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SEPARATION OF PRODUCING AMYLASE BACILLUS FROM SOILS IN THE SHORES OF LAKE URMIA, AND PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF IT

SAHAR MAHDAVI[1*], MAHMOUD REZAZAD BARY[2] AND MAJID NOJAVAN

1: M.Sc. in Microbiology, Urmia Branch, Islamic Azad University, Urmia, Iran

2: Assistant Professor, University of Nazlu Urmia, Urmia, Iran

3: Professor, Urmia Branch, Islamic Azad University, Urmia, Iran

***Corresponding Author**

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ABSTRACT

Amylases are one of the main enzymes used in industry. These enzymes have potential application in a wide number of industrial processes such as food and fermentation and pharmaceutical industries. Amylases can be obtained from plants, animals and microorganisms. However, enzymes from fungal and bacterial sources have dominated applications in industrial sectors. The production of amylase is essential for conversion of starches into oligosaccharides. A large number of microbial amylases have application in different industrial sectors such as food, textile, paper and detergent industries.Identification and classification studies of bacteria through functional properties have been replaced with molecular methods. First, an intensive screening of soil with 20, 40, 60, 80, 100 meters in distance sampling is done on the Urmia salt lake. Samples were spread onto nutrient agar base containing soluble starch. After incubation for 24h, the plates were flooded with a solution of KI. Colonies exhibiting halo of starch hydrolyzing activity were picked for further study. Samples with the highest amylase activity are identified with 16SrRNA Sequences. Among 79 Separated positive amylase bacteria, two cases with the highest amylase activity are chosen to genotypic evaluations. The degree of amylase bacteria activity reduces with Salinity increase. The optimum pH and temperature were 7 and 45

respectively. Efforts have been underway to identify enzymes with new properties. Since these enzymes not only from the point of application, but also from the point of enzyme studies are important. Our separated halaphilic bacteria have its-own prominent properties, needs more molecular, and biochemical evaluations. .

Keywords: Urmia salt lake, Amylase, Bacillus spp, 16SrRNA sequence

INTRODUCTION

Lake Urmia is in the northern part of Iran, between the coordinates of ‘37 37 to '17 38North and the '59 44 to '56 45 West. This lake, which in the past was ChiChest and Kabudan, is as the largest and one of the saltiest permanent lakes of Iran and it is one of the hyper-saline lakes in the world. In this sense, it is similar to America great lake. The approximate area of it is about 6000 square kilometers, its length, from north to south, is about 140 km and its average width is about 40 km. Its average depth is about 6 meters. Construction of the causeway in the Urmia Lake (Shahid Kalantari Highway), has created changes in natural lakes. These changes will not be limited only to the biological and chemical effects, but they have followed by other consequences, such as changes in sedimentation in the lake, and drying parts of the lake (Alavi Panah et al., 2005), which this important matter will require doing research in this area. Physical and chemical properties of the soil have a great impact on soil microbial population (Mishra, 2009)

Enzymes are compounds that are proteins and are found naturally, and act in catalyzed of chemical processes very specifically. In terms of molecular structure, they are in the form of three-dimensional and very complicated. The substance that the enzyme applies its effect on its surface is called the substrate and the reaction conditions, such as temperature, humidity, and pH is essential for catalytic processes. All living organisms, including microorganisms, plants and animals produce enzymes (Ebadati, 2013). Almost all chemical reactions in a biological cell need enzymes, like all catalysts, enzymes act by lowering reaction activation energy, thus, the reaction rate increases. Synthetic molecules called artificial enzymes also have been seen. Some enzymes are used in commercial terms, for example, in the synthesis of antibiotics, in addition to this, in some households, the enzymes are used to speed up chemical reactions (Monteiro Migalhous, 2010).

Bacterial enzymes are highly applicable in industries; producing a group of industrial

enzymes, almost involves 25% of the world, purchase and sale of enzyme (Cairan, 2008). Among these enzymes, amylase has more applications in industries such as pharmaceutical, textile, food and other processes (Pandey et al., 2000; Reddy et al., 2003; Kar et al., 2010). Amylases are important enzymes for using in industry (Wang et al., 2011), these enzymes hydrolyzed molecular starch to composed polymers of glucose units (Amoozegar et al., 2003). Amylases are widely used in many industrial processes, such as food, fermentation and pharmaceuticals (Gupta et al., 2003). The most important polysaccharide that is used in the food industry is Starch. Glucose enzyme production, by using amylase enzyme obtained from *Bacillus subtilis* and Amyloglucosidase, derived from Aspergillus, has been replaced by acid hydrolysis traditional methods. Speed of action, lack of pollution and the possibility of producing dextrose in industrial scale aremajor advantages of enzymatic methods. However, with the development of recombinant DNA technology, stable microbial enzymes generation stable at high temperatures is provided for enzymatic hydrolysis and therefore industrial and extensive production of glucose.

In addition, by using the alpha-amylase enzyme, starch can be turned to syrups with low dextrose equivalent (DE).

Production of extracellular enzymes in microorganisms is under the influence of nutritional factors, such as carbon sources, nitrogen sources and mineral salts (D., et al., 2001; Chahan and Gupta, 2004).

The effect of different concentrations of starchwas determined on amylase activity and the data have shown that the highest enzyme activity was at a concentration of 1 to 1.5 percent of starch (Amouzegar and colleagues, 2003).

Amylase can be achieved from plants, animals and microorganisms (Fogarty, 1983). However, fungal and bacterial enzymes resources have more applicable in the industry (Monteiroand Mygalhous, 2010). Amylases are among the most important enzymes and have great importance of biotechnology; these enzymes play an important role in the carbon cycle (Mohapatra et al., 1998). Producinga group of industrial enzymes involves almost 25% of the world, purchase and sale of enzymes. These enzymes can be achieved from several sources such as plants, animals and microorganisms (Yugo et al., 2000; Yang et al., 2001; Darani, 2004). Today, great deals of commercial microbial enzymes are

available and almost they involve all the chemical hydrolysis conversions of starch to its industrial products. Microorganisms' amylase has an extensive spectrum of industry demands; also, they are much more resistant than animal and plant amylases (zambara, 2011). Among the bacteria, Bacillus is widely used for the production of amylase in the industry (Akjan, 2011). Bacillus is bacilli that are Gram-positive, anaerobic or facultative, positive catalase, generally animated and with Endospore (Abedi, 2001). Convenience cultivation and low food needs of Bacillus are likely the reason for selecting them for enzyme production in the industry (Oilkeh, 2010). Halophilic species are suitable sources for the production of DNase, protease, lipase, amylase and gelatinase enzymes that have shown significant activity at high salt concentrations (Prakash et al., 2009). Two groups of amylaseshave been identified in the microorganisms, named by alpha amylase and Gluco-amylase. Alpha amylases are extracellular enzymes (Pandey et al., 2000) that randomly break the 1 and 4 α D-glucoside linkages between glucose units in the linear amylose chains (Vijayabaskar et al., 2012).

Amylase belongs to a family of Endo amylases that hydrolyzes starch to shorter oligosaccharides by breaking glycosides linkages (Sivakumar et al., 2012).

In some studies, amylase enzymes have been studied. Rezazad (1991) has examined amylase enzyme production by bacteria in the shores of Urmia Lake soils. Positive amylase bacteria were determined by the agar starch medium and by using iodometry method. Bacterial growth was measured by total count.

Demircan (2010) has purified Alpha amylase produced by Bacillus with deposition method by ammonium sulfate, column chromatography TSK, dialysis and Sepharose column chromatography and the molecular weight of purified enzyme was determined by using polyacrylamide gel electrophoresis (SDS-PAGE). The purified enzyme molecular weight was reported 56 kDa. Monteiro and Magalhous (2010) have studied the amylase production by some bacterial species including Bacillus. PH and the optimal temperature for maximum production were reported approximately 7 and 45 ° C respectively. Abdoval et al. (2011) have obtained Bacillus cereus MS bacteria that were capable of producing alpha-amylase from Sewer water in India. Identifying bacteria were performed by sequencing 16SrRNA. The optimum pH of production was reported 7 and the

temperature was 45 ° C. Alpha amylase was purified by the methods of ammonium sulfate precipitation, ion exchange and chromatography.

In this study, we have tried to deal with the isolation of the amylase enzyme generating Bacilli of soils in the shores of Lake Urmia and phenotypic and genotypic characterization.

MATERIALS AND METHODS

Sampling was performed from different parts of the Urmia Lake: dates were19/Feb/2013 - 05/Mar/2013 - 07/Apr/2013 from ten points of the Lake Urmia shorein distances of 20, 40, 60, 80 and 100 m from the shore of the lake, in nylon bags and by considering sterilized conditions.

Each of the samples prepared dilutions prepared by the consecutive dilution method. To do this, one gram of screened soil was mixed with 9 ml of sterile distilled water and we have prepared these suspensions to the dilution of 10^{-8}. From prepared dilutions, we have cultured the amount of 0.1 cc on nutrient agar surface with the surface culture method and have placed plates upside down inside nylon, plastic (to prevent drying).Then, they were incubated for 24 hours at 30 ° C, the plates that contained single colony were cultured to study the amylase enzymes in a nutrient agar environment plus one percent starch in the form of spot and they were incubated for 24 h at 30 ° C. . By using iodine solution, amylase-producing bacteria were detected (Snihs et al., 1986).

Biochemical tests

Gram staining: prepare extensive bacteria from the pure culture environment and after drying, we stabilize by heat. We dye the sample for 30 seconds with violet crystal. As a result, all bacteria come in purple. After washing with water, cover the microbial layer with Gram and wait 30 minutes that stabilize the violet crystal. After this point, all bacteria are almost purple color. After washing slides with water, slides were exposed to decolorizer substances for 15 to 30 seconds such as alcohol, acetone then rinse with water. After this stage, gram-negative bacteria are colorless, but Gram-positive bacteria remain almost purple. In the end, we cover the fruit surface with Safranine or Fuschin for 30 seconds. Then rinse with water and after drying, put Lamella on it and examine it with a light microscope. Colorless bacteria (gram-negative bacteria) come in red or pink and purple or blue bacteria (gram-positive bacteria) remain unchanged color. (To view with the lens 100, immersion oil should be used). Tested bacteria were gram-positive Basil.

Simon citrate test: culture the bacteria in the Simon citrate environment, which is an inclined level, and keep it at 37 ° C for 24 hours. If the initial green turns into blue, the reaction is positive.

Catalase test: we have transferred a great value of a pure organism's culture into clean, dry slides by the loop. A drop of 3 percent hydrogen peroxide (H2O2) has been shed on it, releasing of gas bubbles indicates the presence of catalase in the microorganism that breaks down hydrogen peroxide into water and oxygen.

Spore Coloring: First, a thin spread of bacteria is prepared. After drying at room temperature, and heat stabilizers, we have covered the surface of the slide with a solution of carbon Malachite and have heated it for 5 minutes, so that, green malachite evaporates, but, does not dry and does not boil. Then we washed the slide with water, and covered the glass slides' surface with Safranin solution (or Fuchsin) and then rinsed it after one minute, and after the slide dried, by 100-microscope lens, immersion oil was studied.

The growth study in various pHs: pH ofculturing and the nutrient broth medium was set by NaCl and KOH one molar on 5 to 10. The pure cultures of bacteria were inoculated to them at 37 ° C for 24 to 48 hours maintenance and then the growth rate was investigated.

The growth study at different temperatures: Samples were cultured in agar nutrient medium and were maintained at temperatures 5, 10,20,30,40,50,55,60 ° C for 24-48 hours, the samples growth was compared.

Study of bacterial growth in environments with glucose, mannose, Mannitol and Arabinose: first one percent soluble was prepared from mentioning sugars, in this way that we dissolved one gram of sugar in 100 ml of distilled water and then it was passed through a membrane filter and was moved to sterilizetest pipes, bacteria samples are added to the environment, and after 24 hours, the bacteria were investigated.

Vezer Proskauer test (VP): VP test is composed of two microbiologists who have worked in the twentieth century on the test method. Pyruvic acid is a by-product of glucose fermentation process. The intermediate product can be metabolized to different final products in different Bacteria, depending on their enzyme system. In the Butanen Glycol path, Pyruvic acid turns into acetoin (acetyl methyl carbonyl) which hasa neutral pH and in the presence of oxygen and40% potassium hydroxide converts to

diacetyl and Alpha Naphtholas a catalyst creates a red color composition.

Methyl red Test (MR): This test is a quantitative test for acid production (setting pH). Some organisms have produced more acid than other organisms. Methyl red is a pH reagent in the range of between 6 (yellow) and 4/4 (red). Acidic pH in methyl red is lower than the pH of other reagents used in the bacteriological culture media. So to change the color to red, tested organisms have to produce plenty of acid from carbohydrates. In fact, this test is used for the detection of bacterial strains that produce strong acids from glucose.

Test methodology:

24-18 hour's pure cultures of Bacteria incubated in a screw-cap tube containing the MR / VP and it should be incubated for at least 24 hours at 35 ° C. A drop of5 percent Alpha Naphthol and two drops of 40 percent KOH were added to it. Shake the tube gently for 30 seconds to a minute to expose the environment to the oxygen of the air for Acetoin oxidation and creating a color reaction. Then, place it constantly for 15 minutes. The red color represents VP positive test.

After culturing and incubation at 37 ° C for 48-24 hours, 5 drops of methyl red color reagent were added. A red color indicates the test is positive.

SIM culturing Medium (Sulfide-Indol-Motility): Three features of the bacteria were determined by this test. Because of the tryptophanamino acid in the environment, bacteria that are able to produce tryptophanase enzymes, oxidize the tryptophan and produce Indole acetic, that the addition of one ml Kovacs reagent and one ml chloroform to 24-hour culture creates amethystine color on the culture surface. Hydrogen sulfide by bacteria, create a black color in the cultural environment. Due to the Semi-solid of the environment, moving bacteria will opaque the environment.

Molecular identification of Top strains

Genomic DNA extracted from bacteria: by sterile swabs, take a colony of the bacteria culture and dilute it by buffer PBS. This solution, because neither acidic nor alkaline, does not damage DNA. Take 200 microliters of the diluted sample by pipette, put it in another micro tube, and then add 20 microliters of proteinase K. Then, add 200 microliters Buffer AL, which contains ethanol, chloroform and so on and mix it (we must take care not to add buffer AL and proteinase K together) place it for 10 minutes at 56 ° C in dry bath, so that proteinase K will be activated as a result of buffer AL and

makes the walls of bacteria slippery. Take it out of dry bath and add 200 microliters of 96% ethanol so that the DNA precipitated and then pourthe micro tube solution into the columns micro tubes. Mix it and transfer it for 1 minute to centrifuge round of 8000. After centrifugation, add 500 microliters of solution AW1 (wash buffer) so that the DNA attached to the filter will be more pure. For a minute, we transfer it to the centrifuge round of 8000. After centrifugation, add 500 microliters AW2solution. (The DNA attached to the Filter will be purer) transfer it to centrifuge round of 1300, after centrifugation, discard the lower part of micro tubes and put the filtered part into the new micro tubes, and transfer it to a centrifuge with the final round for one minute. After centrifugation, add 200 ml of buffer AE and keep it for 5 minutes in the external environment. Put it for a minute in a centrifuge round of 8000, take the solution at the bottom of micro tube and transfer it to the new filtered micro tubes, we will wait one minute to stay outdoors. Then, centrifuge it for a minute and throw away the filter, the sediment at the bottom of the micro-tubes is DNA.

Proliferation of 16SrRNA gene sequence

Universal Primers: In this study, below primers have been used that proliferate piece of371bp length from the region within the gene.

The preparation of the PCR reaction mixture: PCR Mixture was prepared for 1 strain and PCR mixture was prepared for 34 strains.

Table 1- Universal primers sequencing

Proliferated segment length	Primer sequences	Primer
371bp	AACTGGAGGAAGGTGGGGAT	Forward primer
	AGGAGGTGATCCAACCGCA	Reverse primer

PCR template		
First approximation	95 ^{0}C	5 minutes
approximation(35cycles)	95 ^{0}C	40 seconds
Connecting primers	56 ^{0}C	30 seconds
Polymerization	72^{0}C	40 seconds
Final Expansion	72^{0}C	5 minutes

To obtain the size of PCR products: In order to view the size of products, the agarose gel stained with ethidium Bromide was used, so that, the expanded parts were placed into the wells in the agarose gel by pipette.

Preparation of agarose gel: we mix 75 g of the agarose, with cc70 buffer TBE (containing 9 grams of Tris base, 5.5 g of

boric acid, and 0.93 grams of EDTA, into a flask. Then we put it in the Microwave and heat it. Heating continues until the flask solution will be completely transparent. Then we wait until it cools. We add one micro liter of ethidium bromide. We transfer this prepared solution to the tray of electrophoresis and put the spacer electrophoresis shoulder inside it. Then put the gel in a dark place and wait until it dries. Then transfer the tray into the electrophoresis device and take the shoulder out. From left to right, empty the first place of the wells, the second place marker (ladder), the third place PCR product of sample 1, the fourth place PCR product of sample 34, the fifth and sixth place derived products without primer, from each one, we transfer 6 micro-liters. Then attach120 watts current to the tank by the power supply and after 45 minutes, we remove the gel and place it in the dock gel. Separated bands were compared with the markers.

View 371 bpbars after electrophoresis: After electrophoresis, electrophoresis gel was put in the gel dock machine and specific bands were observed.

PCR product sequencing of 16s rRNA gen: 45 microliters of the final product of PCR, with Forward primers was sent to South Korea macro gene Company.

Sequence analysis of gene 16 SrRNA Determination: Arsasy sequences of the company were observed by using chromas software.

The results of soil cultivation on nutrient agar environment

In the study, 79 samples of bacteria were isolated.

Table 2- Results of culture in agar nutrient culture medium

20 meters	**9 samples**
40 meters	**10 samples**
60 meters	**19 samples**
80 meters	**21 samples**
100 meters	**20 samples**

Table 3-amount of salinity in sampling soils

(ds m^{-1})amount of salinity	sampling soils
30.1	**20 meters**
25	**40 meters**
23	**60 meters**
15	**80 meters**
10	**100 meters**

Result of Culture in a specific environment

We culture 79samples of the bacteria, which were isolated in the form ofa single colony, on a nutrient agar medium, with one percent starch content.

The results from growth in specific environments of soils with 20 meters distance fromUrmia Lake: In this study, 9 samples of positiveamylase were isolated, that the sample 3 from the Plate 1 had the highest amylase activity with a halo as much as 18 mm.

The results from growth in specific environments of soils with 40 meters distance from Urmia Lake: In this study, a total of 10 positive amylasewere isolated, that the sample 2 from the plate 5 had the highest amylase activity with a halo as much as 20 mm.

The results from growth in soils with 60 meters distance from Urmia Lake: In this study, 19 samples of positive amylase were isolated, that the sample 4 from the plate 5had the highest amylase activity with a halo as much as 24 mm.

The results from growth in soils with 80 meters distance from Urmia Lake: In this study, 21 samples were isolated, that the samples 1 and 4 from plate 5had the highest amylase activity with a halo as much as 24 mm.

The results from growth in soils with 100 meters distance from Urmia Lake: In this study, 20 samples were isolated, that the sample 2 from plate 1had the highest amylase activity with a halo as much as 22 mm.

According to experiments, by reducing the amount of salinity, bacteria of positive amylase increases and there is a direct positive relation between salinity and positive amylase bacteria and the activity.

After culturing, the strains that had more amylase activity than other strains were selected for biochemical tests.

The results of biochemical tests (Table 5)

Table 4 - Effect of salinity on the bacterial amylase activity

Sampling intervals	(ds/m)Salinity level	number of samples
20 meters	30.1	9
40 meters	25	10
60 meters	23	19
80 meters	15	21
100 meters	10	20

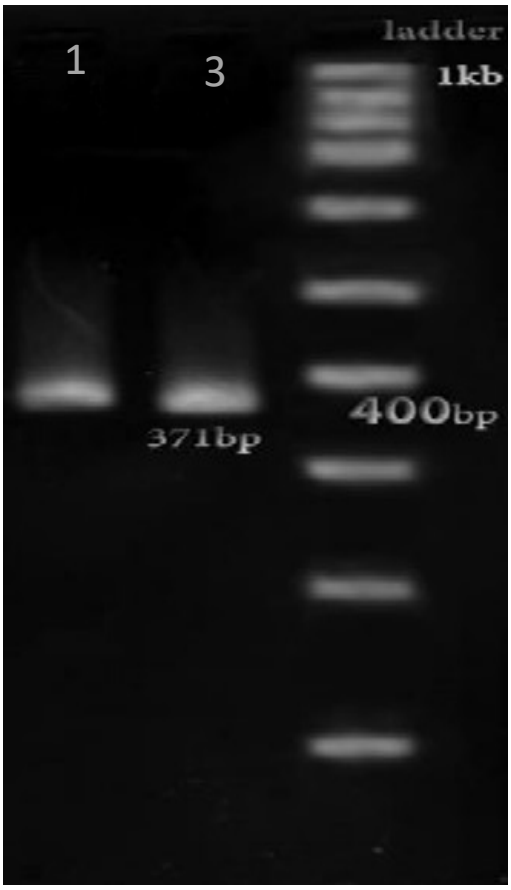
Table 5- The results of biochemical tests

	1	9	18	25	26	30	33	34	35	40
move	+	+	-	+	+	-	-	+	-	-
Gram	+	+	+	+	+	+	+	+	+	+
Creating Spore	+	-	+	+	-	+	+	+	+	+
Aerobic growth	+	-	+	+	-	-	+	+	+	+
Anaerobic growth	+	+	+	+	+	+	+	+	+	+
Vezer Proskauer test	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	-	-	+	-	+	+	+
Pigmented colonies.										
Yellow-pink-red	-	-	-	+	-	-	+	-	-	-
Dark brown / black	-	-	-	-	-	-	-	-	-	-
Simon citrate	+	-	+	+	+	+	-	+	+	+
PH growth in										
5	+	+	+	+	-	-	-	+	+	+
6	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+
8	+	-	-	+	-	-	+	+	+	+
8	-	-	-	+	-	-	-	-	+	+
10	-	-	-	-	-	-	-	-	-	-
NaCl growth in										
2%	+	+	+	+	+	-	+	+	+	+
5%	+	+	+	+	+	-	+	+	+	+
7%	+	+	+	+	+	+	+	+	+	+
7%	+	-	+	+	+	+	+	+	+	-
Sugar consumption										
Mannitol	+	+	-	-	+	+	-	+	+	+
Mannose	+	-	+	+	+	+	+	+	+	+
Arabinose	+	+	-	+	-	-	-	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+
Growth in temperature										

5	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	+	-	-	-	-	-
20	+	+	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+	+	+
40	+	+	+	+	+	+	+	+	+	+
50	+	+	-	-	-	+	+	-	-	-
55	-	-	-	-	-	+	+	-	-	-
65	-	-	-	-	-	-	-	-	-	-

According to the biochemical test table 5, morphological characteristics of these tests show that the strain 1 and 34 belong to *bacillus licheniformis*.

PCR Results: Information obtained by agarose gel from the electrophoresis of amplified samples by PCR shows that the molecular weight of amplified SrRNA 16 of two 1 and 34 strains is bp371.



The molecular identification results of selected strains: sequences obtained by using the BLAST software online database NCBI are compared with the reported sequences in the database and the closest species were extracted from gene data banks' base.

Results of determining sequences and BLAST on them

Table 6-closest species to isolated strains based on the gene sequence 16SrRNA

Sample number	Sample Name	The closest species based on 16S rRNA gene analysis with BLAST	Identity percentage
1	1	*Bacillus licheniformis*	98
2	34	*Bacillus licheniformis*	99

DISCUSSION

This study, with slightly different in details, was similar to research in the area of Urmia Lake, in 1991 by Rezazad. This study can be considered as an important point for further investigation, in the region and identifying the efficiently organism population from the production of an enzyme. The effects of soil salinity of the Urmia Lake shores, at different intervals, on samples were obtained and their production of the enzyme is very clear in this study. Demircan (2010) has purified Alpha amylase produced by Bacillus with deposition method, by ammonium sulfate, column chromatography of TSK, dialysis and column chromatography of Sepharose and has determined the purified enzyme molecular weight by polyacrylamide gel electrophoresis (SDS-PAGE). In this study, unlike the present study, the effects of salt-loving bacteria have not been done in the production of amylase. In addition, their isolated bacteria have been identified as Bacillus cereus. Research conducted by Ozivel et al (2005), molecular Weight of amylase enzyme has not been studied, the positive amylase bacteria, which they have separated, was halophilic Halomonas, and their Bacillus was not reported. In this study, starch has been selected as a carbon source and Peptone was selected as a source of nitrogen. The effect of temperature on amylase production, have been examined. It has been reported that, amylase has not lost its activity at 50 ° C for 2 h of incubation, but, it has lost 70% of its original activity after 2 hours of incubation, at 90 ° C, that is similar to the present research with finite differences. Positive amylase Bacteria separated by Abdoval et al (2011) was Bacillus cereus MS, while, we have separated positive amylase bacillus licheniformis that the difference in bacterial species causes differences in the effects of enzymes on starch. Identify their bacteria was done same as the study, by sequencing of SrRNA16. To purify the enzyme, the method of ammonium sulfate precipitation, ion exchange and chromatography were used.

Amylase enzyme producing bacteria isolated by Babavalian et al (2009) was salt loving as

this study and like our research, at first; agar nutrient culture medium without salt was used to differentiate salt-bearing bacteria from absolute salt-loving bacteria. Halotolerant strains had not been able to grow in these environments. Our research led to the isolation of 79 positive amylase bacteria that we have placedgenotype investigations only on gram-positive by SrRNA 16 method. In their study, 22 gram-negative samples and 61 gram-positive bacteria were isolated and molecular and phylogenetic characteristics of the selected bacteria were identified by the SrDNA16technique and were sequenced. Due to the drying of Urmia in recent years, the sudden salinity of soils can be effective in the growth of bacteria. The results showed that the number of positive isolated amylase samples has a reverse relation to the degree of salinity, so that the number of amylase bacteria produces in a distance of 20 meters shore of Lake Urmia has reached from 9 to 21 in 80 meters shore and in this distance, the salt has reached from30.1 to 15 dsm^{-1}. Among the isolated bacteria, several samples have had very good enzymatic activity. Since this enzyme is widely used in industry, separation of the samples that produce heat resistant enzymes will be important. Separated bacteria had the ability to grow at 50 ° C and therefore are resistant to heat.

Those enzymes that are able to have optimal activity in harsh industrial conditions in terms of temperature and salt are very important and salt-lovers are potential sources of such enzymes. Most of environmental isolates capable of producing hydrolytic enzymes belong to the gram-positive species; especially spore-forming Gram-negative bacilli that such a finding seems to expect with extreme conditions that governing in this salty Lake because of high salt as an extreme factor.

According to the results of this research, the following suggestions are expressed.

1. Purification and characterization of the amylase enzyme of Bacillus
2. Three-dimensional structure of the enzyme through X-ray crystallography to determine the properties listed
3. To clone amylase producing gene from Bacillus species producing amylase
4. Examine the possibility of mutation formation in order to increase the production of amylase by using mutagenic compounds
5. Optimization of amylase production by bacillus *licheniformis* in the scale of semi-industrial and industrial

6. Determining areas with basils with the greatest potential to produce the amylase enzyme

7. Basils optimal characteristics identifying in terms of pH, temperature, food for maximum production of amylase enzyme

8. Indigenous of food glucose product knowledge by using amylase in the country

9. Help and time optimization and reducing the financial costs of industries in the country by producing microbial amylase enzyme

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