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Studies on Optimization of Amylase Production by *Streptomyces cheonanensis* VUK-A Isolated from Mangrove Habitats

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Authors' contributions

This work was carried out in collaboration between all authors. Author KN performed the statistical analysis, wrote the protocol, and the first draft of the manuscript. Author RKM managed the analyses of the study. Author BSSNHB managed the literature searches, author VM designed the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To optimize the cultural parameters for enhanced amylase production by S*treptomyces cheonanensis* VUK-A.
Place and Duration of the Study: Coringa mangrove ecosystem of Andhra Pradesh, India, between June 2012 and July 2013.
Methodology: About 20 actinobacterial strains were isolated and subjected to primary screening for amylase production. The primary screening was carried out by inoculating the strains on Inorganic salts starch agar medium. The amylase assay was done by using the procedure described by Bernfield. The reaction mixture containing 1 ml of starch solution (10 mg/ml) and 1 ml of enzyme extract was incubated at room temperature for 15 min and the level of reducing sugars was determined by Dinitrosalicylate method. Attempts were made to optimize the various cultural parameters such as pH, temperature, carbon and nitrogen sources for enhanced amylase

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productivity of *S. cheonanensis* VUK-A.
Results: Among the 20 actinobacterial strains screened, one isolate found to be potential and was identified by morphological, cultural, physiological, biochemical characteristics and 16S rRNA analysis. The strain was identified as *Streptomyces cheonanensis* VUK-A. The optimum pH and temperature for amylase production by the strain was found to be 7.0 and 30°C respectively. Different carbon and nitrogen sources were amended separately to the production medium to determine their effect on amylase production. The production of amylase by the strain was enhanced when cultured on ISP-4 broth amended with sorghum flour (30 mg/ml) and peptone (10 mg/ml) with pH 7.0 and incubated at 30°C for 72 h.
Conclusion: The present study revealed that *Streptomyces cheonanensis* VUK-A isolated from mangrove sediments yielded high amounts of amylase in the medium (ISP-4) amended with sorghum flour (30 mg/ml) and peptone (10 mg/ml) with initial pH 7.0 at 30°C after 72 h of incubation. The enzyme yield before optimization was 4.3 U/ml while it was 11.2 U/ml after optimization.

Keywords: Actinobacteria; amylase; optimization pattern; Streptomyces cheonanensis VUK-A.

1. INTRODUCTION

Actinomycetes are one of the most important groups that serve as potential source of biotechnologically interesting substances [1]. Starch is an abundant carbon source in nature and amylases which catalyze the hydrolysis of starch or other carbohydrates to sugar, syrups and dextrin are the most important group of enzymes [2]. Amylolytic enzymes have great significance in biotechnological applications ranging from textile to paper industries [3]. The microbial source of amylases are preferred than other sources because of its plasticity and vast availability [4,5]. Very little information is available on the production of amylases by *Streptomyces* sp. when compared to that of bacteria and fungi [6]. In order to improve the amylase production by actinomycete strains, optimization of cultural conditions is a necessary task. An amylase producing actinomycete strain isolated from the Coringa mangrove ecosystem was identified as *Streptomyces cheonanensis* VUK-A by morphological, cultural, physiological and biochemical characteristics along with 16S rRNA analysis [7]. The present study deals with the optimization of cultural conditions for amylase production by S. *cheonanensis* VUK-A.

2. MATERIALS AND METHODS

2.1 Microorganism

Streptomyces cheonanensis VUK-A was isolated from the Coringa mangrove ecosystem located in Andhra Pradesh, India by using soil dilution plate technique on Inorganic salts starch agar medium.

2.2 Identification of the Strain

Identification of the strain VUK-A was carried out by studying micromorphological, cultural, physiological and biochemical characteristics along with the 16S rRNA gene sequence of the strain [7]. The 16S rRNA gene sequence of the strain has been deposited in NCBI genebank.

2.3 Screening of the Strain for Amylase

The screening of the strain for amylase production was studied by inoculating it on Inorganic salts starch agar (ISP-4) [8] and incubated at 30°C for 72h. After incubation, the plate was flooded with Gram's iodine solution and left for 5 min. The strains which produce amylase were identified by zone of clearance or decolorization against the blue color back ground.

2.4 Production Medium for Amylase

The composition of production medium (Inorganic salts-starch broth (ISP-4) include soluble starch (1 mg/ml), K_2HPO_4 (0.1 mg/ml), $MgSO_4.7H_2O$ (0.1 mg/ml), NaCl (30 mg/ml), $(NH_4)_2SO_4$ (0.2 mg/ml), $CaCO_3$ (0.2 mg/ml), $MnCl_2$ (0.01 mg/ml) and $ZnSO_4$. $7H_2O$ (0.01 mg/ml). The pH was adjusted to 7.2 and medium was sterilized for 15 min at 121°C.

2.5 Amylase Assay

The amylase assay was made by using the procedure described by Bernfield [9]. The reaction mixture containing 1 ml of starch solution (10 mg/ml) and 1 ml of enzyme extract was incubated at room temperature for 15 min. Level of reducing sugars was determined by Dinitrosalicylate method [10]. The reaction was terminated by the addition of 2 ml Dinitrosalicylic (DNS) reagent and the tubes kept at zero time

incubation served as control. The solution was heated in a water bath for 5 min followed by the addition of 1 ml potassium sodium tartrate solution. After cooling, the solution was made up to 10 ml and optical density was read at 560 nm with Spectrophotometer and expressed in units. One amylase unit equals to that amount of enzyme needed to release 1 mg of reducing sugar (maltose as standard) for 15 min at 30°C.

3. OPTIMIZATION OF CULTURAL CONDITIONS FOR AMYLASE PRODUCTION BY THE STRAIN

3.1 Effect of Incubation Period on Growth and Amylase Production

The suspension prepared from one week old culture was inoculated into ISP-4 broth and the fermentation was carried at 30°C for 7 days. Biomass was recorded as dry weight (mg/ml) whereas amylase was analyzed from the culture filtrate served as crude enzyme extract.

3.2 Influence of pH on Amylase Production

The effect of initial pH on amylase production by the strain was determined by inoculating the strain in ISP-4 broth with initial pH levels ranging from 5 to 10 and incubated at 30°C in shaking condition. Enzyme production was measured after 72 h of incubation [11]. The optimal pH achieved at this step was used for further study.

3.3 Impact of Temperature on Amylase Production

To determine the impact of temperature on enzyme production, the strain was inoculated in to inorganic salts-starch broth (ISP-4) with initial temperatures ranging from 25°C to 45°C for 72 hours. Temperature at which the strain showed maximum production was fixed for further studies.

3.4 Influence of Carbon Sources on Amylase Production by the Strain VUK-A

The effect of carbon sources on amylase production was studied by supplementing the production medium (ISP-4) with different carbon sources such as rice flour, sorbitol, sorghum flour, starch and sucrose each at a level of 10 mg/ml (w/v). Influence of various levels of best carbon source (10-50 mg/ml w/v) on enzyme production was also examined [12].

3.5 Effect of Nitrogen Sources on Amylase Production by the Strain VUK-A

The impact of nitrogen sources on enzyme production was also investigated by adding different nitrogen sources like ammonium oxalate, ammonium sulphate, beef extract, malt-extract, peptone, potassium nitrate, tryptone, tyrosine, urea and yeast-extract (each at a concentration of 2 mg/ml) to the production medium containing an optimum amount of the superior carbon source. Besides, the concentration of nitrogen source (2-20 mg/ml) supporting optimal yields of amylase was also recorded [11].

3.6 Statistical Analysis

Statistical data are recorded on biomass of the strain and enzyme production by using One-way Analysis (ANOVA).

4. RESULTS AND DISCUSSION

4.1 Identification of the Strain

The strain VUK-A exhibited typical morphological, cultural, physiological and biochemical characteristics of the genus *Streptomyces* sp. Micromorphology of the strain revealed extensively branched aerial mycelium bearing short chain of spores. As the sporophore morphology is of rectus flexibilis type, the strain may be placed in the rectus-flexibilis group of *Streptomyces* [7]. The 16S rRNA sequence of the strain was deposited in NCBI Gene bank with an accession number JN087502.

4.2 Screening of Amylase

Screening of amylase was carried out on Inorganic salts starch agar (ISP-4) plate method. The strain *Streptomyces cheonanensis* VUK-A produced amylase as evidenced by a clear zone around the colony against the dark blue background (Plate 1).

4.3 Effect of Incubation Period on Growth and Amylase Production by *Streptomyces cheonanensis* VUK-A

Growth as well as amylase production of the strain VUK-A was determined in ISP-4 broth. The production of amylase by the strain began after 24 h of incubation and gradually raised up to 72 h and then started declining (Fig. 1). Shang and Wang recorded high yields of amylases after 48 h of incubation in *Streptomyces rimosus* [13]. The optimal incubation period for obtaining high yields of amylases was reported to be 96 h for *Streptomyces albidoflavus* [12], *S. tendae* TK-VL_333 [11] and *Streptomyces* spp. [14] while it was at 72 h for *S. clavifer* [15] and 48 h for thermophilic *Streptomyces* sp. MSC702 [16] and *S. erumpens* [17].

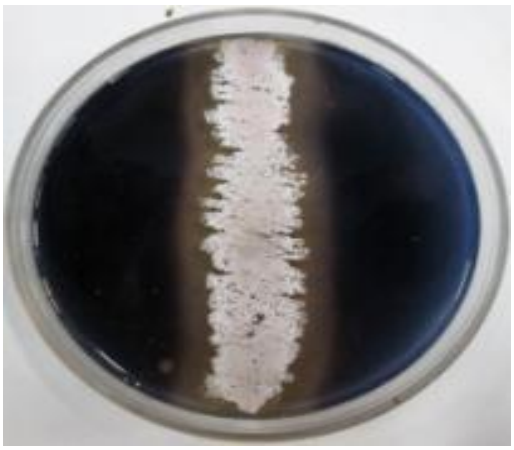

Plate 1. Screening of the strain VUK-A for the production of amylase

4.4 Impact of Initial pH on Amylase Production by *Streptomyces cheonanensis* VUK-A

The optimum pH for amylase production by the strain was found to be 7.0 (Fig. 2) which is consistent with the result of *Streptomyces aureofaciens* 77 [18], *S. erumpens* [17], *S. tendae* TK-VL_333 [11] and *Streptomyces* sp. MSC702 [16]. High yields of amylase were reported at pH 6.7 for *Streptomyces rimosus* [13] and at pH 6.0 for *S. clavifer* [15].

4.5 Effect of Temperature on Amylase Production by *Streptomyces cheonanensis* VUK-A

The strain incubated at 30°C showed maximum yield of amylase production (Fig. 3). The optimal temperature for amylase production was reported at 30°C for *Streptomyces albidoflavus* [12] and *S. tendae* TK-VL_333 [11], while it was 28°C for *S. aureofaciens* 77 [18], 50°C for *S. erumpens* [17] and *Streptomyces* sp. D1 [19]. Shang and Wang [13] recorded high yield of amylases from *Streptomyces rimosus* grown at 40°C.

4.6 Influence of Carbon Sources on Amylase Production by *Streptomyces cheonanensis* VUK-A

Different carbon sources were separately amended to the production medium to determine their effect on amylase production. Sorghum flour was found to be the best carbon source for amylase production by the strain followed by starch and rice flour (Fig. 4). Utilization of carbon sources for the production of amylases by *Streptomyces* sp. was found to vary. High amounts of amylases were reported with starch in *Streptomyces aureofaciens* 77 [18] and *S. albidoflavus* [12], while sorghum flour supported high yields of amylases from *S. tendae* TK-VL_333 [11].

In the present work, maximum yield of amylase was obtained with cheap and easily available carbon source like sorghum flour which may be useful for the development of cost-effective and high quality biotechnological processes for amylase production. As sorghum flour supported high yields of amylases, the effect of different concentrations of sorghum flour was further analyzed.

Optimal productivity of amylase by the strain was observed when cultured in the medium containing 30 mg/ml sorghum flour (Fig. 5). Culture medium amended with 30 mg/ml soluble starch favored high yields of amylase production by *S. aureofaciens* 77 [18] and sorghum flour @ 30 mg/ml by *S. tendae* TK-VL_333 [11].

4.7 Effect of Nitrogen Sources on Amylase Production by *Streptomyces cheonanensis* VUK-A

Nitrogen source appears to be essential for the production of high amount of amylase by *Streptomyces cheonanensis* VUK-A. Amylase biosynthesis by microorganisms has been correlated to the presence or absence of several amino acids and complex nitrogen sources in the medium [16]. In the present study, peptone was found to be the best organic nitrogen source for high amylase production by the strain followed by

yeast extract and beef extract. Ammonium oxalate proved to be good inorganic nitrogen source for the production of amylase by the strain followed by ammonium nitrate, potassium nitrate and ammonium sulphate (Fig. 6). Peptone was reported to be the best organic nitrogen source for high amylase production by *Streptomyces tendae* TK-VL_333 [11] and *Streptomyces* sp. MSC702 [16]. Narayana and Vijayalakshmi [12] reported yeast extract as the best nitrogen source for high amylase production by *S. albidoflavus.*

The production of amylase by the strain was tested by increasing the concentration of peptone in the medium from 1mg/ml to 20 mg/ml. Peptone @10 mg/ml was found to support the production of high levels of the enzyme (Fig. 7). Kavitha and Vijayalakshmi [11] reported peptone @ 5 mg/ml was suitable for high amylase production by *Streptomyces tendae* TK-VL_333 while Narayana and Vijayalakshmi [12] reported yeast extract @ 5 mg/ml as the optimal nitrogen source for amylase production by *Streptomyces albidoflavus.*

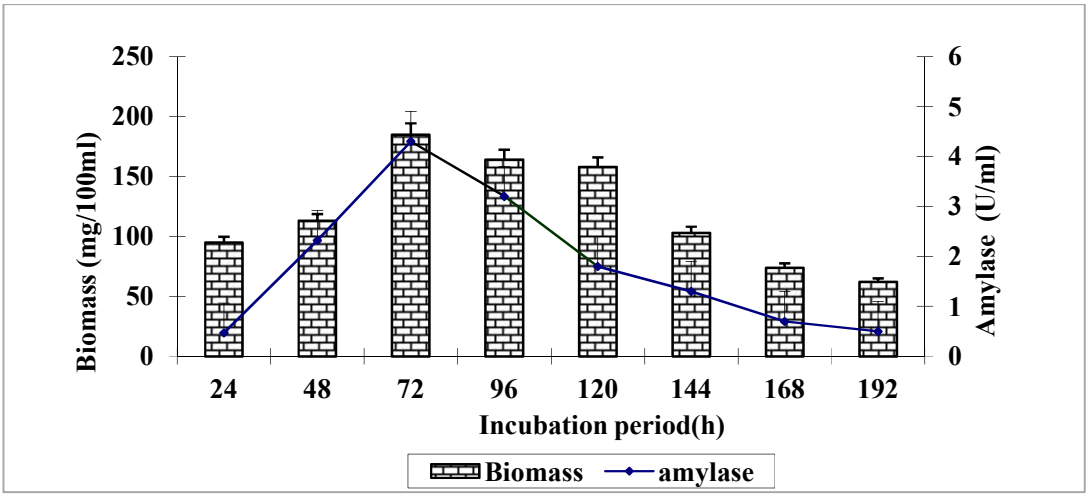

Fig. 1. Effect of incubation period on growth and amylase production by *Streptomyces cheonanensis* VUK-A (Values are the means of three replicates ±SD)

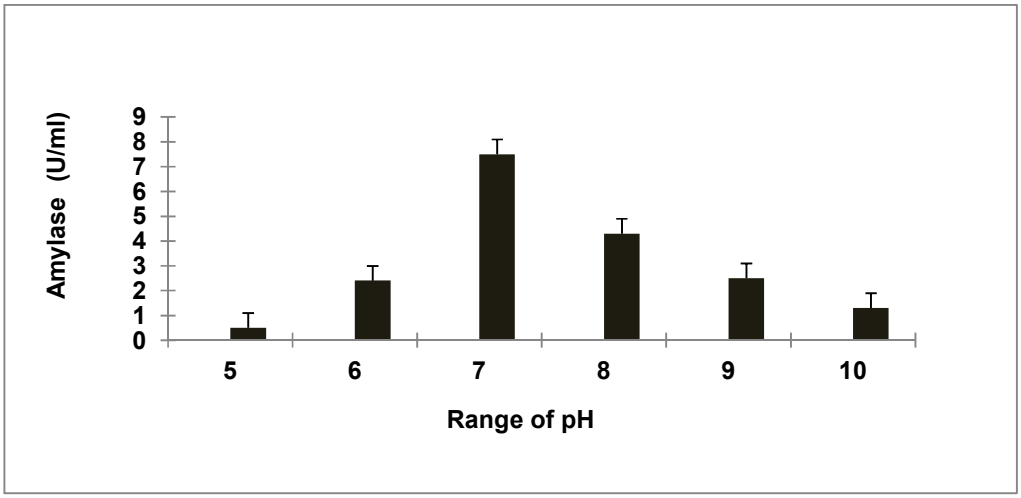

Fig. 2. Impact of pH on amylase production by *Streptomyces cheonanensis* VUK-A (Values are the means of three replicates ±SD)

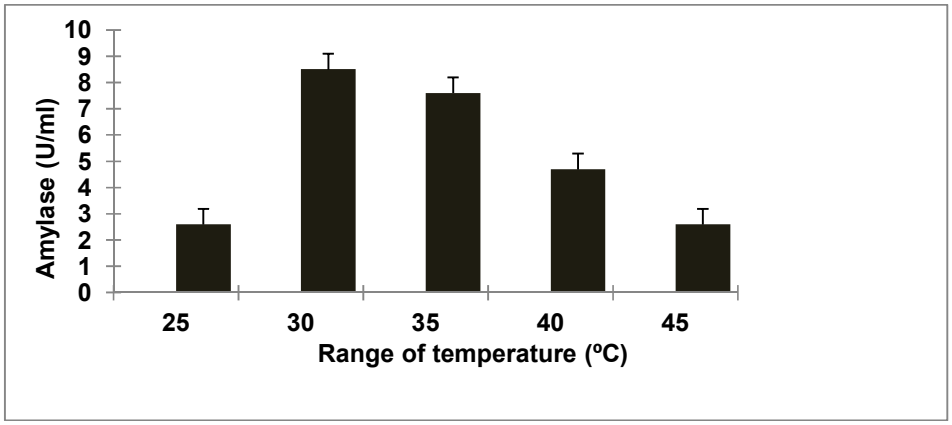

Fig. 3. Effect of temperature on amylase production by *Streptomyces cheonanensis* VUK-A (Values are the means of three replicates ±SD)

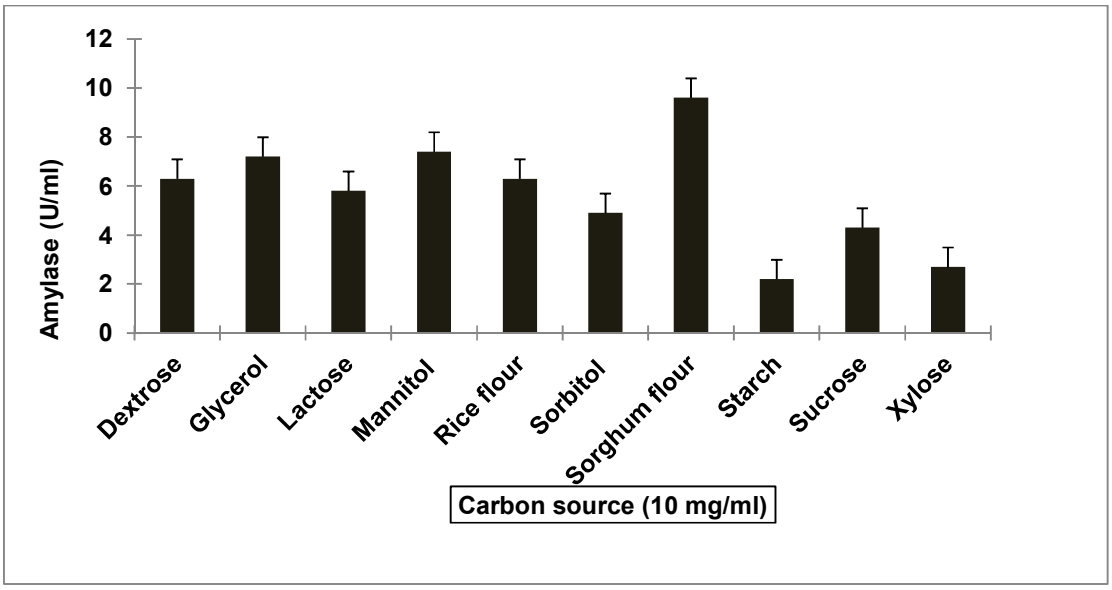

Fig. 4. Influence of carbon sources on amylase production by *Streptomyces cheonanensis* VUK-A (Values are the means of three replicates ±SD)

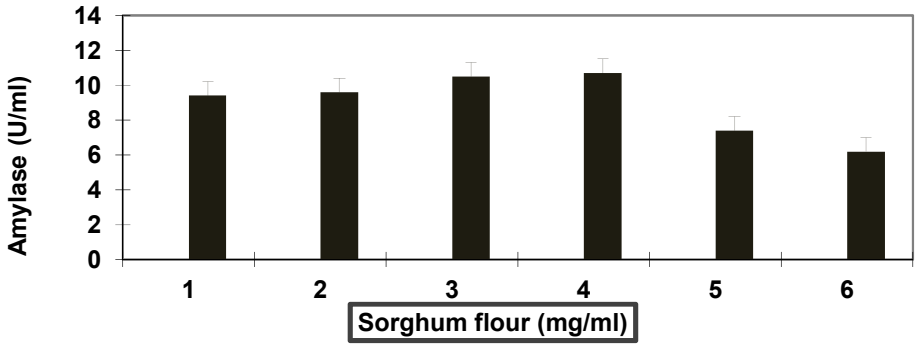

Fig. 5. Effect of concentration of optimized carbon source on amylase production by *Streptomyces cheonanensis* VUK-A (Values are the means of three replicates ±SD)

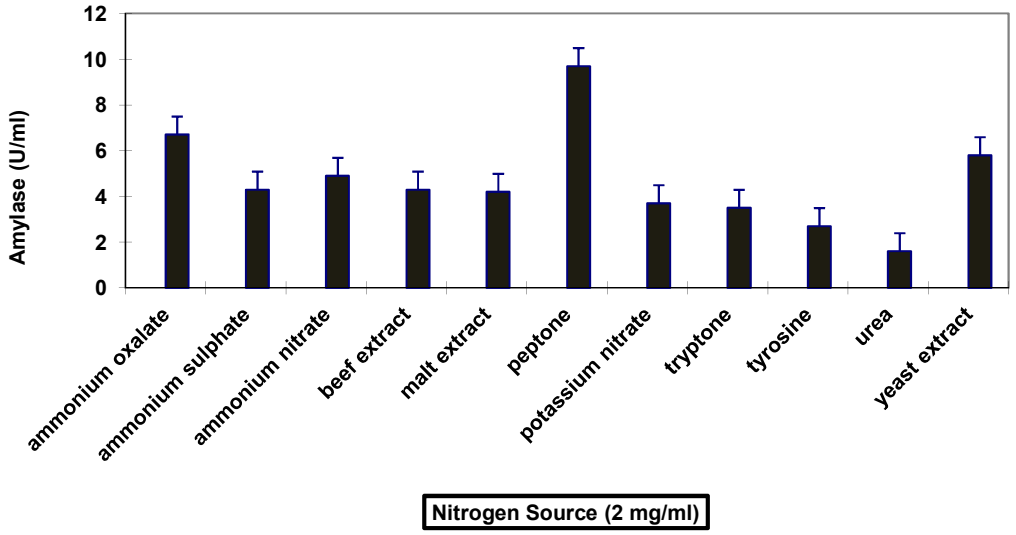

Fig. 6. Effect of nitrogen sources on amylase production by *Streptomyces cheonanensis* VUK-A (Values are the means of three replicates ±SD).

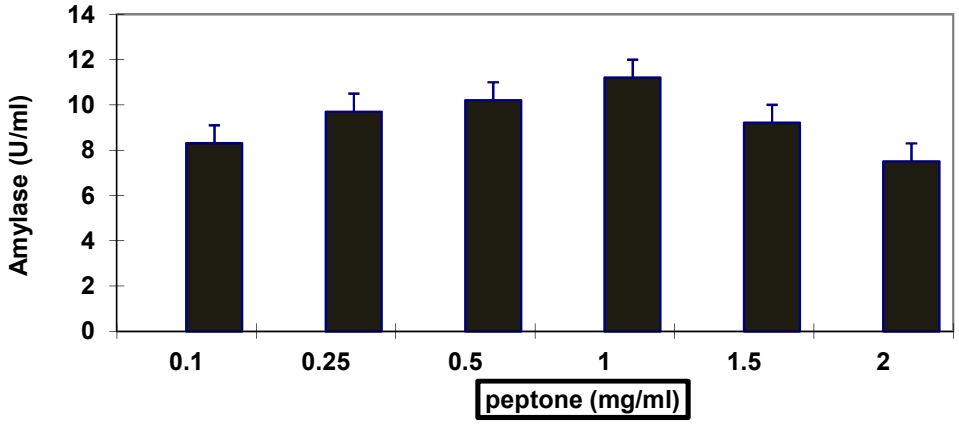

Fig. 7. Impact of concentration of peptone on amylase production by *Streptomyces cheonanensis* VUK-A (Values are the means of three replicates ±SD).

5. CONCLUSION

The present study revealed that *Streptomyces cheonanensis* VUK-A isolated from mangrove sediments yielded high amounts of amylase in the medium (ISP-4) amended with sorghum flour (30 mg/ml) and peptone (10 mg/ml) with initial pH 7.0 at 30°C after 72 h of incubation. The enzyme yield before optimization was 4.3 U/ml while it was 11.2 U/ml after optimization. This is the first report on production of amylase by *Streptomyces cheonanensis* VUK-A isolated from mangrove sediments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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