different temperature conditions were studied.

2. Materials and Methods

2.1. Inoculation and culturing of Pseudomonas stutzari K-1

Pseudomonas stutzari strain K-1 was collected from damp soil and sub cultured on Agar plates. LB medium (40 mL) was prepared as starting inoculum in 250 mL Erlenmeyer flask and autoclaved for 15 minutes at121°C. A loopful culture of *Pseudomonas stutzari* from plates was transferred into the flask. It was then incubated on orbital shaker at 180 rpm for 18 hours at 37°C. To take the cultures of *Pseudomonas stutzari* K-1at different temperatures; 120 µL samples was taken in triplicate flasks and placed in orbital shaker at 180 rpm for 18 hours at 20°C, 30°C, 37°C, 42°C, 50°C and 60°C.

2.2. Protein extraction, characterization and estimation

After 18 hours growth cultures were harvested, centrifuged at 10,000 *xg* for 20 min and the bacterial strain pellets were washed twice with sterile 0.01M phosphate-buffered saline (PBS; pH 7.2). The suspension for each strain was mixed with the same volume of a sample buffer with 1.5% β. mercaptoethanol containing 6% SDS. The mixture was incubated for 1 hour at room temperature and then stored at -20°C. To characterize and quantify the proteins of *Pseudomonas stutzari* strain K-1, cultured at different temperatures e.g. 20°C, 30°C, 37°C, 42°C, 50°C and 60°C and proteins estimation was done for the detection for the amount of proteins present in the sample solutions by the Lowry method [12]. Egg albumin (30mg/100mL) solution was added in 11 test tubes as 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mL, respectively in each tube. Distilled water upto 1.0 mL volume 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.0 mL was added in each test tube. Then reagent C was added (0.5 mL) and the solutions were mixed well and incubated for 15 min at room temperature. The reagent (E) Folin's solution was added (0.5 mL) to each tube and incubated for 30 min. The spectrophotometric absorbance of each standard sample was taken at 450 nm to establish the standard curve after plotting protein concentration against absorbance. The absorbance of unknown sample was checked by interpolation using the standard curve and recorded the concentration of proteins in samples (Fig. 1).

2.3. SDS-PAGE

For the quantitative estimation of proteins, discontinuous gel system was used [13], by using acrylamide-bisacrylamide 30:0.8% powder. Dissolved the resolving gel buffer (pH 8.8) and stacking gel buffer (pH 6.8) with SDS 12.5% and coupled with Bromophenol blue dye (1%). Ammonium persulphate (APS 10%) was added with running gel (X-tris-Glysine-SDS buffer). Loading dye was prepared by dissolving 1.5 mL mercaptoethanol and 2g of SDS in 8 mL of Tris buffer 1mL (pH 6.8). Proteins were stained with brilliant coomassie blue G250; fixed and washed using buffer. Vertical gel electrophoresis systems was used for the separation of proteins of different molecular weight with SDS-PAGE which was prepared by dissolving 12.5% separating gel after the addition of ammonium persulphate and TEMED (N,N,N,N-tetramethylene diamine), then to flatten the surface ethanol was over layered to remove bubbles from stacking gel. Now following the polymerization of separating gel in about 10 min, poured ethanol and when stacking gel was prepared poured immediately on the top of resolving polymerized gel and wells were formed before the polymerization of stacking gel.

2.4. Electrophoresis

Proteins samples (25µL) were prepared with loading dye (5µL) to maintain quantity up to 30 µL and protein marker having molecular weight markers (10-170 kDa). The wells were washed by gel buffer 4-5 times with the help of syringe, then checked by adding buffer in upper spaces, after loading 20 µL of protein marker and 30µL samples in wells, the electrophoresis chamber was fixed with power supply of 15mA and after 30 min was changed to 25mA for 180 min. Electrophoresis was stopped immediately when tracing dye reached 1-2 cm above the bottom of gel in lower chamber. By employing gel documentation system, photograph obtained having different bands reflecting protein pattern of *P. stutzari* strain K1. Gel was removed, fixed, stained to get clear color and destined as well to observe bands results.

3. Results and Discussion

Psuedomonas stutzari strain K1 proteins were isolated and growth was measured by optical density; these proteins were quantified by Lowry method and characterized by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

3.1. Temperature optimization

To find the optimum temperature for maximum growth of *Pseudomonas stutzari strain K-1*, the protein concentration of this strain at various temperature 20°C, 30°C, 37°C, 42°C, 50°C and 60°C at 600nm were studied. The absorbance curve describes the increase of growth (0.8, 1.5, 1.7, 2.0, and 1.3) with the temperature variation and concussively best at 42°C which is (2.0) and minimum at 20°C and 60°C respectively as shown in Fig. 1.

3.2. Protein estimation

The protein estimation of P. *stutzari* was carried out by Lowry method and egg albumin was used as standard. Protein samples were isolated after growth of *P. stutzari* at predefined temperature conditions (20°C, 30°C, 37°C, 42°C, 50°C and 60°C) and standard curve (Fig. 2) was used for the estimation of unknown proteins. Protein concentrations at 30°C, 37°C, 50°C were 6.930, 8.635 and 6.180 mg/mL, respectively. A significant difference in protein concentration was observed at different temperature conditions; maximum was observed at 42°C which was 11.325 mg/mL and no growth was detected at 20°C and 60°C as shown in Table 1.

3.3. Protein characterization by SDS-PAGE

For the determination of molecular weight of *P. stutzari* proteins samples were loaded on 12.5% resolving gel and 4% stacking gel. Four types of electrophoretic profiles were observed at temperature 30°C, 37°C, 42°C, and 50°C.

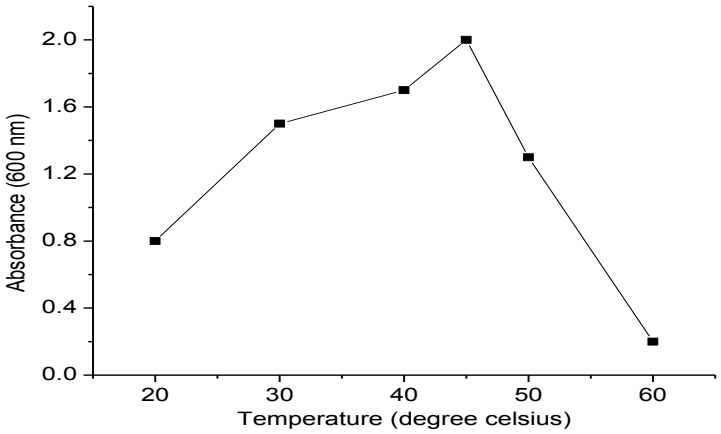

Fig.1. Effect of temperature on the growth of *P. stutzari* in LB growth medium after 18 h

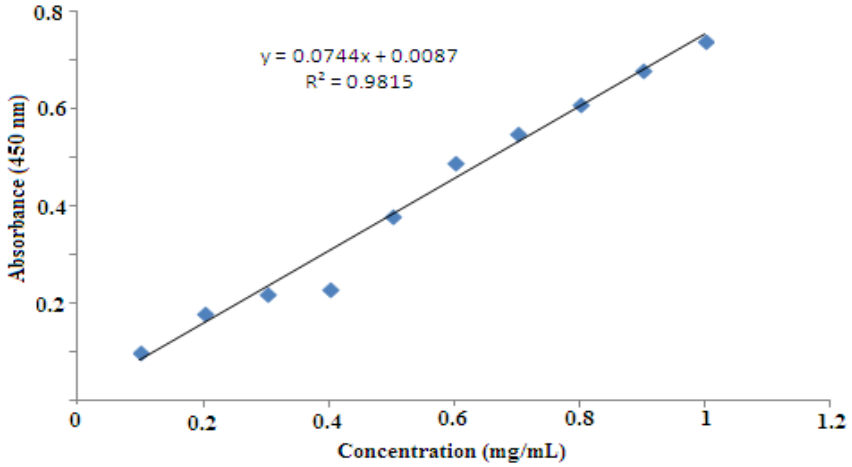

Fig. 2. Standard curve for protein estimation by Lowry method

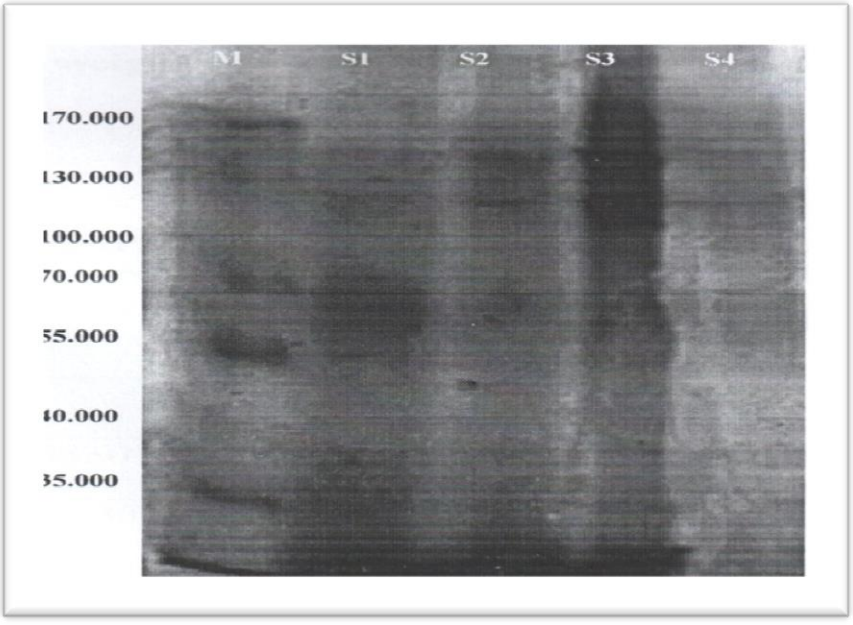

Fig. 3. Patterns of different proteins isolated from *P. stutzari* strain K-1 separated by SDS-PAGE.

(M=Marker, S1=30°C, S2=37°C, S3=42°C, S4=50°C)

Table 1: Protein concentration (mg/mL) of *P. stutzari* by Lowry method

Sr No	*Pseudomonas stutzari* samples	Temperature Variable (Range 20-60°C)	Protein Concentration (mg/mL)
1	Sample 1	20°C	No growth
2	Sample 2	30°C	6.930
3	Sample 3	37°C	9.655
4	Sample 4	42°C	11.325
5	Sample 5	50°C	6.180
6	Sample 6	60°C	No growth

Table 2: Molecular weight of proteins isolated from *P. stutzari* by SDS-PAGE

Markers MW (kDa)	Sample MW (kDa) (30°C)	Sample MW (kDa) (37°C)	Sample MW (kDa) (42°C)	Sample MW (kDa) (50°C)
			220.909	
170.00			171.212	
	148.915	146.433	144.009	146.34
130.00	130.00			
	123.847	121.474	121.473	121.948
			115.258	109.441
100.00				
	90.223	94.836		
70.00	74.067			
55.00		58.015		
		53.015	54.660	53.335
	40.00	43.737		
35.00				36.361

At temperature 30°C, 37°C, 42°C, and 50°C the occurrence of common bands of 53kDa, 121kDa, and 140 kDa was observed (Fig. 3). Different protein patterns were observed at temperature 30°C, 50°C and protein bands were observed to be of 148kDa,130kDa, 123kDa, 90kDa,74kDa, and 43kDa as shown in Table 2. Predominantly *Pseudomonas stutzari* group divided into two sub-biotypes: One clustered around 62% G+C content does not tolerate temperature more than 43°C[13], and a second ranges from 65% to 66% G+C content near 43°C and we observed the optimum temperature growth maximum at 42°C[14].

Protein results showed highest protein concentration at 42°C which was 11.325 mg/mL in comparison to 30°C, 37°C and 50°C as 6.930 mg/mL, 8.635 mg/mL and 6.180 mg/mL, respectively. No growth was observed at 20°C and 60°C. From protein contents, it is suggested that 42°C is the optimum temperature condition for the growth of *P. stutzari* K-1 strain. SDS-PAGE analysis also showed a wide range of protein bands at this temperature and most common bands of 121kDa, 140kDa, and 153kDa were observed at four out of six temperature conditions but the distinct bands of proteins were observed at 42°C. These results were found to be in line with Leang *et. al*. [15], who discovered L-Rh1 protein at 42°C, also revealed that chitinases played an important role in antifungal and antagonistic pathogen control and resulted in plant growth. It is assumed that proteins expressed at 42°C could be one of these Chitinase or L-Rh1 proteins.

4. Conclusion

From results it was concluded that the optimum growth temperature for *Pseudomonas stutzari* strain K-1 was 42°C. At 42°C maximum protein concentration was observed versus all other temperature ranges (20-60°C) studied. The protein contents at 30°C, 37°C and 50°C were found to be 6.930, 8.635 and 6.180 mg/mL, respectively as compared to 42°C where protein contents was 11.325 mg/mL which indicate most suitable temperature for this organism growth.

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