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Original Article

Biochemical and molecular identification of the isolated bacteria from the beach soil and saline water of Sawa Lake, Iraq

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Abstract: This work was conducted to study the biochemical characteristics of the bacterial species isolated from the beach soil, and water of Sawa Lake using molecular and morphological markers. Samples were collected from the water, and surrounding beach area. The results showed the presence of isolates, including *Proteus mirabilis*, *Providencia vermicola, Alcaligenes aquatilis, Raoultella planticola, Enterobacter cloacae, Klebsiella pneumonia,* and *P. rettgeri*. The molecular diagnosis confirmed those of Biochemical traditional markers using 16S gene primers.

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Introduction

Natural lakes have different origins; however, those artificial lakes are created by dams on rivers or other water systems (Abu Samour and al-Khatib, 1999). Sawa Lake is located southwest of Samawah city, with a surface area of about 3-5 km and a length of 10 km, a depth of 3-5.5 m surrounded by lime, and a salinity of up to 35 g/l as TDS (Jamil, 1977). It has no inlet or outlet system, but it gets water from the Euphrates through joint cracks and fissures transporting water to aquifers beneath it. Its water level fluctuates during dry and wet seasons but does not dry up.

The presence of their special microorganisms characterizes most lakes. Some bacteria grow optimally on media containing 3-15% salt and are widely distributed in salt lakes. These bacteria receive great attention because of their potential for producing of solutes and degrading enzymes (Ventosa and Oren, 1998). The salt-tolerant bacteria form a heterogeneous physiological group that includes Gram-positive or Gram-negative bacteria (Arahal and Ventosa, 2002). They also can be classified based on their need for sodium chloride.

Bacteria possess several characteristics that helped them to exist in saline media, such as enzymes that work in saturated salts, the purple membrane that allows light growth, and gas vesicles that help their buoyancy, and high osmosis in saline conditions can be harmful. In cells, the water goes out to the external medium to achieve an osmotic balance to prevent cellular water loss (Galinski, 1993). Some molecular characteristics of the different bacteria present in saline environments have an effective role in helping bacteria to overcome such a problem. Therefore, this work aimed to study the biochemical characteristics of such bacterial species isolated from the soil, and water of Sawa Lake using molecular and morphological markers.

Materials and Methods

A total of 50 samples were collected from the water and soil of Sawa Lake from randomly selected sites. The samples were diluted using CHROM agar medium. The Petri dishes were incubated at 28℃ for 48 hours (Saravanan et al., 2004). The colonies were purified and the streaking method was used for culturing nutrient agar under sterilization conditions. The Petri dishes were incubated for 24-48 hours until

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Table 1. Initiator program of 27 F, and 1492 R (Green and Sambrook, 2012).

PCR step	Repeat cycle	Temperature	Time
Initial Denaturation	1	94°C	3 min.
Denaturation		94°C	45 sec.
Annealing	35	56°C	45 sec.
Extension		72°C	60 sec.
Final extension	1	72°C	7 min.

Table 2. The results of biochemical tests for the isolated bacteria from the water and sediment of Sawa Lake.

Tests \ Isolations	*P. mirabilis*	*P. vermicola*	*A. aquatilis*	*R. planticola*	*E. cloacae*	*K. pneumoniae*	*P. rettgeri*
Gram stain	-	-	-	-	-	-	-
Motility	+	+	+	+	+	-	+
Voges proskauer	-	-	-	+	-	+	-
Starch hydrolysis	+	-	-	+	+	-	+
Nitrate reduction	-	-	-	-	+	+	+
Indol	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+
Oxidase	+	-	+	-	-	-	-
Urease test	+	+	+	+	-	+	+
Methyl Red	+	-	+	+	-	-	+
Gelatin hydrolysis	-	-	-	-	-	-	-
VitalizationCitrate	+	+	-	+	+	-	-
T.S.R	K/A/-/H2s	K/A/-/-	K/A/-/ H2s	K/A/-/-	A/A/-/-	A/A/+/-	K/A/-/ H2s

the appearance of single colonies.

Biochemical diagnosis of bacterial isolates: The isolates were diagnosed based on morphological characteristics (Baron and Finegold, 1990; Collee et al., 1996), and biochemical tests (Cruickshank et al., 1975). The tests include Gram stain, motility, Voges proskauer, starch hydrolysis, nitrate reduction, Indol, Oxidase, Catalase, Urease, methyl red, gelatin hydrolysis, citrate vitalization, and H_2S.

Molecular diagnosis of bacterial isolates: Bacterial isolates were diagnosed using PCR technique. The DNA of the seven isolates was extracted using an extraction kit for Gram-negative and Gram-positive bacteria (Cat. No. D6005, USA; Zymo Research Company) according to its protocol. DNA concentration was measured using a spectrophotometer with a wavelength of 260 nm, and calculated using the equation of DNA concentration (µg/ml) = light absorption at a wavelength of 260 nm × 50 × dilution factor. To determine the purity of DNA, the following equation was applied (William et al., 1997): DNA purity = DNA purity of absorption at a wavelength of 260 nm / Absorption at a wavelength of 280 nm.

Polymerase chain reaction to double 16s rRNA gene was carried out under aseptic conditions for isolates using the Maxime PCR PreMix (i-Taq, Cat. No. 25026) kit (iNtRoN, South Korea) using the non-specialized primers of 16S gene including. rRNA. 27F: 5'-AGA GTT TGA TCC TGG CTC AG-3' and 1492R: 5'- GGT TAC CTT GTT ACG ACT T-3' (1492) (Islam and Sar, 2011). The PCR steps and conditions are shown in Table 1. The PCR products were examined using 1% agarose gel for one hour until the blue-colored loading solution reached approximately 2 cm before the end of the gel. Then the gel containing the PCR product was examined using UV light to determine the product and match it with the measurement scale used kb.

The multiplexed bacterial isolates' DNA products (PCR amplicons) were sequenced (Macrogen Company, South Korea) in the forward and reverse directions. All sequences of nitrogenous bases were analyzed by BLAST Basic Local Alignment Search Tool) to compare with the available data in Genbank (National Center for Biotechnology Information, NCBI). The neighbor-joining phylogenetic tree was drawn using MEGA7 software (Kumar et al., 2018).

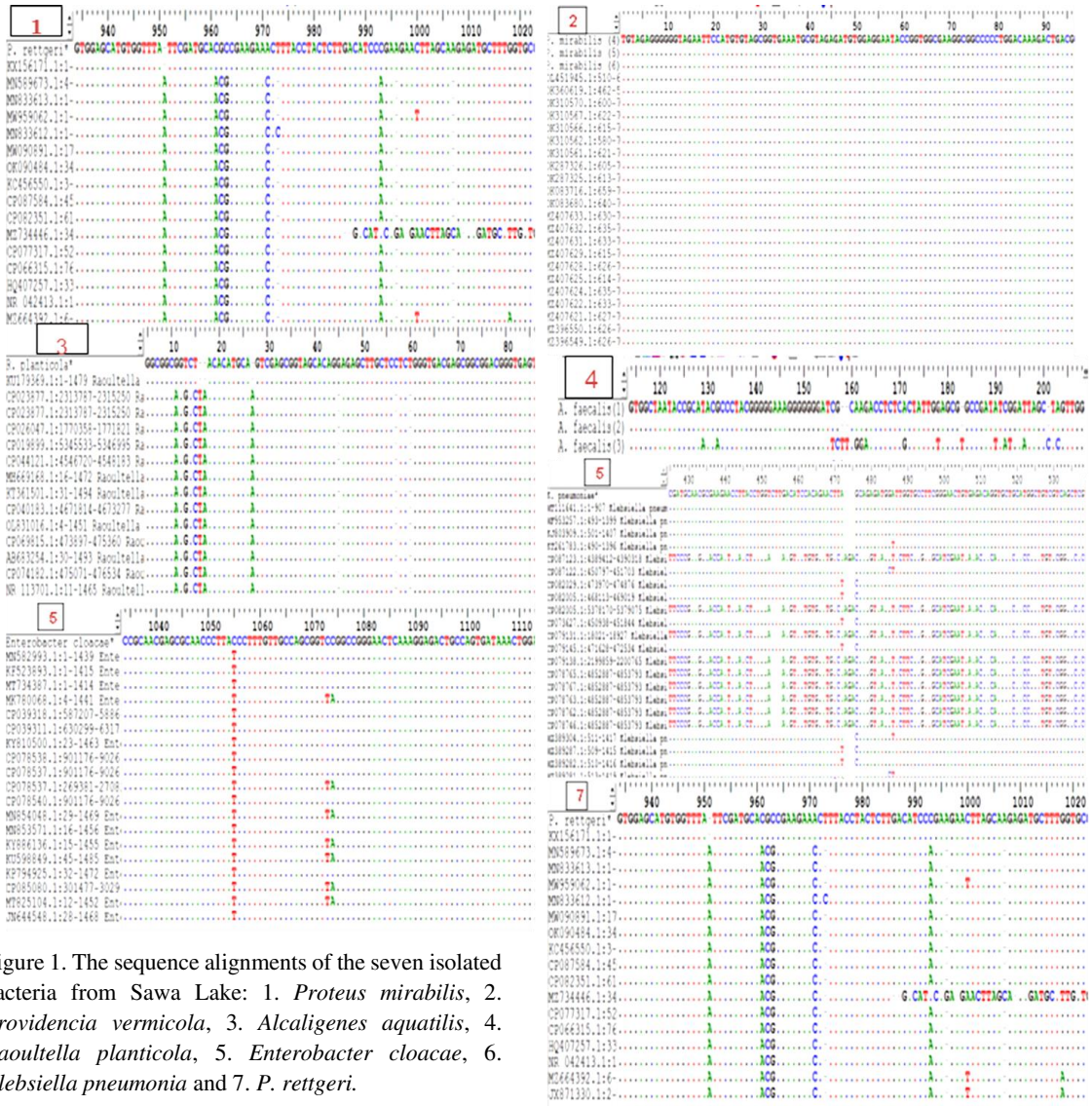

Figure 1. The sequence alignments of the seven isolated bacteria from Sawa Lake: 1. *Proteus mirabilis*, 2. *Providencia vermicola*, 3. *Alcaligenes aquatilis*, 4. *Raoultella planticola*, 5. *Enterobacter cloacae*, 6. *Klebsiella pneumonia* and 7. *P. rettgeri.*

Results

Biochemical tests: The results of biochemical tests are presented in Table 2, showing bacteria isolates from the water and sediment of Sawa Lake that describes their colony's morphology.

Proteus mirabilis: A Gram-negative bacterium, its medium-sized colonies form transparent white concentric circles on moving nutrient agar.

Providencia vermicola: A Gram-negative bacterium, its colonies form circular, opaque from the inside and transparent from the edges, mucous viscous on nutrient agar.

Alcaligenes aquatilis: Gram-negative bacteria with spherical colonies appear in smooth mucous yellow color with flat, regular, motile edges on nutrient agar.

Raoultella planticola: Gram-negative bacteria whose colonies are small in size, flat, yellow, transparent, and with irregular edges on the nutrient agar.

Enterobacter cloacae: Its colonies are large, circular, convex, translucent, smooth, mucous, and motile, as Gram-negative bacilli on the nutrient agar.

Klebsiella pneumonia: Non-motile Gram-negative *Bacillus*, as clear, round, convex, and mucous colonies on the nutrient agar.

Providencia rettgeri: It was shown as a small, opaque, convex, mucous, and Gram-negative bacilli on the nutrient agar.

Molecular diagnostics: The results of the molecular analysis showed the presence of seven isolates viz. *P. mirabilis, P. vermicola, A. aquatilis, R. planticola, E. cloacae, K. pneumonia*, and *P. rettgeri* in the sediment and soil of lake Sawa confirm the results of

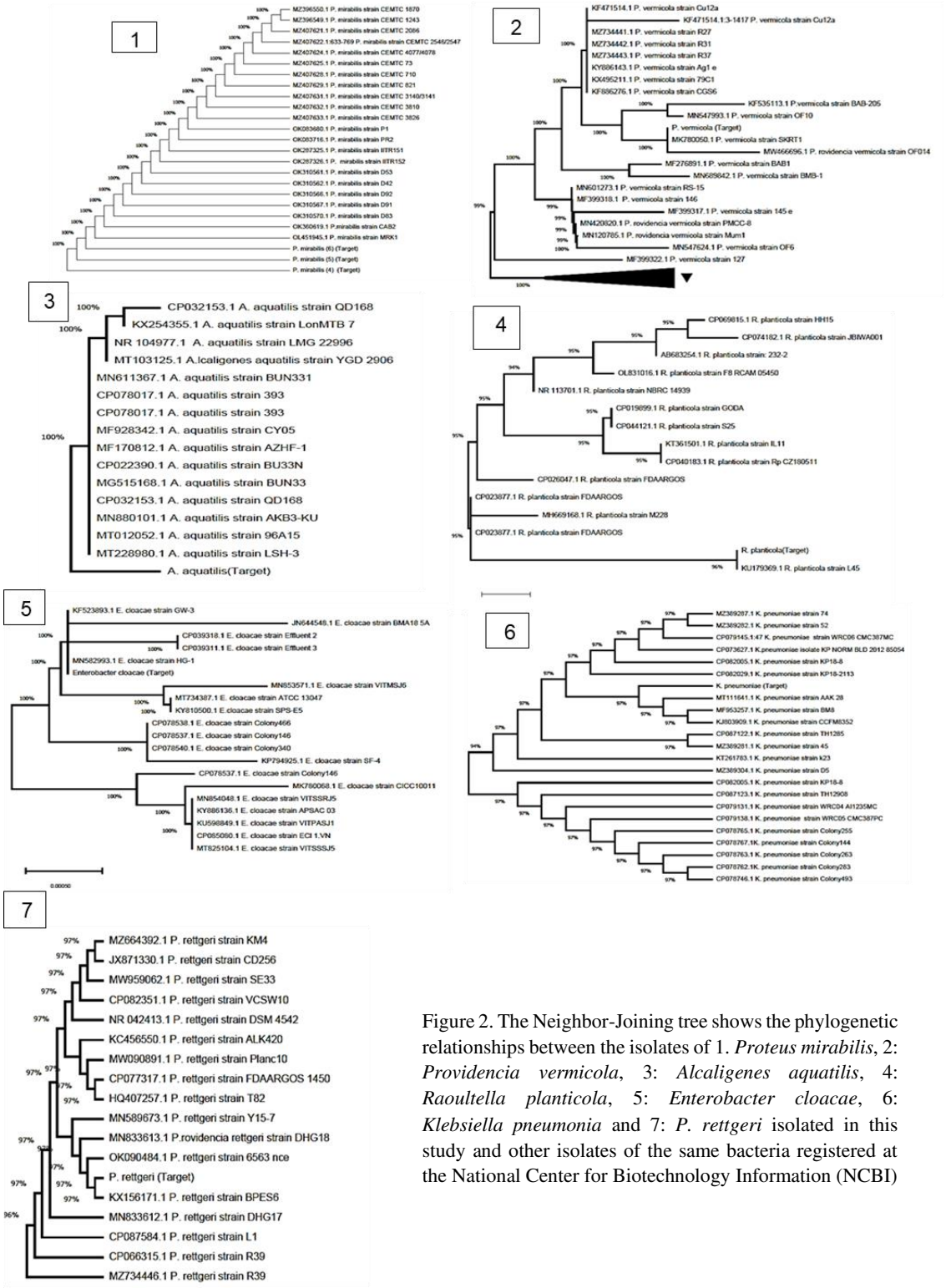

Figure 2. The Neighbor-Joining tree shows the phylogenetic relationships between the isolates of 1. *Proteus mirabilis*, 2: *Providencia vermicola*, 3: *Alcaligenes aquatilis*, 4: *Raoultella planticola*, 5: *Enterobacter cloacae*, 6: *Klebsiella pneumonia* and 7: *P. rettgeri* isolated in this study and other isolates of the same bacteria registered at the National Center for Biotechnology Information (NCBI)

the traditional biochemical methods (Figs. 1, 2).

Discussions

The gene of rRNA 16s sequences is globally used to identify bacteria isolates (Sambrook and Maniatis, 1989). Anzai et al. (2000) indicated the presence of similarities between the bacterial isolates in many regions of the world. Our results showed some differences in the bases of the isolated bacteria. The reconstructed Neighbor-Joining tree using registered genes in the National Center for Biotechnology Information NCBI (Wisnieweski, 2008; Zakaria et al., 2005) revealed their differences. However, the differences in the sequence of *Proteus mirabilis* were

significant compared to others (Fig. 1). This may be due to their evolution to adapt to environmental features of their habitats, such as contamination with heavy metals, which prompted the genetic change to tolerate pollution to ensure avoiding their toxicity, or the adapting to the high level of salinity of the water and sediments of Lake Sawa (Naik and Dubey, 2011).

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