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ISOLATION AND CHARACTERIZATION OF NOVEL EXTREMELY HALOTHERMOPHILIC BACTERIUM, *HALOMONAS CASEINILYTICA WN.1B.S,* FROM WADI AN NATRUN, EGYPT

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Abstract

The purpose of the present study was to isolate microbial halothermophiles from hyper saline Al- Hamra Lake at Wadi An Natrun, Egypt. Twenty eight bacterial isolates were obtained and the morphological and physiological properties in addition to enzyme activities were studied. Amongst those isolates, WN.1B.s was selected as the most potent isolate based on growth at high temperature (up to 65ºC) and at high salt concentration (up to 34%, near saturation state). A phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate WN.1B.s had the highest sequence similarity with respect to type strains of *Halomonas caseinilytica* (97 %), *Halomonas elongata* (96 %), *Halomonas eurihalina* (95 %), *Halomonas koreensis* (95 %) and *Halomonas halmophila* (95 %). Based on physiological characteristics and 16S rRNA sequence analysis this isolate was identified as *Halomonas caseinilytica* WN.1B.s which belonged to bacterial domain, class *Gammaproteobacteria*, order *Oceanospirillales*, *family Halomonadaceae, Halomonas species.* Enzyme screening for strain WN.1B.s showed that, the isolate secrete amylases, lipases, cellulases and pectinase enzymes under harsh conditions that may be useful in different industrial processes.

Introduction:

Poly-extremophilic microorganisms adapted to more than one extreme conditions among those organisms, halothermophilic microorganisms that adapted to two environmental stress conditions of high salt concentration and high temperature. This organisms found in most aerobic halophilic Archaea of the order Halobacteriales *such as, Haloarcula quadrata, Halobacterium salinarum and Haloferax volcanii* **(Grant, 2001),** and extremely halophilic bacteria with high temperature equal to or greater than 50°C such as *Dichotomicrobium thermohalophilum, Halonatronum saccharophilum, Halothermothrix orenii* and *Natranaerobius thermophilus* **(Bowers *et al.*, 2009).** Another group of poly-extremphiles, halo-alkalothermophiles are a novel physiological group of bacteria that required to three extreme conditions; high salt concentration, alkaline pH values and elevated temperature for growth. Very few extreme halophiles that are able to grow under this conditions for instance, strain *Natranaerobius thermophilus* is the first true anaerobic, halo-alkalothermophile isolated from sediments of solar-heat, alkaline, and hypersaline soda lakes of the Wadi An Natrun (**Mesbah *et al.*, 2009; Bowers and Wiegel, 2011).**

Wadi An Natrun is a depression in Sahara desert located in Egypt and about 80 Km northwest of Cairo. Along the valley stretches a chain of seven large alkaline, solar heated and hypersaline lakes supplied by underground seepage water from the river Nile and occasional winter precipitation. The depth of lakes ranges between 0.5−2 m. High evaporation rates and arid climatic conditions during the summer months cause the salinity to rise above 30% (w/v).

Wadi An Natrun lakes are extreme in more than one condition; high salt concentrations between 91.0 and 393.9 g/l and alkaline pH in addition to increasing in lakes temperature due to sun action. Salinity and temperature are the same throughout the water column. **(Imhoff *et al.*, 1979; Taher, 1999).** Wadi An Natrun lakes are populated by dense number of novel prokaryotic species, *Archaea* as well as *Bacteria* that have the ability to adapte to more than one stress condition.

Halothermophilic microorganisms have great potential applications in various biotechnology fields including bioremediation of contaminated hypersaline brine, fermentation of soy and fish sauce, and production of poly hydroxy alkanoates, compatible solutes, and β-carotene., as in addition they are valuable sources of microbial enzymes that can be used in many harsh industrial processes due to their tolerance to high temperature and high salinity conditions **(Gomes and Steiner, 2004; Dodia *et al.*, 2006; Namwong *et al*., 2006; Alqueres *et al*., 2007).**

The purpose of this research was to explore any novel extremely halothermophilic aerobic or facultative anaerobic bacteria, and to examine their phenotypic, physiological and biochemical characteristics. It was also aimed to assess the bacterial biodiversity of halophilic bacteria in Al- Hamra lake at Wadi An Natrun using preliminary description.

Materials and methods:

Sampling:

Twenty three soil samples were collected from different localities of Al- Hamra lake, Wadi An Natrun, Beheria governorate under aseptic conditions.

Isolation media:

Two different media were used to isolate halothermophilic microorganisms; medium (A) containing the following ingredients (g/l); NaCl, 125; $MgCl_2.6H_2O$, 50; K_2SO_4, 5; $CaCl_2.6H_2O$, 0.2; Tryptone, 5; Yeast extract, 5; and Agar, 20 **(Mullakhanbhai and Larsen, 1975)**, pH of the medium was adjusted at 6.8 and sterilized at 121˚C for 15 min. and medium (B) containing the following ingredients (g/l); NaCl, 220; $MgSO_4.7H_2O$, 10; KCl, 5; $CaCl_2.2H_2O$, 0.2; KNO_3, 1; Disodium

citrate, 3; Casein hydrolysate, 5; Yeast extract, 1; and Agar, 20 **(Post, 1977),** pH of the medium was adjusted at 7.2 and sterilized at 121˚C for 15 min.

Isolation of halothermophiles:

Isolation procedures were performed to recover halothermophilic microorganism by dilution plate technique on two previous agar media (A& B). An appropriate volume (0.1 ml) of diluted samples were streaked on agar media and incubated at 46ºC. The isolated strains were sub-cultured several times under same conditions to obtain pure cultures. Pure isolates were sub- cultured on slants of agar media and kept for further investigation at 4˚C **(Johnson *et al.*,1959; Atlas, 1993).**

Morphological studies for microbial isolates:

Pure colonies were characterized for color and shape. Microbial isolates were also classified on the basis of Gram's stain to Gram's positive or negative confirmed by using 3% KOH reaction.

Physiological studies for microbial isolates:

Microbial isolates were cultivated at different temperatures (46-65ºC), different pH values (6−10), and different sodium chloride concentrations of 12.5% to 35% with medium A and 22% to 35% with medium B.

Preliminary survey for enzymes production:

For primary screening of enzymes; proteases, amylases, pectinases, lipases, dehydrogenase and cellulases microbial isolates were inoculated in the form of regular spots on different agar medium (A) and (B) supplemented with respective substrate. (i) For proteases: on gelatin agar. proteases production was detected on the basis of gelatin hydrolysis around the colony after addition of acid mercuric chloride solution reagent. (ii) For amylases: on starch agar plates, for the detection of amylase production, plates were flooded with the iodine solution to detect the clear zone surrounding the colony against blue background.(iii) For pectinases: on pectine agar plate. Appearance of clear zone surrounding the colony after addition of iodine solution indicated the secretion of the pectinases by the corresponding organisms. (iv)For lipases: on tributyrin agar medium. The detection of lipases was done on the basis of the appearance of clear zone surrounding the colonies. (v) For cellulases: on cellulose agar medium. Secretion of cellulases was detected with clear zone around the colony against dark background by adding iodine solution. (vi)For dehydrogenase: on methylene blue agar medium. Secretion of dehydrogenase was detected with reduction of methylene blue around microbial colony. All experiments performed at 50ºC for 48-72 h.

Identification of the most potent halothermophilic isolates:

Identification of most potent halothermophilic isolates were based on 16S rRNA sequence analysis and also by study their morphological, physiological and biochemical characteristics using the identification keys described by **(Collins and lyne, 1985; Cowan, 1993)**. Partial 16S rDNA sequence of bacterial isolate were carried out in Sigma Research Company, Cairo, Egypt. DNA was extracted using protocol of GeneJet genomic DNA purification Kit (Fermentas) and amplified using Maxima Hot Start PCR Master Mix (Fermentas). PCR product was purified using Gene JET PCR Purification Kit (fermentas). The forward and reverse primers used for PCR amplification were 27^{f} (5′-AGAGTTTGATCCTGGCTCAG -3′) and 1492^{r} (5′-GGTTACCTTGTTACGACTT-3′) (16S rDNA universal primer). Sequencing of the PCR product was carried out in GATC (Guanin Adenin Thymin Cytosin) German Company using ABI 3730xl DNA sequencer.

Phylogenetic analysis of bacterial isolates:

By using 16S rRNA gene sequences, the strains were identified by BLAST search (blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences of closely related type strains were retrieved for constructing the phylogenetic trees to confirm similarities of most potent strains with other related groups.

Result and Discussion:

Description of obtained isolates:

Twenty eight halothermophilic isolates were obtained from Al-Hamra Lake, Wadi An Natrun, Beheria governorate. Of those, 18 isolates were grown on medium (A) containing 125 g/l sodium chloride and 10 isolates were grown on medium (B) containing 220 g/l sodium chloride. All isolates are catalase positive and can be divided according to Gram stain and cell shape into; Gram-positive bacilli (4 isolates), Gram-negative bacilli (10 isolates), Gram-positive cocci (10 isolates), and Gram -negative cocci (4 isolates) as shown in Table [1].

The morphological and physiological characteristics of all isolates shown in table (1). These isolates showed good growth at neutral and slightly alkaline pH (7-8). Most of isolates grow at temperature up to 50°C, ten isolates grow at temperature up to 60°C (WN.3A.s, WN.14A.s, WN.15A.s, WN.3B.s, WN.4B.s, WN.6B.s, WN.7B.s, WN.9B.s, WN.10B.s, WN.11B.s, and only two isolates WN.1B.s and WN.2B.s were able to grow at higher temperature up to 65°C (Table 1). As have been described in Table [1], the isolates were categorized into 3 categories based on tolerance to different NaCl concentrations, namely moderately-, borderline-, and extremely-halophiles as described by many researchers **(Kushner, 1978; Kushner and Kamekura, 1988)**. Moderate halophiles; that adapted to grow at salt concentration up to 150 g/l, including isolates WN.10A.s and WN.13A.s., borderline

halophiles; that adapted to grow at salt concentration up to 200 g/l, including isolate WN.12A.s., and extreme halothermophiles; that adapted to grow at salt concentration up to or above 300 g/l, including the other twenty five isolates**.**

While the results that reported by **Aneela *et al*., (2012),** during isolation of extremophile organisms from environments with very high concentrations of salt of Karak region of Pakistan were; higher frequencies of moderately-, slightly halotolerant and halophilic bacteria compared to lower frequencies of extremely halophilic bacteria in saline environments. This work performed on Tryptic Soya Agar medium containing various concentrations (5-20 %) of NaCl and incubated at 28ºC.

Isolation occurred on medium A that contain 50 g/l of $MgCl_2.6H_2O$ and medium B that contain 10 g/l of $MgSO_4.7H_2O$ so the most probably isolates shloud be extremophilies therefore our results were full agreement with **Grant *et al*., (2001)** who reported that; The growth of extremely halophiles requires relatively high NaCl concentration and the majority of them require magnesium ion (Mg^{2+}) for their growth whereas slightly and moderately-halophiles do not require magnesium ion for growth**.**

Enzyme screening:

Halothermophilic microorganisms secreted different enzymes although the presence of these harsh conditions of high salt concentration in addition to elevated temperature. All isolates were screened for six enzymes: proteases, amylases, pectinases, lipases, dehydrogenase, and cellulases (Table 2). All isolates haven't the ability to secrete Dehydrogenase enzyme, 13 isolates were produced amylase, 11 isolates were produced protease, 10 isolates were produced cellulase, 9 isolates were produced lipase, and 5 isolates were produced pectinase. High potence was found in: (i) isolates (WN.14A.s& WN.2B.s) they produced amylase, cellulase and pectinase enzymes. (ii) isolates (WN.16A.s& WN.7B.s) they produced amylase, protease, cellulase, and lipase enzymes. (iii) isolates (WN.3B.s& WN.11B.s) they produced amylase, protease, and lipase enzymes. (iv) isolate WN.3A.s produced amylase, protease, and cellulase enzymes. (v) isolate WN.1B.s produced amylase, cellulase, lipase, and pectinase enzymes. (vi) isolate WN.6B.s produced amylase, protease, cellulase, and pectinase enzymes.

Table (1): Morphological and physiological characteristics of halothermophilic isolates obtained from Al- Hamra Lake, Wadi An Natrun, Egypt.

No.	Isolate code	Morphological characteristics			physiological characteristics		
		Gram's reaction	3% KOH reaction	Shape	Maximum Temp. (°C)	Maximum NaCl conc.(g/l)	pH range
1	WN.1A.s	+	-	Bacilli	50	250	6−8
2	WN.2A.s	-	+	Bacilli	50	250	6−8
3	WN.3A.s	-	+	Bacilli	60	300	6−8
4	WN.4A.s	-	+	Bacilli	50	250	6−8
5	WN.5A.s	-	+	Cocci	50	300	6−8
6	WN.6A.s	-	+	Bacilli	50	250	6−8
7	WN.7A.s	+	-	Bacilli	50	300	6−8
8	WN.8A.s	+	-	Bacilli	50	300	6−8
9	WN.10A.s	-	+	Cocci	50	140	6−8
10	WN.11A.s	-	+	Bacilli	50	250	6−8
11	WN.12A.s	+	-	Bacilli	50	200	6−8
12	WN.13A.s	-	+	Bacilli	50	140	6−8
13	WN.14A.s	-	+	Bacilli	60	250	6−8
14	WN.15A.s	-	+	Bacilli	60	250	6−8
15	WN.16A.s	-	+	Bacilli	50	300	6−8
16	WN.18A.s	-	+	Cocci	50	300	6−8
17	WN.19A.s	+	-	Cocci	50	300	6−8
18	WN.21A.s	-	+	Bacilli	50	300	6-8
19	**WN.1B.s**	-	**+**	**Cocci**	**65**	**340**	**6−8**
20	WN.2B.s	-	+	Cocci	65	325	6−8
21	WN.3B.s	-	+	Cocci	60	250	6−8
22	WN.4B.s	-	+	Cocci	60	325	6−8
23	WN.5B.s	-	+	Cocci	50	220	6−7
24	WN.6B.s	-	+	Cocci	60	325	6−8
25	WN.7B.s	+	-	Cocci	60	220	6−8
26	WN.9B.S	-	+	Cocci	60	325	6−8
27	WN.10B.s	+	-	Cocci	60	250	6−8
28	WN.11B.s	+	-	Cocci	60	325	6−8

WN; Wadi An Natrun A; Isolation medium (A) B; Isolation medium (B)

Table (2): Primary screening test for enzyme production by the isolated halothermophilic isolates obtained from Al-Hamra lake,Wadi An Natrun, Egypt .

No.	Isolate code	Amylase	Protease	Cellulase	Lipase	Dehydrogenase	Pectinase
1	WN.1A.s	−	+++	+	−	−	−
2	WN.2A.s	−	−	−	−	−	−
3	WN.3A.s	+++	+++	++++	−	−	−
4	WN.4A.s	−	−	−	−	−	−
5	WN.5A.s	−	−	−	−	−	−
6	WN.6A.s	−	−	+	+	−	−
7	WN.7A.s	−	++++	−	−	−	−
8	WN.8A.s	−	++++	−	−	−	−
9	WN.10A.s	−	−	−	−	−	−
10	WN.11A.s	−	−	−	−	−	−
11	WN.12A.s	−	−	−	−	−	++
12	WN.13A.s	−	−	−	−	−	−
13	WN.14A.s	+++	−	++++	−	−	++
14	WN.15A.s	−	+++	−	−	−	−
15	WN.16A.s	+	+	+	++	−	−
16	WN.18A.s	−	−	−	−	−	−
17	WN.19A.s	++	−	−	−	−	−
18	WN.21A.s	+	−	−	+	−	−
19	**WN.1B.s**	**+++**	−	**+++**	**++**	−	**+**
20	WN.2B.s	+++	−	+++	−	−	+++
21	WN.3B.s	++	+++	−	+	−	−
22	WN.4B.s	+	++++	−	−	−	−
23	WN.5B.s	+	−	+	−	−	−
24	WN.6B.s	+	+++	+	−	−	+
25	WN.7B.s	++	+	+	+	−	−
26	WN.9B.S	−	−	−	+	−	−
27	WN.10B.S	−	−	−	+	−	−
28	WN.11B.S	+	++	−	++	−	−

-, No activity; +**,** low enzyme production: clear zone diameter between (0.5−1cm); ++, moderate enzyme production: clear zone diameter between (1−1.8cm)**;** +++**,** high enzyme production: clear zone diameter between (1.8−2.5cm); ++++**,** Very highly enzyme production: clear zone diameter between (2.5−3.3cm).

Selection of most potent halothermophilic isolate.

Most potent halothermophilic isolate was selected according to growth at high temperature and high salinity. Among all isolates, WN.1B.s selected to be most potent isolate because it have the ability to grow at high temperature up to 65°C and high salt concentration reached to 340 g/l. This strain was found to produce four kinds of enzymes, amylase, cellulase, lipase and pectinase with relatively high potency so this strain have a potential candidate for different biotechnological processes required such this enzymes.

Identification of halothermophilic isolate *WN.1B.s*.

Morphological, physiological and biochemical characteristics *of* strain *WN.1B.s:*

Isolate WN.1B.s is Gram-negative with oval to cocci shape (Fig. 1). It appears on agar plate with yellow to orange color. This isolate is aerobic and non-spore former. The isolate grow at elevated temperature from 46˚C up to 65˚C and NaCl concentration up to 340 g/l (saturation state). This isolate grow well at slightly alkaline pH (7-8). Isolate WN.1B.s was found to produce amylase, cellulase, lipase and pectinase enzymes which can be used in many application such as surfactant, fish sauce, food industry, antifouling, and other useful applications.

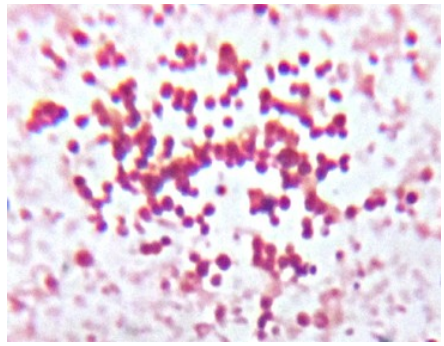

Fig (1): Photograph showing shape of halothermophilic isolate *WN.1B.s* under light microscope (X: 1500).

Phylogenetic and 16S rRNA sequence analysis of halothermophilic isolates *WN.1B.s*.

Strain WN.1B.s was closely related to *Halomonas* species with high similarity (97 %) to *Halomonas caseinilytica* according to 16S rRNA gene that was amplified and analyzed in which partial 16S rRNA gene sequence (1274 bp) of strain WN.1B.s was determined. The sequence was compared with closely related sequences of reference organisms from NCBI network service (blast.ncbi.nlm.nih.gov/Blast.cgi). PCR product of I6S rDNA gene for the isolate WN.1B.s shown in Fig. (2).

Strain WN.1B.s showed the highest levels of sequence similarity with respect to type strains of *Halomonas caseinilytica* (97 %), *Halomonas elongata* (96 %),

Halomonas eurihalina (95 %), *Halomonas koreensis* (95 %) and *Halomonas halmophila* (95 %) and showed less than (95.0 %) sequence similarity with respect to other *Halomonas* species that belong to bacterial domain, class *Gammaproteobacteria*, order *Oceanospirillales*, *family Halomonadaceae*, Strain WN.1B.s isolated from a soil samples from Al- Hamra lake, Wadi An Natrun, Beheria governorate, Egypt. Dendrogram tree was illustrated in (Fig. 3) showing the phylogenetic relationship of WN.1B.s with related groups.

This result was showed partial agreement with the finding recorded by **Hong *et al* (2008),** who isolated novel halophilic bacterium (designated strain AJ261^{T}), which belongs to the genus is *Halomonas*, for which the name is *Halomonas caseinilytica* proposed. This strain showed the highest levels of sequence similarity

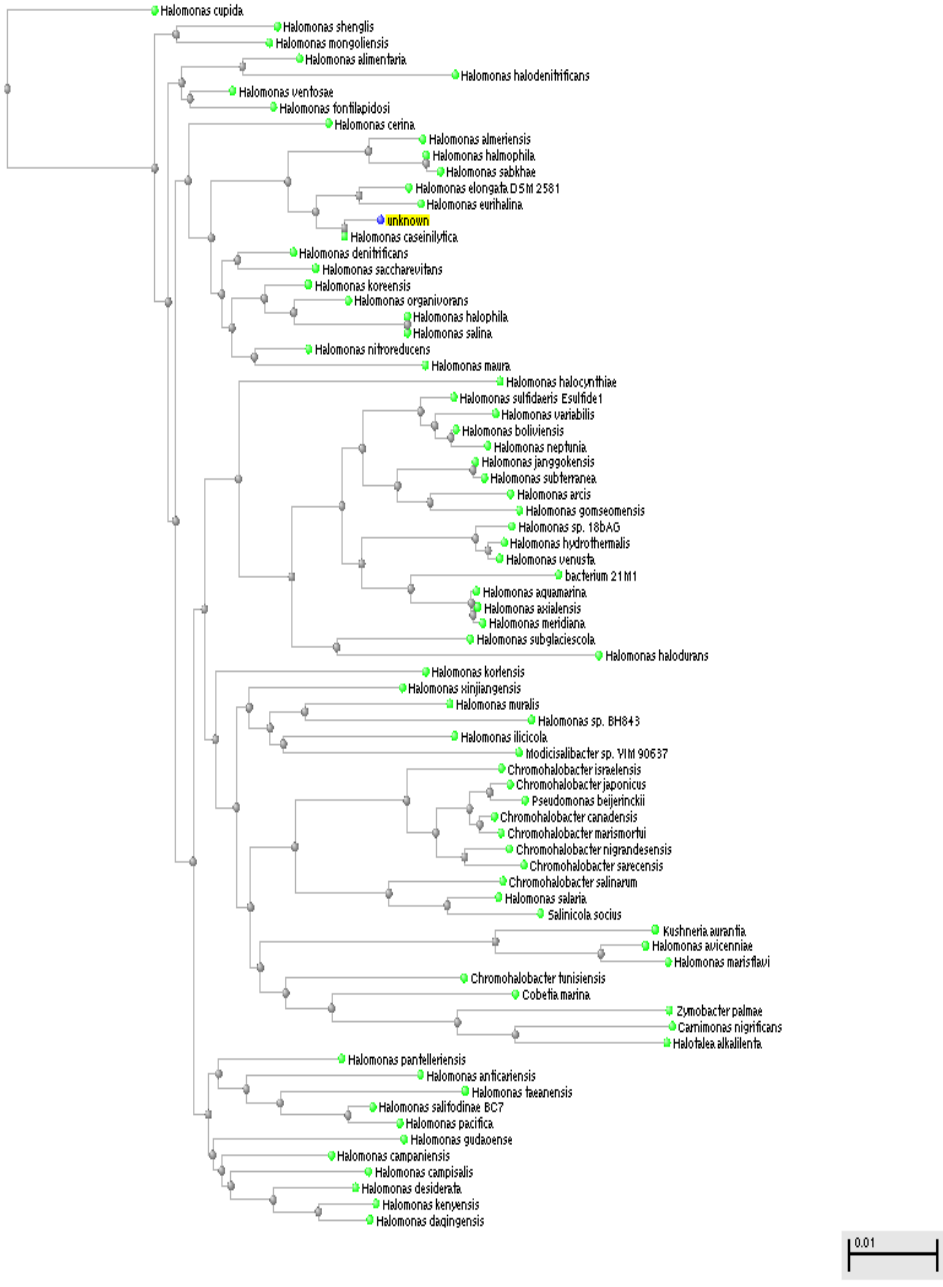

Fig. (3): Neighbor-joining tree based on 16s rRNA gene sequences, showed the Phylogenetic relationships of the isolate WN.1B.s and related taxa.

Table. (3): A comparative studies of the identification properties for isolate *WN.1B.s* in relation to the reference strain *Halomonas caseinilytic*.

Organism Character	*WN.1B.s*	*Halomonas caseinilytic*

Cell shape	oval	short rod or oval
Colony pigmentation	yellow-orange	light yellow
Gram reaction	−	−
KOH (3%)	+	+
Motility	Motile	Motile
Spore formation	−	−
Catalase	+	+
Oxidase	+	+
Relation to oxygen	+	+
Salt range (%, w/v)	up to 34	0.5−15
Temp. range (˚C)	up to 65	4-48
pH range	5−8	5−9
Indol production	−	−
Methyl red	+	+
Voges-Proskuaer	−	−
Citrate utilization	−	−
Nitrate reduction	+	+
Urease	−	−
H2S formation	−	−
Carbohydrate fermentation		
Glucose	D	+A
Galactose	D	+A
Fructose	+A	+A
Xylose	+A	+
Arabinose	+A	+A
Manitol	−	+A
Lactose	−	+A
Maltose	−	+A
Sucrose	−	+A
Mannose	D	+A
O/F test	O	ND
Extracellular Enzymes		
Amylase	+	ND
Lipase	+	ND
Cellulase	+	ND
Dehydrogenase	−	ND
Pectinase	+	ND

(+): positive, (−): Negative, (O/F): Oxidation Fermentation test, (D); Doubtful, (+A): Acid production**. ND, No data.**

Conclusion:

Twenty eight halothermophilic isolates were obtained from Al- Hamra lake, Wadi An Natrun, Egypt, one of this isolates was identify as *Halomonas caseinilytic WN.1B.s* that, have the ability to grow at harsh conditions of extreme salt

concentration and elevated temperature. This strain secreted useful enzymes that can be used in various fields of biotechnology includes fermentation of soy, fish sauce, β-carotene production, compatible solutes production, enhanced oil recovery and degradation of toxic chemicals that can pollute hypersaline habitats.. Further studies are recommended on the remaining halothermophilic isolates to more identification and searching for new novel organisms that may be used for any biotechnological fields.

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الملخص العربي

عزل وتوصيف هالومونس كازينيليتكا كعزلة بكتيرية جديدة محبة للملوحة والحرارة العالية المعزولة من وادي النطرون, مصر

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• يهدف البحث إلى عزل بعض الكائنات المحبة للملوحة ودرجة الحرارة المرتفعة من وادي النطرون ومن ثم دراسة الخواص الظاهرية (المورفولوجية) وبعض الخواص الفسيولوجية لهذه العزلات واختيارأفضل عزلة لها القدرة علي تحمل أعلى درجة حرارة وأعلى تركيز للملح وكذلك تعريف هذه العزلة باستخدام الخواص البيوكيميائية والمورفولوجية والفسيولوجيةوكذلك باستخدام 16s rRNA sequence analysis.

ويمكن تلخيص نقاط البحث كالتالي:

• تم عزل 28 عزلة من بحيرة الحمرا من وادي النطرون محافظة البحيرة علي نوعين من الأوساط الغذائية, الوسط الأول(A) وكان يحتوي على نسبة 12.5 % من ملح كلوريد الصوديوم والوسط الثاني(B) وكان يحتوي على نسبة 22 % من ملح كلوريد الصوديوم وكانت درجة حرارة العزل 46 درجه مئوية .

• بالإضافة إلى دراسة الخواص المورفولوجية تم دراسة الخواص الفسيولوجية لهذه العزلات من:

1- درجة حرارة: لمعرفة أعلى درجة حرارة يمكن أن تتحملها كل عزلة وذلك برفع درجة الحرارة من 46 إلي 65 درجه مئوية.

2- نسبة كلوريد الصوديوم : لمعرفة أعلى ملوحة يمكن أن تتحملها كل عزلة وذلك برفع تركيز الملح لكلا الوسطين الغذائيين حتى درجة التشبع (35%).

3- الأس الهيدروجيني: لمعرفة الأس المناسب الهيدروجيني لكل عزلة وذلك بتدرج الأس الهيدروجيني من 6 إلي 10.

- تم دراسة بعض النشاطات الإنزيميه لهذه العزلات ممثلة في الإنزيمات الآتية (السيلليلاز و البروتياز والأميلاز والليباز والبيكتينايزوالديهيدروجينايز) فوجد ان هذه الكائناتـلها القدرة على إنتاج الإنزيماتـبكميات متباينة من كائن لآخر تحت ظروف قاسية من الملوحة العالية ودرجات الحرارة المرتفعة.
- تم اختيارأفضل عزلة من هذه العزلات وكانت تتحمل درجة حرارة 65 درجه مئوية وتركيز لملح كلوريد الصوديوم 34% وهي العزلة WN.1B.s وكان الاس الهيدروجيني المثالي للنمو هو 8.
- تم تعريف هذه العزلة معتمدا علي (rRNA sequence analysis) ثم تم تاكيد التعريف بواسطة الاختبارات البيوكيميائيه والمورفولوجية والفسيولوجيةفوجد أن هذه العزلة مشابهه بنسبة 97% للكائن هالومونس كازينيليتكا *Halomonas caseinilytic* وكانت هذه العزلة لها القدرة على إنتاج الإنزيماتـالآتية: السيليلاز والأميلاز والليباز والبيكتينايز تحت ظروف غير مواتيه يمكناستخدامها في الصناعات المختلفة التي تجرى في وجود تركيزات عالية من الأملاح ودرجات الحرارة العالية.