ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Isolation and screening of amylase producing *Bacillus* species from soil

Aneela Rehman and Asma Saeed*
Department of Microbiology, University of Karachi, Karachi-75270, Pakistan

Manuscript Info

Manuscript History:

Received: 12 February 2015
Final Accepted: 15 March 2015
Published Online: April 2015

Key words:

Amylase, Bacillus species, Starch hydrolysis , enzyme assay

Corresponding Author

Asma Saeed

Abstract

Amylases are one of the most important industrial enzymes, which hydrolyze starch molecules to fine products such as dextrins, maltose etc. In recent years, interest in the microbial production of enzyme has increased dramatically due to its wide spread use in baking, food, textile, and detergent industry. Amylase production by bacteria esp. by *Bacillus* species is of great importance nowadays. The purpose of current investigation is to isolate amylase producing *Bacillus* species from soil. Soil samples were collected from different areas of Karachi, Pakistan. Samples were analyzed for bacteriological study. 89 different bacterial species were isolated from different soil samples. Morphological and biochemical identification revealed 39 *Bacillus* species. These species were screened for amylase production. Five species were selected as highest enzyme producers in starch hydrolysis test. Among the five, AS-2 was found to be the highest enzyme producing specie in enzyme assay. The enzyme activity was found to be 3179.62 IU/ml/min. Such specie could be used as the auspicious source of amylase in industry.

INTRODUCTION

Enzymes are chemical substances produced by living cells that are capable of initiating a chemical reaction without themselves being used in that reaction. They enhance the rate of a chemical reaction (Oyeleke et al., 2009). Enzymes are produced by plants, animals but microbial enzyme production is of great importance as these are more economical to produce, calculable, tractable and stable (Burhan et al., 2003). Amylases are very important enzymes now days. They hydrolyze starch into dextrins and small polymers of glucose. There are two major classes of amylases, mostly identified among microorganisms are α- amylase and gluco-amylase (Vijayabaskar et al., 2012). β-amylase, mostly from plant origin, has been recorded from microbial sources (Pandey et al,. 2000). Amylases have been isolated from bacteria, fungi and actinomycetes (Kathiresan et al., 2006). Bacterial amylases are mostly reported from acidophilic, alkalophilic and thermophilic bacteria (Boyer et al., 1972). Most Microbial enzymes are commercially available and have replaced chemical hydrolysis of starch in industry (Pandey et al., 2000). *Bacillus subtilis*, *Bacillus stearothermophilis*, *Bacillus licheniformis* and *Bacillus amyloliquifaciens* are most prominent among the bacterial sources of amylase (Sani et al., 2014). Amylase are very important in recent days with application ranging from food, textile, paper, and fermentation such as baking, brewing, digestive acid’s production, chocolate cake’s production, fruit juices and starch syrups (Lin et al., 1997; Pandey et al., 2000; Verma et al., 2011). There are several reports on starch degrading microorganisms from different sources and respective amylase activity (Ryan et al., 2006; Serin et al 2012). Soil is one of the rich sources of starch degrading microorganisms as it contains mostly starchy substances. The purpose present investigation is to isolate *Bacillus* species from different soil samples and their screening for amylase production.

MATERIALS AND METHODS

Sample Collection

Soil samples were collected from different areas of Karachi. Soil samples were store at 4◦c in sterile glass tubes.

Medium for isolation

Medium used for isolation of Bacillus species was L. B agar (Luria Basal Agar). The medium containing per liter (10 g starch; 1 g yeast extract; 11 g tryptone; 8.9 g Nacl; 8 g agar; PH 7).

Isolation of Bacillus species

Serial dilution technique was used for the isolation of bacteria. 1g of soil was added to the glass tube containing 10 ml sterilized distilled water, sample was serially diluted and plated on L.A medium by spread plate method. Plates were incubated at 37◦c for 24 hours.

Identification of Isolated Organisms

The isolated colonies were subjected to Gram staining and the colonies showing Gram positive reaction were picked and maintained on L.B agar slants. The Isolates were subjected to different physiological and biochemical parameters and were identified by using Bergey's Manual of Determinative Bacteriology. These parameters are shown in Table I and II.

Starch Hydrolysis Test

Starch hydrolysis was observed by using iodine solution. Iodine with starch gives blue colour. Clear zone around colony shows starch hydrolysis (Mishra et al., 2008). 1% starch was incorporated in L.B agar. Plates were inoculated with the isolated Bacillus species and incubated at 37◦c for 24 hours. Colonies were exposed to 2% iodine solution and observed for the zone of hydrolysis around each colony (Nusrat et al., 2007).
Enzymatic index was calculated as:

$$\text{Enzyme index} = \frac{\text{Diameter of zone of degradation}}{\text{Diameter of colony}}$$

Crude enzyme preparation and enzyme assay

100 ml of L.B broth with 8% (w/v) inoculum was incubated for 48 hours with continuous agitation (150 rpm). 48 h old culture was transferred to micro centrifuge tubes and centrifuged at 4000 rpm for 15 min. Cell were discarded and supernatant was used as crude enzyme for enzyme assay (Mishra et al., 2008). Substrate solution(1% w/v soluble starch in sodium phosphate buffer pH 6.0) was also centrifuged at 4000rpm for 15 minutes. One ml of substrate solution and 1 ml of enzyme solution (cell free supernatant) were incubated at 55◦C. After 10 minutes of incubation, reaction was stopped by adding 3,5-dintrosaliclic acid reagent. The tube was boiled for 15 minutes and then cooled on ice to room temperature. Absorbance was measured at 540 nm using spectrophotometer (Bernfeld, 1955). Enzyme Assay was performed for five highest starch hydrolyzing strains. The amylase activity was determined in IU/ml/min by applying the following formula:
IU/ml/min = Activity of enzyme **x** 1000 / Molecular wt. of maltose **x** time of incubation

RESULTS :

Sample collection and isolation of Gram positive bacteria

Soil samples were collected from 15 different areas of Karachi, Pakistan. 89 different organisms were isolated from these samples. Morphological and biochemical characteristics revealed 39 isolates belonging to genus *Bacillus*. All the 39 isolates were Gram positive, rod shaped, spore former and starch hydrolyzers (Table I).

Identification of *Bacillus* species

All 39 isolates were found to be VP, citrate and catalse positive, capable of producing acid from glucose, xylose, mannitol, and sucrose. All the isolates showed motility using SIM medium. Bacilli are thermotolerant, the isolated species were also capable of growing at 55◦C. The results are presented in Table II.

Starch Hydrolysis Test

Starch hydrolysis was performed with all the 39 isolates, zone of hydrolysis was measured and enzyme index was calculated for each colony (Table III). Five isolates showing high values include AS-2 (2.11), AS-25 (1.9), AS-32 (1.87), AS-82 (1.9) and AS-85 (1.87) as shown in Table III.

Enzyme Assay

Five isolates with high enzyme index were analyzed for enzyme activity. Among the five, AS-2 showed highest enzyme activity i.e., 3179.6 IU/ml/min. AS82 was the second highest enzyme producer (2904.4 IU/ml/min). Enzyme activities for AS25, AS32, and AS85 were 1440.74 IU/ml/min, 2018.51 IU/ml/min and 1851.85 IU/ml/min respectively (Table IV and Graph I).

Table I

Morphological and biochemical characteristics of bacterial isolates

Isolate no*	Source of isolates	Gram reaction	Microscopic characteristics	Starch hydrolysis	Catal-ase	Heamo-lysis
AS 1	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 2	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 3	Garden	Gm +ve	Bacilli, short rods, scattered	+	+	+
AS 4	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 5	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 6	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 7	Garden	Gm -ve	Bacilli, short rods, scattered	-	-	-

AS 8	Garden	Gm -ve	Bacilli, short rods, scattered	-	+	-
AS 9	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 10	Garden	Gm +ve	Coccid in bunches	+	+	+
AS 11	Garden	Gm -ve	Cocci, in chains	-	-	+
AS 12	Ground	Gm -ve	Bacilli, long rods, in chains	-	+	+
AS 13	Ground	Gm -ve	Bacilli, short rods, scattered	-	+	-
AS 14	Ground	Gm +ve	Cocci, in chains	+	+	+
AS 15	Ground	Gm +ve	Cocci, in bunches	+	+	+
AS 16	Ground	Gm +ve	Bacilli, long rods, scattered	+	+	+
AS 17	Ground	Gm -ve	Cocci, in chains	-	-	+
AS 18	Garden	Gm -ve	Cocci, in chains	-	+	-
AS 19	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 20	Garden	Gm -ve	Cocci, Scattered	-	-	+
AS 21	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 22	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 23	Garden	Gm -ve	Bacilli, long rods, scattered	+	+	-
AS 24	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+

AS 25	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 26	Ground	Gm -ve	Cocci, in chains	+	-	-
AS 27	Ground	Gm -ve	Cocci, in chains	+	-	+
AS 28	Ground	Gm -ve	Cocci, in bunches	-	+	+
AS 29	Ground	Gm -ve	Cocci, scattered	-	-	+
AS 30	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 31	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 32	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 33	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 34	Ground	Gm +ve	Cocci, in bunches	-	+	+
AS 35	Ground	Gm -ve	Cocci, in pair, diplococcic	-	+	+
AS 36	Ground	Gm +ve	Cocci, in chains	+	+	+
AS 37	Ground	Gm -ve	Cocci, Scattered	-	-	+
AS 38	Ground	Gm -ve	Cocci, Scattered	+	+	+
AS 39	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 40	Garden	Gm +ve	Bacilli, short rods, scattered	+	-	+
AS 41	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+

AS 42	Garden	Gm -ve	Bacilli, short rods, scattered	-	+	+
AS 43	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 44	Garden	Gm +ve	Coccid, in bunches	+	-	+
AS 45	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 46	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 47	Garden	Gm -ve	Cocci, in chains	+	+	+
AS 48	Garden	Gm -ve	Cocci, in chains	+	+	+
AS 49	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 50	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 51	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 52	Ground	Gm +ve	Cocci, in bunches	+	+	+
AS 53	Ground	Gm +ve	Cocci, in bunches	+	+	+
AS 54	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 55	Ground	Gm +ve	Cocci in chains	+	+	+
AS 56	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 57	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 58	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+

AS 59	Ground	Gm -ve	Bacilli, short rods, scattered	+	-	+
AS 60	Ground	Gm -ve	Bacilli, short rods, chains	-	-	+
AS 61	Garden	Gm -ve	Cocci, Scattered	-	-	+
AS 62	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 63	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 64	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 65	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 66	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 67	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 68	Ground	Gm -ve	Bacilli, short rods, scattered	+	-	+
AS 69	Ground	Gm -ve	Bacilli, short rods, scattered	+	-	+
AS 70	Ground	Gm -ve	Cocci, Scattered	-	-	+
AS 71	Ground	Gm -ve	Cocci, Scattered	-	+	+
AS 72	Ground	Gm -ve	Bacilli, short rods, scattered	-	-	+
AS 73	Ground	Gm -ve	Bacilli, short rods, scattered	-	-	+
AS 74	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 75	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+

AS 76	Ground	Gm -ve	Bacilli, long rods, in chains	+	+	+
AS 77	Ground	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 78	Ground	Gm -ve	Bacilli, long rods, scattered	-	-	+
AS 79	Ground	Gm -ve	Bacilli, long rods, scattered	-	-	+
AS 80	Ground	Gm +ve	Cocci, in bunches	-	+	+
AS 81	Garden	Gm +ve	Cocci, in bunches	-	+	+
AS 82	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 83	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 84	Garden	Gm -ve	Cocci, Scattered	-	-	+
AS 85	Garden	Gm +ve	Bacilli, long rods, in chains	+	+	+
AS 86	Garden	Gm +ve	Cocci, in chains	+	+	+
AS 87	Garden	Gm -ve	Cocci, in pair, diplococcic	+	+	+
AS 88	Garden	Gm -ve	Cocci, Scattered	+	-	+
AS 89	Garden	Gm +ve	Cocci, in bunches	+	+	+

***Bacillus* species are highlighted**

Table II

Morphological and biochemical characteristics of bacterial isolates

Isolate no.*	VP	7% Nacl	Mobility	Temperature (°C)				Production of acid *				Citrate
				35	45	55	65	G	S	X	M	
AS- 1	+	+	+	+	+	+	-	+	+	+	+	+
AS -2	+	+	+	+	+	+	-	+	+	+	+	+
AS -3	-	-	+	+	-	-	-	+	-	+	-	+
AS -4	+	+	+	+	+	+	-	+	+	+	+	+
AS -5	+	+	+	+	+	+	-	+	+	+	+	+
AS -6	+	+	+	+	+	+	-	+	+	+	+	+
AS -7	-	-	+	+	+	-	-	+	-	+	+	-
AS -8	-	-	+	+	+	-	-	-	+	+	-	-
AS -9	+	+	+	+	+	+	-	+	+	+	+	+
AS -10	+	-	-	+	+	+	-	+	+	-	+	+
AS -11	-	-	+	+	-	-	-	+	-	-	+	+
AS -12	-	-	+	+	-	-	-	+	+	-	+	-
AS -13	+	-	-	+	-	-	-	+	-	-	-	+
AS -14	+	-	-	+	+	-	-	-	-	+	-	+
AS -15	+	-	-	+	+	-	-	+	-	-	-	+
AS -16	-	-	-	+	-	-	-	+	+	-	+	+
AS -17	+	-	+	+	-	-	-	+	+	+	+	+
AS -18	+	-	-	+	-	-	-	+	+	-	+	-
AS -19	+	+	+	+	+	+	-	+	+	+	+	+
AS -20	-	-	+	+	-	-	-	-	+	-	-	+
AS -21	+	+	+	+	+	+	-	+	+	+	+	+
AS -22	+	+	+	+	+	+	-	+	+	+	+	+
AS -23	+	-	+	+	+	+	-	-	-	+	-	+
AS -24	+	+	+	+	+	+	-	+	+	+	+	+
AS -25	+	+	+	+	+	+	-	+	+	+	+	+
AS -26	-	-	-	+	+	-	-	+	-	-	+	-
AS -27	-	-	-	+	-	-	-	-	-	+	-	-
AS -28	+	-	-	+	+	-	-	-	-	-	+	-
AS -29	-	-	+	+	-	-	-	-	-		+	-
AS -30	+	+	+	+	+	+	-	+	+	+	+	+
AS -31	+	+	+	+	+	+	-	+	+	+	+	+
AS -32	+	+	+	+	+	+	-	+	+	+	+	+
AS -33	+	+	+	+	+	+	-	+	+	+	+	+

AS -34	+	-	-	+	+	+	-	-	+	-	-	+
AS -35	+	-	+	+	+	-	-	-	-	-	+	+
AS -36	+	-	-	+	+	+	-	+	+	-	-	+
AS -37	-	-	-	+	+	-	-	-	+	+	-	+
AS -38	-	-	-	+	-	-	-	-	+	-	+	+
AS -39	+	+	+	+	+	+	-	+	+	+	+	+
AS -40	+	-	-	+	+	+	-	-	+	-	+	+
AS -41	+	+	+	+	+	+	-	+	+	+	+	+
AS -42	-	-	+	+	-	-	-					
AS -43	+	+	+	+	+	+	-	+	+	+	+	+
AS -44	+	-	-	+	+	+	-					
AS -45	+	+	+	+	+	+	-	+	+	+	+	+
AS -46	+	+	+	+	+	+	-	+	+	+	+	+
AS -47	-	-	-	+	+	-	-	+	-	+	-	-
AS -48	-	-	-	+	+	-	-	+	+	-	-	-
AS -49	+	+	+	+	+	+	-	+	+	+	+	+
AS -50	+	+	+	+	+	+	-	+	+	+	+	+
AS -51	+	+	+	+	+	+	-	+	+	+	+	+
AS -52	+	-	+	+	+	+	-	+	+	+	+	+
AS -53	+	-	+	+	+	+	-	+	+	+	+	+
AS -54	+	+	+	+	+	+	-	+	+	+	+	+
AS -55	+	-	+	+	-	-	-	+	+	+	+	+
AS -56	+	+	+	+	+	+	-	+	+	+	+	+
AS -57	+	+	+	+	+	+	-	+	+	+	+	+
AS -58	+	+	+	+	+	+	-	+	+	+	+	+
AS -59	-	-	+	+	-	-	-	-	-	+	+	-
AS -60	-	-	+	+	+	-	-	+	+	+	-	+
AS -61	-	-	+	+	-	-	-	+	+	-	+	-
AS -62	+	+	+	+	+	+	-	+	+	+	+	+
AS -63	+	+	+	+	+	+	-	+	+	+	+	+
AS -64	+ +	+	+	+	+	+	- -	+	+	+	+	+
AS -65	+	+	+	+	+	+	-	+	+	+	+	+
AS -66	+	+	+	+	+	+	-	+	+	+	+	+
AS -67	+	+	+	+	+	+	-	+	+	+	+	+
AS -68	-	-	+	+	+	-	-	+	-	+	-	+
AS -69	-	-	+	+	+	-	-	-	+	-	-	-

AS -70	-	-	+	+	-	-	-	+	+	+	+	-
AS -71	-	-	+	+	-	-	-	-	-	-	+	-
AS -72	+	-	+	+	-	-	-	-	-	+	+	+
AS -73	+	-	+	+	+	-	-	+	+	+	+	+
AS -74	+	+	+	+	+	+	-	+	+	+	+	+
AS -75	+	+	+	+	+	+	-	+	+	+	+	+
AS -76	+	-	+	+	+	-	-	+	-	+	-	-
AS -77	+	+	+	+	+	+	-	+	+	+	+	-
AS -78	+	-	+	+	+	-	-	+	-	-	-	-
AS -79	+	-	+	+	+	-	-	+	+	+	-	+
AS -80	+	-	-	+	+	+	-	+	+	+	+	+
AS -81	+	-	-	+	+	+	-	+	+	+	+	+
AS -82	+	+	+	+	+	+	-	+	+	+	+	+
AS -83	+	+	+	+	+	+	-	+	+	+	+	+
AS -84	+	-	-	+	+	-	-	+	+	-	-	-
AS -85	+	+	+	+	+	+	-	+	+	+	+	+
AS -86	+	-	-	+	+	+	-	+	-	+	+	+
AS -87	+	-	+	+	-	-	-	-	-	+	-	+
AS -88	+	-	+	+	+	-	-	-	-	+	+	-
AS -89	+	-	-	+	+	+	-	+	+	-	+	+

******Bacillus*** species are highlighted**

****G = Glucose, S = Sucrose, X = Xylose, M = Mannitol**

Table III

Amylase production by bacterial isolates

Isolate number	Diameter of colony (mm)	Diameter of halo (mm)	Enzyme index
AS- 1	13	20	1.53
*AS -2	9	19	2.11
AS -4	14	24	1.72
AS -5	17	20	1.17
AS -6	10	12	1.2
AS -9	8	9	1.12
AS -19	8	13	1.62
AS -21	16	18	1.12
AS -22	9	15	1.67
AS -24	8	16	2
*AS -25	10	19	1.9
AS -30	18	19	1.05

AS -31	10	12	1.09
*AS -32	8	15	1.87
AS -33	15	20	1.33
AS -39	6	10	1.67
AS -41	10	12	1.2
AS -43	13	15	1.15
AS -45	15	20	1.33
AS -46	10	12	1.2
AS -49	12	20	1.67
AS -50	9	13	1.44
AS -51	13	18	1.38
AS -54	12	4	1.167
AS -56	2	2.6	1.33
AS -57	10	11	1.1
AS -58	14	17	1.21
AS -62	15	21	1.4
AS -63	10	14	1.4
AS -64	11	17	1.54
AS -65	13	15	1.15
AS -66	10	15	1.5
AS -67	15	20	1.33
AS -74	8	13	1.62
AS -75	13	23	1.77
AS -77	18	14	1.28
*AS -82	10	19	1.9
AS -83	10	12	1.2
*AS -85	8	15	1.87

***Bacillus* species showing high enzyme index and are selected for determining enzyme activity**

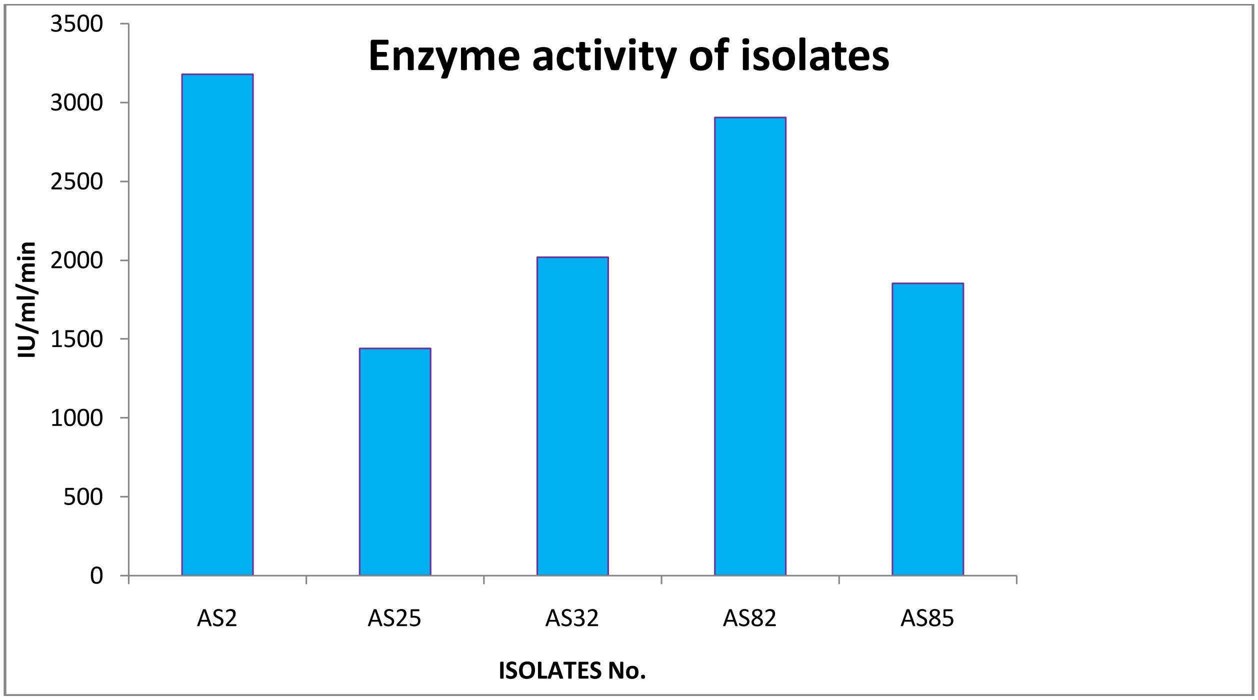
Table IV

Enzyme activity of bacterial isolates

Isolate number	IU/ml/min
AS-2	3179.62
AS-25	1440.74
AS-32	2018.51
AS-82	2904.4
AS-85	1851.85

Graph 1

Enzyme activity of bacterial isolates



DISCUSSION :

The occurrence of amylolytic organisms from the soil agrees with the earlier report that the soil is an enriched repository of amylase producers (Madhav 2011). Various species of bacteria, fungi and actinomycetes are most prominent enzyme producers. Among various amylase producers *Bacillus* species are most prominent (Pandey et al., 2000). Present investigation shows the potential of soil organisms to produce highly commercially important enzyme capable of starch degradation. Many isolates showed starch hydrolysis. Five isolates with high enzyme index are shown in Table III. Enzyme assay for all five high enzyme producing strains showed that AS-2 gives highest enzyme activity3179.6 IU/ml/min. AS-82 is the second highest enzyme producer (2904.4 IU/ml/min).Enzyme activity for AS-25, AS-32, and AS-85 is 1440.74 IU/ml/min, 2018.51 IU/ml/min and 1851.85 IU/ml/min respectively as shown in Table IV. The study revealed that soil harbors amylolytic bacilli and that the amylase production by these bacteria may in future, be used in different industrial sectors as well as other research areas. For further studies, the *Bacillus* specie AS-2 with highest enzyme activity has been selected for optimization , characterization and purification of enzyme.

ACKNOWLEDGEMENTS
The present study was partially supported by the research grant from Dean, Faculty of Science to Dr. Asma Saeed.

REFERENCES

1. Bernfeld, P. (1955): Amylases alpha and beta. Methods Enzymol., 1:140-146.

2. Burhan, A., Nisa, U., Gokhan, C., Ashabil, A. and Osmair, G. (2003): Enzymatic properties of a novel thermostable thermophilic alkaline and chelator resistant amylase from an alkaphilic *Bacillus* sp. isolate ANT-6. Proc. Biochem., (38): 1397–1403.

3. Boyer E.W. and Ingle, M. B. (1972): Extracellular alkaline amylase from *Bacillus* sp. J. Bacteriol., 110 : 992-1000.

4. Kathiresan, K. and Manivannan, S. (2006): Amylase production by *Penicillium fellutanum* isolated from mangrove rhizosphere soil. Afr. J. Biotech., 5 : 829-832.

5. Lin, L. -L., Hsu, W. -H. and Chu, W. -S. (1997): A gene encoding for an α -amylase from thermophilic *Bacillus* sp. strain TS-23 and its expression in *Escherichia coli.* J. Appl. Microbiol. , 82: 325-334.

6. Mishra, S. and Behera, N. (2008): Amylase activity of starch degrading bacteria isolated from soil receiving kitchen wastes. Afr. J. Biotech., 7:3326-3331.

7. Madhav, K., Verma, S. and Tanta (2011). Isolation of amylase producing *Bacillus* species, from soil sample of different regions in Dehradun and to check the effect of pH and temperatures on their amylase activity. J. Pharm. Biomed. Sci., 12 (03) : 8.

8. Nusrat, A. and Rahman, S. R. (2007): Comparative studies on the production of extracellular α-amylase by three mesophilic *Bacillus* isolates. Bang. J. Microbial., 24(2):129-132.

9. Oyeleke, S. B. and Oduwole, A. A. (2009): Production of amylase by bacteria isolated from a cassava dumpsite in minna, Nigerstate, Nigeria. Afr. J. Microbiol. Res., 3(4): 143-146.

10. Pandey, A., Nigam, P., Soccol, C. R., Soccol, V. T., Singh, D. and Mohan, R. (2000): Advances in microbial amylases. Biotechnol. Appl. Biochem., 31: 135- 152.

11. Ryan, S. M., Fitzgerald, G. F. and VanSinderen, D. (2006): Screening for and identification of starch-, amylopectin-, and pullulan-degrading activities in bifidobacterial strains. Appl . Envir. Microbiol., 72: 5289- 5296.

12. Sani, I., Abdulhamid, A., Bello, F., Yahaya, M. and Bagudo, A. I. (2014): Isolation, partial purification and characterization of α-amylase from *Bacillus subtilis.* J. Microbiol. Biotech. Res., 4:49-54.

13. Serin, B., Akcan, N. and Uyar, F. (2012). Production and optimization of α-amylase from *Bacillus circulans* ATCC 4516 with solid state fermentation. J. Biol. & Chem.40:393-400.

14. Verma, V., Avasti, M. S., Gupta, A. R., Singh, M. and Kushwaha, A. (2011): Amylase production and purification from bacterial isolates from a waste potato dumpsite in district Farrukhabad U.P State India. Eur. J. Experimental Bio., 1(3): 107-113.

15. Vijayabaskar, P., Jayalakshmi, D. and Shankar, T. (2012): Amylase production by moderately halophilic *Bacillus cereus* in solid state fermentation. Afr. J. Microbiol., 6: 4918–4926