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# Identification and diagnosis of several halophilic bacteria in Sawa Lake, Southern Iraq

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## ABSTRACT

Six halophilic bacteria were isolated from Sawa Lake water, during the periods from February, 2016 till March, 2017. The most halophilic bacteria recorded during present study were not isolates from the lake water before; the success of isolation was due to the employed special culture media. All isolated bacteria were gram negative included: *Aeromonas salmonicida, Vibrio vulnificus, Chrysiceobacterium indologenes, Shewanella algae, Sphingomonas paucimobilis*, and *Acinetobacter lwoffii*. The most frequently culture species belong to *Shewanella algae* has been frequently shown in all stations and seasons. All isolated bacteria were pathogenic for aquatic and humans, thus lake water body was not suitable for swimming or cure dermatologist as believed by public.

**Keywords:** halophilic bacteria, Sawa Lake, Southern Iraq.

## 1. INTRODUCTION

Hypersaline waters are defined as those containing salt concentrations in excess of sea water, and can be classified to thalassohaline, which have a marine origin, if the composition similar to that of sea water, and athalassohaline, the composition of such waters varies widely, and reflects the composition of the surrounding geology, topography and climatic conditions, often influenced by the dissolution of mineral deposits [1]. Most inhabitant of these environments are micro-organisms that are called "halophiles" which are extremophile organisms that survive in the environments with very high salt concentrations. The name derived from the Greek for "salt-loving" [2]. Halophilic micro-flora has been well study from hypersaline regions around the world. However, Sawa Lake, singularity by not only it does contain the highest salt concentration of all natural water in Iraq, but the unique ionic composition of its water which highly inhibitory even to those microorganisms best adapted to life in this lake.

Only limited data has been given on the microbial community of the lake. The past observational studies by Abdul bari and Aqeel [3] was shown the close connection between the components of the lake and quantitative types of microbes present. Their results mention that 58% of total bacteria is the bacterium *E.coli*, while 33% *Bacillus*, and 5%, 4% of *Klebsiella*, and *Pseudomonas*, respectively. Makki [4] isolated 19 species of bacteria from Sawa lake and its coastal soil including *Staphylococcus aureus, Staphylococcus saprophyticus, Closteridium tetanii, Salmonella typhi, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Citrobacter freundii, Enterobacter cloaca, Vibrio alginolyticus, V. parahaemolyticus, V. cholera, V. vulnificus, Enterococcus spp., Shigella spp., Aeromonas spp, Corynebacterium spp*. and *Bacillus cereus*.

Najum and Hasan [5] stated that from 10 water samples of Sawa lake in different period, only 5 (50%) were positive for isolation of *vibrio cholera* where cultured on specific medium, in addition to isolates

other type of Vibrio as *V. parahemolyticas* (70%), *V. valnificus* (90%) and *V. alginolyticus* (80%) also *Areomonas hydrophila* (90%).

## 2. MATERIALS AND METHODS

Sawa Lake located at the eastern edge of the southern desert of Iraq, 22 Km to the west of the Euphrates river, about 23 km to the west of Al-Samawa city, southern of Iraq between longitudes 44° 59 29 E and 45° 01 46.61 E and Latitudes 31° 17 43.10 N, and 31° 19 49.79N. The maximum length of the lake is about 4.74 km but the maximum width of 1.77 km isolated by gypsum barrier with total path 12.5 Km surrounded the lake. It doesn't have a clear geometrical shape, but it tends to be pear-like [6, 7]. Water samples were collected in different seasons and different areas of Sawa Lake, during morning hours, through 8.30 a.m. to 1.30 p.m. of day, from February, 2016 to March, 2017, 100 ml of sample in sterilized bottles were collected from the water at a depth one meter, and stored at 4ºC till used for isolation of bacteria according to [8].

In this present study it was tested several media A, B, C, D, and E for isolation of halophilic bacteria belonging to different genera inhabiting Sawa lake water [9]. Also, Luria Bertani broth was used, prepared by dissolved 10g tryptone, 5g yeast extract, and 10g NaCl. One ml of Sawa lake water was added to 9 ml of sterile media in plates and broth in glass test tubes incubated at 37 Cº. Growth was determined by turbidity in the broth after 4-5 days, and colony formed in plate were picked using a sterile wire loop and streaked on sterile nutrient agar, sub-culturing was done by streaking for several time to obtain pure culture. The bacteria isolated were identified based on some biochemical tests such as, gram stain, catalase, oxidase test. Morphological characteristics was observed, identification was done, and confirmed by Vitek 2 compact system.

## 3. RESULTS AND DISCUSSION

In spite of adverse conditions to live in Sawa Lake, its support unique and varied community of halophilic bacteria. It is interesting to note that none of the genera (Table.1), that isolated in present study was previously described in Sawa Lake before except *Vibrio vulnificus*, and *Aeromonas sp.*, succeeded in isolating more probable that these isolation happened because of the used enrichment media or another possibility is the organisms have been introduced from many species of birds that migrate among wetland, visit the lake, and act as microbial dispersers.

**Table 1:** Halophilic bacteria in Sawa Lake

| | Seasons | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Winter | | Spring | | Summer | | Autumn | |
| | Feb. 2016 | Jan. 2017 | Apr. 2016 | Mar. 2017 | May 2016 | Aug. 2016 | Oct. 2016 | Nov. 2016 |
| ***Aeromonas salmonicida*** | | | | | | | | |
| **ST.1** | _ | _ | + | + | + | + | + | _ |
| **ST.2** | _ | _ | + | + | + | + | + | _ |
| **ST.3** | _ | _ | + | + | + | + | + | _ |
| **ST.4** | _ | _ | + | + | + | + | + | _ |
| **ST.5** | _ | _ | + | + | + | + | + | _ |
| ***Vibrio vulnificus*** | | | | | | | | |
| **ST.1** | _ | _ | + | _ | + | + | + | _ |
| **ST.2** | _ | _ | + | _ | + | + | + | _ |
| **ST.3** | _ | _ | + | _ | + | + | + | _ |
| **ST.4** | _ | _ | + | _ | + | + | + | _ |
| **ST.5** | _ | _ | + | _ | + | + | + | _ |
| ***Chryseobacterium indologenes*** | | | | | | | | |
| **ST.1** | _ | _ | _ | _ | + | + | + | _ |
| **ST.2** | _ | _ | _ | _ | + | + | + | _ |
| **ST.3** | _ | _ | _ | _ | + | + | + | _ |
| **ST.4** | _ | _ | _ | _ | + | + | + | _ |
| **ST.5** | _ | _ | _ | _ | + | + | + | _ |
| ***Sphingomonas paucimboilis*** | | | | | | | | |
| **ST.1** | + | + | _ | + | _ | _ | + | + |
| **ST.2** | + | + | _ | + | _ | _ | + | + |
| **ST.3** | + | + | _ | + | _ | _ | + | + |
| **ST.4** | + | + | _ | + | _ | _ | + | + |
| **ST.5** | + | + | _ | + | _ | _ | + | + |
| ***Shewanella algae*** | | | | | | | | |
| **ST.1** | + | + | + | + | + | + | + | + |
| **ST.2** | + | + | + | + | + | + | + | + |
| **ST.3** | + | + | + | + | + | + | + | + |
| **ST.4** | + | + | + | + | + | + | + | + |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **ST.5** | + | + | + | + | + | + | + | + |
| ***Acinetobacter lowffii*** | | | | | | | | |
| **ST.1** | _ | + | _ | + | _ | _ | _ | _ |
| **ST.2** | _ | + | _ | + | _ | _ | _ | _ |
| **ST.3** | _ | + | _ | + | _ | _ | _ | _ |
| **ST.4** | _ | + | _ | + | _ | _ | _ | _ |
| **ST.5** | _ | + | _ | + | _ | _ | _ | _ |

+Present, -Absent

**Scientific Classification of *Aeromonas salmonicida***
Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Aeromonadales
Family: Aeromonodaceae
Genus*: Aeromonas* [10]

It was used media E for isolation, on grams staining, gram negative bacilli with no specific arrangement was seen (Figure 1. A).The organism was catalase, and oxidase positive reaction. No growth was observed on MacConkey agar, on blood agar plate creamish to whitish colonies, not easily emulsifiable, and having entire margin grew (Figure.2). Identification was done and confirmed by Vitek 2 compact system. This result probably confirmed the observation of massive death of small fish Day's goby at the shore of lake during (March- May, 2016) through this study. It is considered as primary pathogen in variety of fishes, and not in humans as they cannot grow at 37C optimal temperature required for its growth has been reported as 22-25 [11], however nonpathogenic organisms for human may change their host preference and virulence when leg's skin infection was observed due to contact with soil and water of the lake were these organisms have been reported to be present in aqueous environments during samples collecting (Figure 1. B). These results were in confirm with the study of [12] who reported the first case of furunculosis infected on both legs by *Aeromonas salmonicida* in a 67 year old immunocompetent male in India.

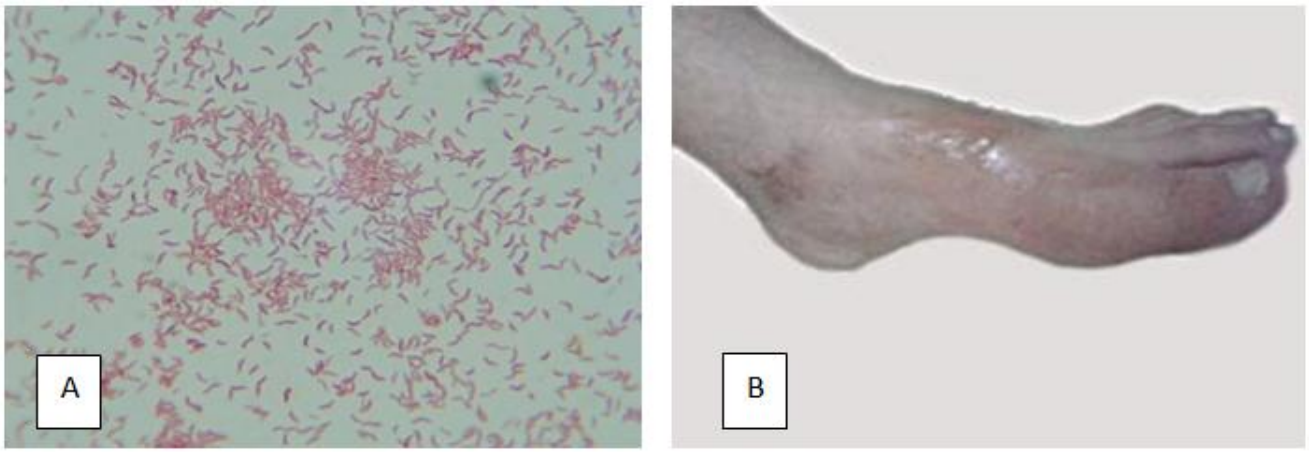


**Figure 1:** A: *Aeromonas salmonicida* negative bacilli, B: skin infection

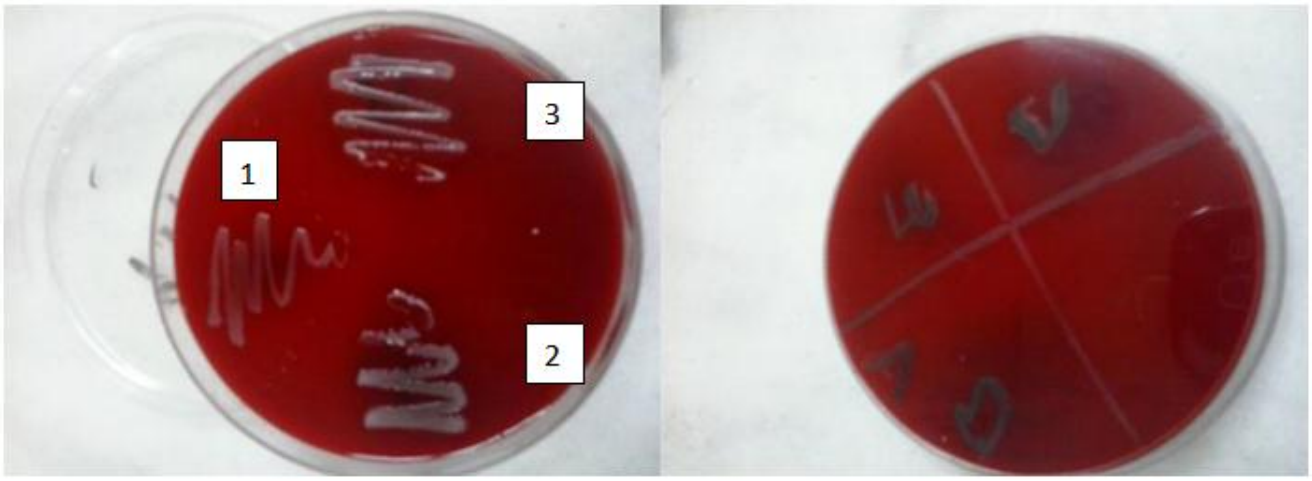


**Figure 2:** Blood agar plate 1*: Aeromonas salmonicida*, 2: *Vibrio vulnificus*, 3: *Chryseobacterium indologenes*

**Scientific classification of *Vibrio vulnificus***
Domain: Bacteria
Phylum: Protebacteria
Class: Gammaprotebacteria
Order: Vibrionales
Family: Vibrionaceae
Genus: *vibrio* [13]

Media B which proposed by [9] used for isolation of gram negative bacilli (Figure. 3), catalase and oxidase negative reaction according to [14]. Samples were subjected to culture on blood agar and MacConkey agar. No growth was observed on MacConkey agar, on blood agar plate creamish colonies were observed (Figure.2). Identification was done and confirmed by Vitek 2 compact system.

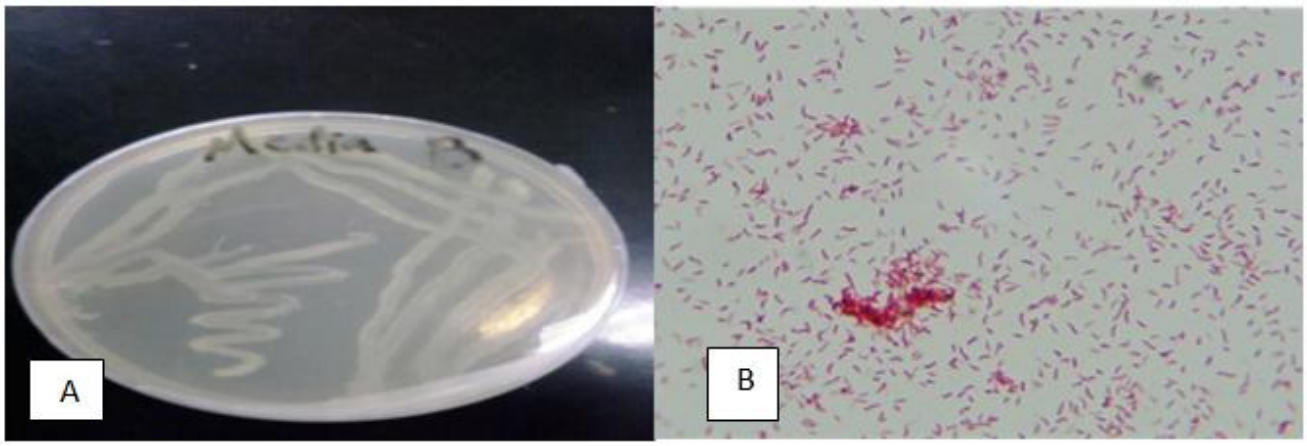


**Figure 3:** *Vibrio vulnificus*, A: growth on media B, B: gram negative bacilli

**Scientific classification of *Chrysiceobacterium indologenes***
Domain: Bacteria
Phylum: Proteobacteria
Class: Flavobacteria
Order: Flavobacteriales
Family: Flavobacteraceae
Genus: *Chryseobacterium* [15]

The organism isolated from media C was rod shaped, gram negative (Figure.4), colonies are white – pale yellow in color, circular, convex or low, smooth, with entire edges, catalase, and oxidase positive reaction, no growth was observed on MacConkey agar, on blood agar plate, cream, mucoid, smooth colonies, and have entire margin (Figure.2). Identification was done and confirmed by Vitek2 compact system.

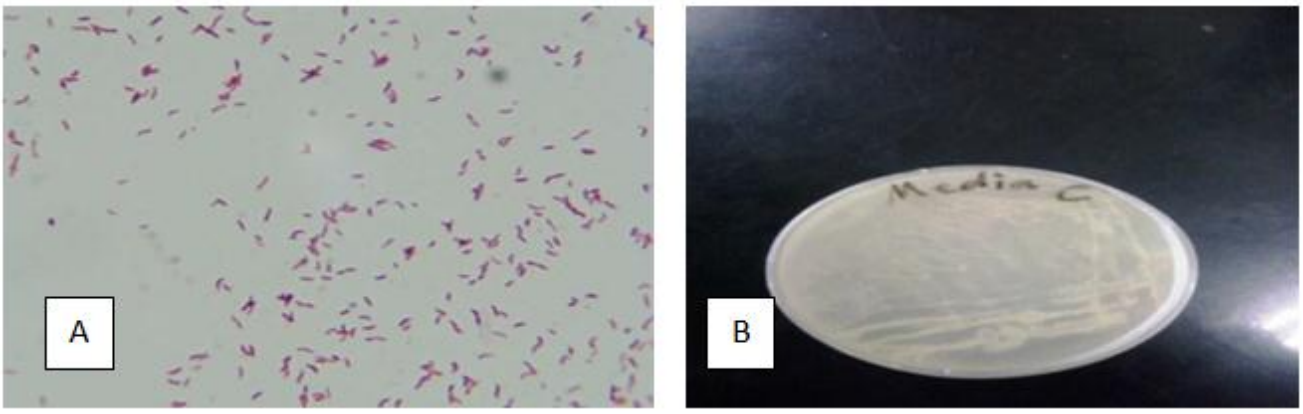


**Figure 4:** *Chryseobacterium indologenes*, A: gram negative bacteri, B: bacterial growth on media C

**Scientific classification of *Shewanella algae***
Domain: Bacteria
Phylum: Protobacteria
Class: Gammaproteobacteria
Order: Altermonadales
Family: Alteromondaceae
Genus: *shewanella* [16]

In the present study available data indicated that *S. algae* was a predominant isolation in media E in all season especially during summer (Table.2), The finding of this study in complete agreement with [17] they are reported that *Shewanella* species are found throughout the world in marine environments and most reported human infections occur in countries with warm climates. It is gram negative, rod shape, catalase, and oxidase positive reaction. On nutrient agar the colonies are smooth, mucous not easily emulsion (Figure.5).

Identification was done and confirmed by Vitek 2 compact system, orange or white in color pigmented when used media E (Figure.6), due to strong accumulation of cytochrome proteins [16]. Skin lesions with ulcer are common caused by *Shewanella algae* because of both the cytolysin and the protease are able to increase vascular permeability and cause tissue damage, which may enhance bacterial invasiveness [18]. A case of skin and soft tissue manifestations (i.e., lower leg cellulitis) occurring after raw seafood ingestion has been described in a 58-yr-old man living in the southern area of Korea, both lower legs were erythematous and edematous, and hemorrhagic bullae were noted on the anterior aspect of the left lower leg [19]. The result and study of Jampala *et al* [20] probably confirmed our observation of leg's skin infection when summing in the lake during sampling time probably associate with *Aeromonas salmonicida* (Figure 1. B).

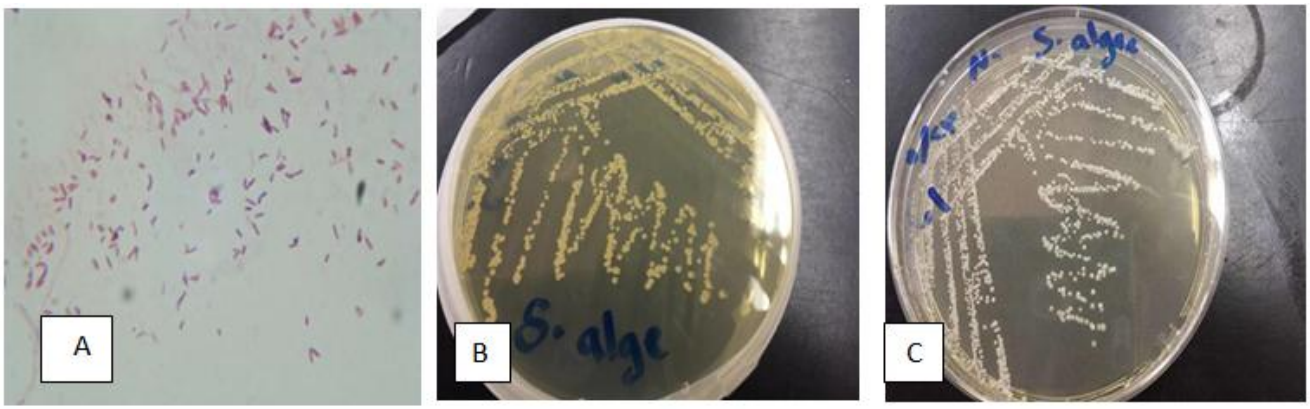


**Figure 5:** *Shewanella algae* A: gram negative reaction, B: orange color growth culture of *S .algae*, C: white color growth culture of *S. algae*

**Figure 6:** *Shewanella algae* culture with orange color in E media broth.

**Scientific classification of *Sphingomonas paucimobilis***
Domain: Bacteria
Phylum: Proteobacteria
Class: Alphaproteobacteria
Order: Sphingomonadales
Family: Sphingomonadaceae
Genus: *Sphingomonas* [21]

Media E broth used for isolation, gram negative rod shaped bacteria, pale yellow color colonies appears on agar plate which turn to deep yellow after several day (Figure.7), catalase positive, oxidase negative reaction. No growth was observed on MacConkey agar, identification was done and confirmed by Vitek 2 compact system. The organism can be cultured on a variety of employing media A, B, C, D, and E, including blood agar, but not on MacConkey.

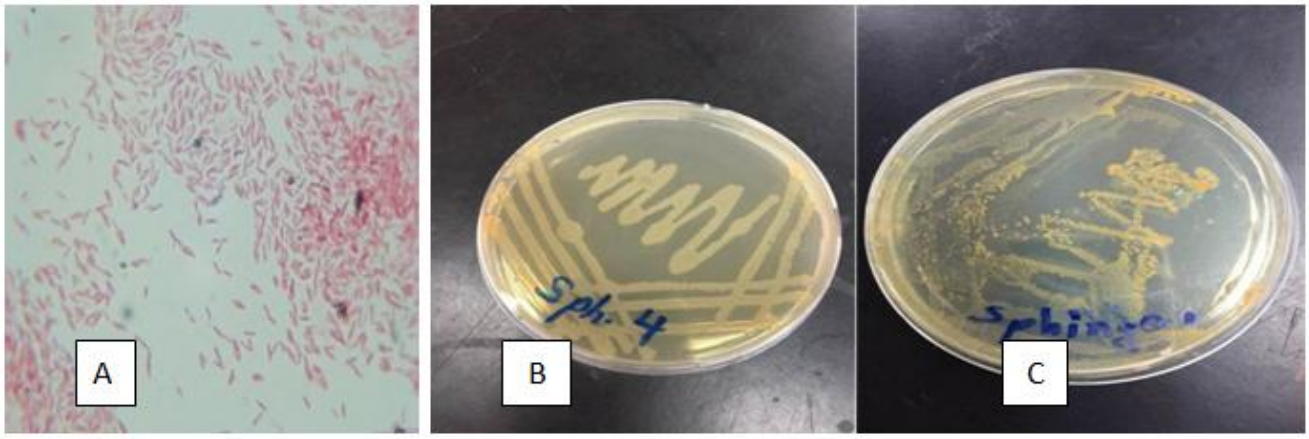


**Figure 7:** *Sphingomonas puacimobilis* , A: bacteria in gram stain, B: pale yellow colony growth on nutrient agar, C: old colony on nutrient agar.

**Scientific classification of *Acinetobacter lwoffii***
Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Pseudomonadales
Family: Moraxellaceae
Genus: *Acinetobacter* [22]

Luria Bertani broth was used for isolation, gram negative short rods bacterium, colonies circular, convex, smooth, with entire margins, beige on nutrient agar (Figure.8). It is with catalase positive, and oxidase negative reactions .Identification was done and confirmed by Vitek 2 compact system. Vaneechoutee *et al*., [23] stated that the organisms can form a pink colour on MacConkey agar. However, no growth was

observed on MacConkey agar in present study suggested may be other strain of *Acinetobacter lowffii* depended on [24] who stated that most members of *Acinetobacter* show good growth on MacConkey agar with the exception of some *A. lwoffii* strains.

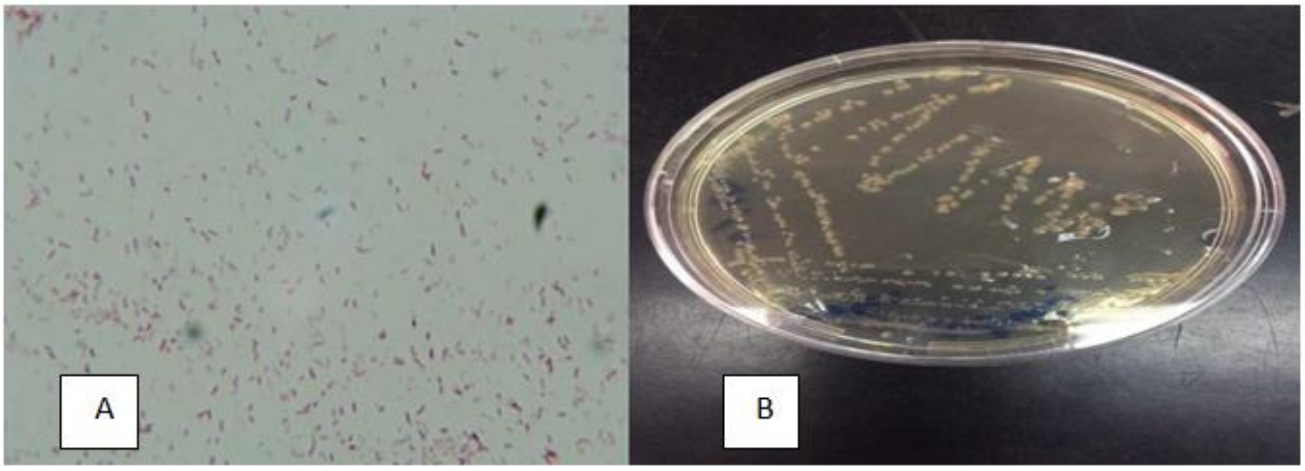


**Figure 8:** A: gram negative reaction, B: *Acinetobacter lowffii* growth on nutrient agar.

## 4. CONCLUSION

The halophilic bacteria isolated in present study can be cultured on a variety of employing media A, B, C, D, and E, but not on MacConkey. The present research mainly found that media E was more suitable to isolate the halophilic bacteria compared with other media used.

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