

# Hydrocarbons Distribution in Shatt Al-Arab River Bacteria and Fungi

Wasen Abdul Ameer Ali, Wisam Abdul Ameer Farid, Abdul Muttalib Abdullah Al-Eed

**Abstract :** The hydrocarbons in bacteria and fungi of Shatt Al-Arab River were estimated. The bacteria contained n-alkanes from C<sub>13</sub> to C<sub>33</sub>. While, the n-alkanes in fungi ranged from C<sub>13</sub> to C<sub>35</sub>. The two patterns of carbon atoms numbers of n-alkanes were observed in bacteria, the low molecular weight (<20) with the predominance of C<sub>16</sub> to C<sub>19</sub> and the high molecular weight (>20) with the predominance of C<sub>21</sub>, C<sub>22</sub>, and C<sub>24</sub> to C<sub>29</sub>. In fungi, the carbon atoms numbers of n-alkanes were characterized by the other two patterns, the first in the range C<sub>13</sub> to C<sub>23</sub> with the predominance of C<sub>13</sub>, C<sub>14</sub>, C<sub>16</sub> and C<sub>19</sub> to C<sub>22</sub>, and the second in the range >23 with the predominance of C<sub>27</sub> to C<sub>30</sub>. The pristane compound was only revealed in fungi samples. The distribution patterns of carbon atoms numbers of n-alkanes and the carbon preference index (CPI) values of bacteria and fungi suggested the biogenic origin of hydrocarbons.

**Keywords:** Shatt Al-Arab River, biogenic hydrocarbons, bacterial hydrocarbons, fungal hydrocarbons, n-alkanes distribution

## I. INTRODUCTION

Hydrocarbons are chemical compounds composed mainly of the elements carbon and hydrogen. They are the principal components of fossil fuels. Crude oil and oil products are the major sources of hydrocarbons in Shatt Al-Arab River environment but they are not the only source. Other sources also exist, such as these derive from natural origin (Al-Saad, 1995). The hydrocarbons with natural origin are commonly referred to as natural or biogenic hydrocarbons. The diversity of the composition of biogenic hydrocarbons is due to the wide variety of natural biochemical processes (Klenkin *et al.*, 2010).

Many microorganisms are known to synthesize a wide range of hydrocarbons. These include cyanobacteria, bacteria, yeasts, and fungi (Ladygina *et al.*, 2006). Among hydrocarbons, the aliphatic hydrocarbons are synthesized by these organisms. Olefins occur frequently at relatively high concentrations, whereas aromatic hydrocarbons are rare. The first report on the microorganisms production of C<sub>10</sub> to C<sub>25</sub> aliphatic hydrocarbons was made by Jankowski and ZoBell (1944). Stone and ZoBell (1952) isolated hydrocarbon fractions from marine bacteria *Serratia marino rubrum* and *Vibrio ponticus*. Several biochemical mechanisms have been proposed for hydrocarbons biosynthesis, most notably the head-to-head condensation and elongation-decarboxylation pathways from fatty acid precursors (Ladygina *et al.*, 2006).

Due to the specificity of biosynthetic pathways, the number of individual hydrocarbons synthesized by organisms is very much smaller than that found in crude oil or oil products (Ladygina *et al.*, 2006).

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Based on the estimated primary production and the proportion of hydrocarbons to cell constituents, the global input of biogenic hydrocarbons may be far exceeding that of petrogenic hydrocarbons. However, not only the molecular composition of the two sources is different, but, also the kinetics input. Biogenic hydrocarbons are generated slowly and over vast areas, and their input is thus matches the degradative capacity of hydrocarbons utilizing microorganisms (GESAMP, 1993).

Hydrocarbons is difficult to differentiate in terms of their origin, since individual hydrocarbons are components of both, oils and biogenic hydrocarbons. However, there still exists difference between "biogenic" and "oil" hydrocarbons (Klenkin *et al.*, 2010).

Oil contains approximately equal amounts of n-alkanes with even and odd numbers of carbon atoms; organisms mostly contain n-alkanes with an odd number of carbon atoms. Oil contains a large amount of aromatic hydrocarbon; organisms contain no homological series of mono-, di-, tri-, and tetramethylbenzol and mono-, di-, tri-, and tetramethylnaphthalenes. Oil contains some naphtho-aromatic hydrocarbons and heterocompounds, containing S, N, O<sub>2</sub>, metals, and heavy asphaltenic substances, which do not occur in organisms. Based on these distinctions, some authors suggest criteria allowing one to establish the presence of biogenic hydrocarbons in the total sum of detected hydrocarbons. These are pristane to phytane ratio (Pr/Ph), heptadecane to pristane ratio (C<sub>17</sub>/Pr), pentadecane to hexadecane ratio (C<sub>15</sub>/C<sub>16</sub>), the ratio of peak areas of separated compounds S<sub>sep</sub> to the "hump" area S<sub>sep</sub>/S<sub>hump</sub> and the ratio of hydrocarbons with even and odd numbers of carbon atoms, carbon preference index (CPI) (NRC, 2003).

In the recent years, biogenic hydrocarbons have gained importance for the Shatt Al-Arab River because of growing biomass of phytoplankton and microorganisms, which are assumed to accumulated the majority of these hydrocarbons. This may result in imitation of river water pollution by oil and oil products. Therefore, the study of natural hydrocarbons in Shatt Al-Arab River are of interest not only in the solution of the complex problem of studying organic compounds in the river, but also in knowledge the patterns of hydrocarbons produced by the organisms to determine what role if any these hydrocarbons played in the hydrocarbons spectrum of the water. Thus, the present study was initiated to determine the levels, distribution pattern, and origin of hydrocarbons in bacteria and fungi isolated from Shatt Al-Arab River, and identify bacterial and fungal genera in the collected samples.

## II. DESCRIPTION OF SHATT AL-ARAB RIVER

The Shatt Al-Arab River originates from the confluence of the two major rivers of Iraq (Tigris and Euphrates) at Qurna. Karun River, the only tributary of the Shatt Al-Arab River, joins its eastern bank south of Basrah City. The

length of the Shatt Al-Arab River from Qurna i.e. its place of origin, to its mouth in Arabian Gulf, extends about 175 km. It's with varies at different points, ranging from 0.4 km at Basrah City to 1.5 km at its mouth. The water depth increases in general towards the Gulf with a maximum of 12.2 m. The water level is, however, affected by the high and low tides of the Arabian Gulf where the average tidal range is about 1.7 m. Shatt Al-Arab water characterized as being well mixed with limited vertical stratification of temperature and chlorinities. The water of Shatt Al-Arab mouth may be traced as far as 5 km into the Arabian Gulf. The discharge of this river reaches the waters of Kuwait Bay during the flood season.

### III. MATERIALS AND METHODS

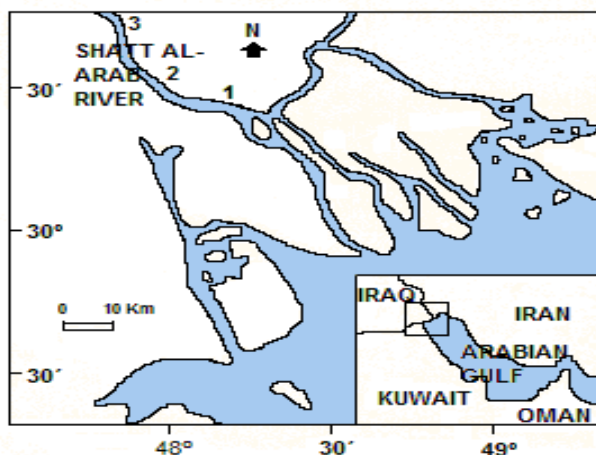
Three sampling locations were selected on Shatt Al-Arab River along the region extending from Abu-Al-Khasib to Garmat-Ali in Basrah governorate, southern Iraq (Figure 1). Replicate water samples were collected by water collector device from each station during the Summer of 2012. The samples were then transferred to laboratory in sterile bottles.

One ml of water sample was inoculated in Erlenmeyer flasks containing tryptone soya broth media (Oxoid) (TSB) adjusted to pH 7.2 for bacteria and pH 5.0 for fungi with added 300 mg l<sup>-1</sup> of cycloheximide for bacteria medium and 100 mg l<sup>-1</sup> chloramphenicol for fungi medium. The flasks were incubated at 22±2 °C in shaker incubator for 24-72 hr. to facilitate the growth of microorganisms.

Isolation of bacteria and fungi was performed by plated 0.1 ml of appropriate dilution of starter broth cultures onto nutrient agar (Difco) for bacteria and sabouraud dextrose agar (Difco) for fungi containing respective antimicrobial agents. The plates were incubated at 22±2 °C for 24-72 hr., after which each type of colony appearing on the agar was recorded and picked up. The grown colonies were purified, enumerated, and examined microscopically.

Pure cultures were further inoculated onto tube slants for bacteria and Petri dishes for fungi containing respective media and were kept stock cultures. These cultures were maintained at 4 °C and subcultured every 6–8 weeks.

Identification of fungal isolates were carried out according to general principles of fungal classification (Bessey, 1950; Hoog de and Goarro, 1995). Bacteria were characterized on the basis of their morphological characteristics and biochemical tests (Cowan and Steel, 1975; Holt *et al.*, 1994). The list of parameters used in characterization of bacteria is represented in Table 2.



**Figure 1.** Map of sampling locations on Shatt Al-Arab River.

The bacterial cells were harvested from broth cultures by centrifugation, and the filamentous fungi were harvested by filtration, washed six times with deionized water and pooled product was freeze-dried. The method used to extract, fractionate, and analyzed the hydrocarbons from the microorganisms was based upon that of Han and Calvin (1969) and Tuteja *et al.* (2011) with some modification as indicated. A 1 g amount of freeze-dried cells were placed in soxhlet apparatus and extracted with 150 ml benzene: methanol mixture (3:1) for 8 hr. The extract was storage and the cells was further extracted with fresh solvents. The combined extract was then reduced in volume to 10 ml by a rotary vacuum evaporator and was saponified for 2 hr. with a solution of 4N KOH in 1:1 benzene: methanol. Then the extract was dried by anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated by a stream of nitrogen.

The concentrated extract was separated into three fractions by column chromatography. The column contained 8g of 5 % deactivated alumina (100-200 mesh) on the top and silica (100-200 mesh) in the bottom which were extracted with methylene chloride for 36 hr., dried at 130 °C for 24 hr., and deactivated with deionized water.

The extract was applied to the head of column. The first fraction containing the aliphatic hydrocarbons (alkanes, olefins, etc.) was eluted from column with n-hexane, the second with benzene, and the third with methanol. The second and third fractions saved for further analysis and the composition of only the first fraction will be discussed. After most of the solvent was removed from the n-hexane fraction, the sample was then analyzed in a Perkin-Elmer Sigma 300 capillary gas chromatography (GC) in which the helium gas was used as a carrier gas with a linear velocity of 1.5 ml min.<sup>-1</sup>. The operating temperatures for detector and injector were 350 °C and 320 °C, respectively. The silica capillary column was operated under initial, final and rate temperatures that programmed as follows: Initial temperature was 60 °C for 4 min. while final temperature was 280 °C for 30 min and rate was 4 °C/min. Quantification of peaks and identification of hydrocarbons were done by a Perkin-Elmer computing integrator model LC-100. The Odd and Even n-alkane Predominance Index (OEPI) and the Carbon Preference Indices (CPI) were used to indicate the general source of hydrocarbons whether their origin was biogenic or anthropogenic (API, 2001; Askari and Pollard, 2005).

### IV. RESULTS AND DISCUSSION

At least sixteen and fourteen genera of bacteria and fungi respectively were isolated from water samples collected from the geographical investigated in this study. The genera were listed in Table 1. The distinguishing characteristics of bacterial genera were shown in Table 2.

The n-alkanes are among the most stable of all biogenic organic compounds and are thought to be diagnostic of biologically produced organic matter which can be derived from the decarboxylation of fatty acids (Han and Calvin, 1969).

The gas chromatography analysis of mixed cultures of bacterial hydrocarbons revealed that the present bacterial samples contained n-alkanes chain length ranged from C<sub>13</sub> to C<sub>33</sub>, with a total concentration from 35.86 µg g<sup>-1</sup>

<sup>1</sup> in location 3 to 36.50  $\mu\text{g g}^{-1}$  in location 1 (Table 3).

A great bulk of information was available concerning bacterial hydrocarbons biosynthesis (Ladygina *et al.*, 2006). The range of the present bacterial n-alkanes (C<sub>13</sub> to C<sub>33</sub>) was similar to those reported by Jones (1969) for *Arthrobacter* sp. (C<sub>15</sub> to C<sub>34</sub>), *Micrococcus* sp. (C<sub>17</sub> to C<sub>30</sub>), *Corynebacterium* sp. (C<sub>15</sub> to C<sub>33</sub>), and *Mycobacterium* sp. (C<sub>17</sub> to C<sub>31</sub>); Al-Saad (1995) for bacteria of north west of Arabian Gulf (C<sub>13</sub> to C<sub>30</sub>); Oppenheimer (1965), Bagaeva and Chernova (1994), and Bagaeva (1998) for *Desulfovibrio desulfuricans* (C<sub>11</sub> to C<sub>35</sub>); and Bagaeva and Zinurova (2004) for *Clostridium pasteurianum* (C<sub>11</sub> to C<sub>35</sub>). A short n-alkanes chains length within the range of the recent n-alkanes chain were also reported by Calvin (1969), and Han and Calvin (1969) for *Rhodospirillum rubrum*, *Rhodomicrobium vannielii* (C<sub>15</sub> to C<sub>21</sub>), *Rhodopseudomonas spheroids*, *Chlorobium* sp. (C<sub>15</sub> to C<sub>20</sub>), and *E. coli* (C<sub>13</sub> to C<sub>23</sub>); Han and Calvin (1969) for *C. tetanomorphum* (C<sub>15</sub> to C<sub>28</sub>), and *C. acidurici* (C<sub>15</sub> to C<sub>26</sub>); Jones (1969), and Tornabene *et al.* (1970) for *V. furnissii* (C<sub>15</sub> to C<sub>24</sub>); and Tornabene *et al.* (1970) for *M. lysodeikticus* (C<sub>24</sub> to C<sub>29</sub>) and *Sarcina lutea* (C<sub>23</sub> to C<sub>30</sub>).

The two patterns of carbon atoms numbers of n-alkanes were observed in the present bacterial samples, the low molecular weight (<20) with the predominance of C<sub>16</sub> to C<sub>19</sub> and the high molecular weight (>20) with the predominance of C<sub>21</sub>, C<sub>22</sub>, and C<sub>24</sub> to C<sub>29</sub> (Figure 2). The peak in range C<sub>16</sub> to C<sub>19</sub> was similar to that reported by Calvin (1969), and Han and Calvin (1969) for *E. coli* (C<sub>16</sub> to C<sub>18</sub>); and Al-Saad (1995) for bacteria of north west of Arabian Gulf (C<sub>17</sub> to C<sub>19</sub>). Han and Calvin (1969) found the predominance of C<sub>17</sub> in *C. acidurici*. A modest C<sub>21</sub> to C<sub>29</sub> n-alkanes was similar to that reported by Han and Calvin (1969) for *C. tetanomorphum* (C<sub>18</sub> to C<sub>27</sub>); Jones (1969) for *Mycobacterium* sp. (C<sub>25</sub> to C<sub>29</sub>), and *Bacillus* sp. (C<sub>27</sub> to C<sub>29</sub>); Oppenheimer (1965), Bagaeva and Chernova (1994), and Bagaeva (1998) for *D. desulfuricans* (C<sub>25</sub> to C<sub>35</sub>); Jones (1969), and Tornabene *et al.* (1970) for *V. furnissii* (C<sub>22</sub> to C<sub>24</sub>); and Bagaeva and Zinurova (2004) for *C. pasteurianum* (C<sub>25</sub> to C<sub>35</sub>). Jones (1969) noted the predominance of C<sub>21</sub> and C<sub>22</sub> in *Arthrobacter* sp., C<sub>27</sub> and C<sub>28</sub> in *Micrococcus* sp., and C<sub>27</sub> in *Corynebacterium* sp.

The hydrocarbons content of the present mixed cultures of fungi ranged from C<sub>13</sub> to C<sub>35</sub>, with a total concentration from 35.01  $\mu\text{g g}^{-1}$  in location 1 to 36.20  $\mu\text{g g}^{-1}$  in location 2 (Table 4). In fungi, the hydrocarbons accumulated in mycelia or in spores was evidenced by various investigations (Ladygina *et al.*, 2006). In this study, the fungal n-alkanes showed a slight stable pattern than bacteria. This did not, however, agree with the results of Jones (1969) who reported that fungal hydrocarbons showed a more stable pattern than bacteria except *T. viride*.

The n-alkanes content of the present fungal samples was similar to those reported by Jones (1969) for *Penicillium* sp., *Aspergillus* sp. and *T. virida* (C<sub>15</sub> to C<sub>36</sub>); Oro *et al.* (1966), and Weete *et al.* (1969) for *Ustilago maydis* and *Sphacelotheca reiliana* spores (C<sub>19</sub> to C<sub>33</sub>); and Weete *et al.* (1969) for *U. agropyri* spores (C<sub>19</sub> to C<sub>31</sub>). A n-alkanes chains length shorter than that of the recent fungal n-alkanes chain but were within the normal distribution range reported by Merdinger *et al.* (1968) for *Pullularia pullulans* (C<sub>16</sub> to C<sub>28</sub>); Laseter *et al.* (1968) and Weete *et al.* (1969) for *Tilletia foetida*, *T. caries* and *T. controversa* spores (C<sub>21</sub> to C<sub>33</sub>). However, Walker and Cooney (1973)

obtained from *C. resinae* on n-alkanes chain length (C<sub>7</sub> to C<sub>36</sub>) longer than that the recent one.

The carbon atoms numbers of n-alkanes in fungal samples were characterized by two patterns, the first in the range C<sub>13</sub> to C<sub>23</sub> with the predominance of C<sub>13</sub>, C<sub>14</sub>, C<sub>16</sub> and C<sub>19</sub> to C<sub>22</sub>, and the second in the range >23 with the predominance of C<sub>27</sub> to C<sub>30</sub> (Figure 3). The similar range of peak (C<sub>13</sub> to C<sub>23</sub>) was reported by Merdinger *et al.* (1968) for *P. pullulans* (C<sub>19</sub> to C<sub>22</sub>). Walker and Cooney (1973) observed the predominance of C<sub>13</sub>, C<sub>14</sub> and C<sub>16</sub> n-alkanes in *C. resinae*. The peak in the range from C<sub>27</sub> to C<sub>30</sub> was similar to those reported by Jones (1969) for *Penicillium* sp., *Aspergillus* sp. and *T. virida* (C<sub>27</sub> to C<sub>30</sub>); and Oro *et al.* (1966), and Weete *et al.* (1969) for *U. maydis* spores (C<sub>27</sub> to C<sub>29</sub>). Oro *et al.* (1966), and Weete *et al.* (1969); Weete *et al.* (1969); and Laseter *et al.* (1968), and Weete *et al.* (1969) demonstrated the predominance of C<sub>29</sub> n-alkane in *S. reiliana* spores; *U. agropyri* spores; and *T. foetida*, *T. caries* and *T. controversa* spores respectively.

The isoprenoid compound, pristane was only revealed in fungal samples with concentration ranged from 1.20  $\mu\text{g g}^{-1}$  in location 1 to 1.41  $\mu\text{g g}^{-1}$  in location 3. (Table 4). This implies the fungi had the enzymatic system which produces the isoprenoid hydrocarbons. This indicated that the fungal activity may play an important role in isoprenoid hydrocarbon diagenesis. Walker and Cooney (1973) revealed unusual composition of the hydrocarbon fraction in *C. resinae* grown on the glucose- or glutamate-containing media. It includes pristane, which is typical of nonphotosynthetic and photosynthetic bacteria.

The bacterial and fungal n-alkanes showed the predominance of odd carbon numbered alkanes than even carbon numbered alkanes. These abundance in fungal samples was slightly than in bacterial samples (Tables 3 and 4). The same conclusion arrived by Jones (1969).

The CPI values ranged from 1.10 in location 2 to 1.13 in location 3 for bacterial samples and from 1.03 in location 1 and 3 to 1.05 in location 2 for fungal samples (Tables 3 and 4). Klenkin *et al.* (2010) reported that the CPI was the most effective criteria to establish the presence of biogenic hydrocarbons in the total sum of detected hydrocarbons since that the other criteria (i.e. C<sub>15</sub>/C<sub>16</sub>, C<sub>17</sub>/Pr and Pr/Ph) sometimes given ambiguous responses because of the high volatility and relatively rapid degradation of those hydrocarbons. The CPI values of the present bacterial and fungal samples tended to unity. This result was agreed with Jones (1969) who obtained on CPI values of around unity from chromatographic analysis of soil bacterial and fungal hydrocarbons. The high CPI values reflect the biogenic nature of n-alkanes in the recent bacterial and fungal samples. Ehrhard and Petrick (1993) reported that, if CPI was greater than one, the sources of hydrocarbons were biogenic, and if it was smaller than one, the sources were anthropogenic.

Obviously that the distributions patterns of n-alkanes of bacteria and fungi are very different from that reported for n-alkanes synthesized by other organisms (i.e. algae and higher plants) which demonstrated previously by Eglinton and Hamilton (1963), Han and Calvin (1969), Jones (1969), and Ladygina *et al.*, (2006). This differences between the n-alkanes patterns of the organisms is striking, and there appears to be a future for taxonomic correlation based on

this approach (Han and Calvin, 1969).

In conclusion, a large numbers of bacteria and fungi species are characterized by their ability of biosynthesis of specific composition of hydrocarbons which may be served as a chemotaxonomic criterion. The synthesis of hydrocarbons by bacteria and fungi varied in dependence on the growth conditions that provides a way for their physiological regulation (Jones, 1969). The hydrocarbons biosynthesis bacteria and fungi may play the role of carbon and energy supply in the future because they possess significant potential advantages over green microalgae, such as the higher growth rate, the capability for the growth in large scale fermentors, great metabolic flexibility and high accessibility to genetic engineering.

**Table 1. Bacterial and fungal genera isolated from water of Shatt Al-Arab river**

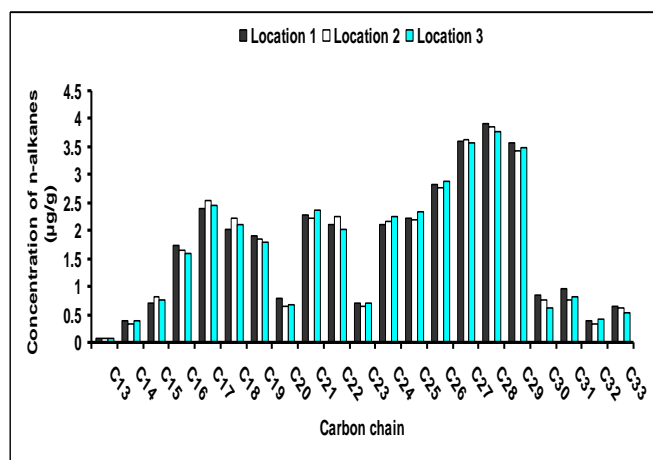
| Location | Bacteria  | Fungi   |
|----------|---|---|
| 1        | <i>Pseudomonas</i> sp., <i>Aeromonas</i> sp., <i>Micococcus</i> sp., <i>Bacillus</i> sp., <i>Eschrichia</i> sp., <i>Corynebacterium</i> sp., <i>Klebsiella</i> sp., <i>Flavobacterium</i> sp., <i>Alcaligenes</i> sp., <i>Arthrobacter</i> sp., <i>Streptococcus</i> sp., and <i>Enterobacter</i> sp. | <i>Penicillium</i> sp., <i>Trichoderma</i> sp., <i>Chaetomium</i> sp., <i>Mortierella</i> sp., <i>Aspergillus</i> sp., <i>Humicola</i> sp., <i>Cheysosporium</i> sp., <i>Eurotium</i> sp., and <i>Gymnoasc</i> sp.                          |
| 2        | <i>Pseudomonas</i> sp., <i>Staphylococcs</i> sp., <i>Micococcus</i> sp., <i>Bacillus</i> sp., <i>Eschrichia</i> sp., <i>Chromobacterium</i> sp., <i>Klebsiella</i> sp., <i>Flavobacterium</i> sp., and <i>Proteus</i> sp.   | <i>Penicillium</i> sp., <i>Chaetomium</i> sp., <i>Gillmanila</i> sp., <i>Cladosporium</i> sp., <i>Mortierella</i> sp., <i>Aspergillus</i> sp., <i>Fusarium</i> sp., <i>Trichurus</i> sp., <i>Cheysosporium</i> sp., and <i>Eurotium</i> sp. |
| 3        | <i>Pseudomonas</i> sp., <i>Aeromonas</i> sp., <i>Micococcus</i> sp., <i>Bacillus</i> sp., <i>Eschrichia</i> sp., <i>Proteus</i> sp., <i>Arthrobacter</i> sp., <i>Streptococcus</i> sp., and <i>Enterobacter</i> sp.   | <i>Penicillium</i> sp., <i>Acrmonium</i> sp., <i>Gillmanila</i> sp., <i>Cladosporium</i> sp., <i>Rhizopus</i> sp., <i>Aspergillus</i> sp., <i>Fusarium</i> sp., and <i>Humicola</i> sp.   |

**Table 2. Distinguishing characteristics of bacterial isolates.**

| Character                   | <i>Pseudomonas</i> sp. | <i>Micrococcus</i> sp. | <i>Staphylococcus</i> sp. | <i>Arthrobacter</i> sp. | <i>Corynebacterium</i> sp. | <i>Bacillus</i> sp. | <i>Flavobacterium</i> sp. | <i>Enterobacter</i> sp. | <i>Aeromonas</i> sp. | <i>Chromobacterium</i> sp. | <i>Klebsiella</i> sp. | <i>Escherichia</i> sp. | <i>Streptococcus</i> sp. | <i>Proteus</i> sp. | <i>Alcaligenes</i> sp. | <i>Rhodococcus</i> sp. |
|-----------------------------|------------------------|------------------------|---------------------------|-------------------------|----------------------------|---------------------|---------------------------|-------------------------|----------------------|----------------------------|-----------------------|------------------------|--------------------------|--------------------|------------------------|------------------------|
| Cell shape                  | Rods                   | Spherical              | Spherical                 | Irregular rods          | Diplobacillus              | Rods                | Rods                      | Rods                    | Rods                 | Rods                       | Rods                  | Rods                   | Spherical                | Rods               | coccal rods            | Spherical              |
| Motility                    | +                      | -                      | -                         | +or-                    | -                          | +                   | +or-                      | +                       | +or-                 | +                          | -                     | +                      | +or-                     | +                  | +                      | +                      |
| Gram stain                  | -                      | +                      | +                         | +                       | +                          | +                   | -                         | -                       | -                    | -                          | -                     | -                      | +                        | -                  | -                      | +                      |
| Albert's stain              | -                      | -                      | -                         | -                       | -                          | +                   | -                         | -                       | -                    | -                          | -                     | -                      | -                        | -                  | -                      | -                      |
| Spores formation            | -                      | -                      | -                         | -                       | -                          | +                   | -                         | -                       | -                    | -                          | -                     | -                      | -                        | -                  | -                      | -                      |
| Pigmentation                | +                      | -                      | -                         | -                       | -                          | +                   | +                         | +                       | +                    | +                          | +                     | +                      | +                        | +                  | +                      | +                      |
| Oxidase test                | +                      | +                      | +                         | +                       | +                          | +                   | +                         | +                       | +                    | +                          | +                     | +                      | +                        | +                  | +                      | +                      |
| Catalase test               | +                      | +                      | +                         | +                       | +                          | +                   | +                         | +                       | +                    | +                          | +                     | +                      | +                        | +                  | +                      | +                      |
| Arginine dihydrolase        | -                      | -                      | -                         | -                       | -                          | -                   | -                         | -                       | +                    | +                          | +                     | +                      | +                        | +                  | +                      | +                      |
| Glucose fermentation        | -                      | -                      | +                         | -                       | +                          | +                   | +                         | +                       | +                    | +                          | +                     | +                      | +                        | +                  | +                      | +                      |
| Lactose fermentation        | -                      | -                      | -                         | -                       | +                          | +                   | +                         | +                       | +                    | +                          | +                     | +                      | +                        | +                  | +                      | +                      |
| Maltose fermentation        | -                      | -                      | -                         | -                       | +                          | +                   | +                         | +                       | +                    | +                          | +                     | +                      | +                        | +                  | +                      | +                      |
| Hydrolysis of starch        | -                      | -                      | -                         | -                       | +                          | +                   | +                         | +                       | +                    | +                          | +                     | +                      | +                        | +                  | +                      | +                      |
| Gelatin hydrolysis          | -                      | -                      | -                         | -                       | +                          | +                   | +                         | +                       | +                    | +                          | +                     | +                      | +                        | +                  | +                      | +                      |
| Urea hydrolyzed             | -                      | -                      | -                         | -                       | +                          | +                   | +                         | +                       | +                    | +                          | +                     | +                      | +                        | +                  | +                      | +                      |
| Hydrogen peroxide formation | -                      | -                      | -                         | -                       | +                          | +                   | +                         | +                       | +                    | +                          | +                     | +                      | +                        | +                  | +                      | +                      |
| Gas production              | -                      | -                      | -                         | -                       | +                          | +                   | +                         | +                       | +                    | +                          | +                     | +                      | +                        | +                  | +                      | +                      |
| Nitrate reduction           | -                      | -                      | -                         | -                       | +                          | +                   | +                         | +                       | +                    | +                          | +                     | +                      | +                        | +                  | +                      | +                      |
| Indol                       | -                      | -                      | -                         | -                       | +                          | +                   | +                         | +                       | +                    | +                          | +                     | +                      | +                        | +                  | +                      | +                      |

**Table 3. Concentrations of n-alkanes (µg/g dry weight ±SD), Odd and even n-alkanes values, and CPI values in bacteria of Shatt Al-Arab river.**

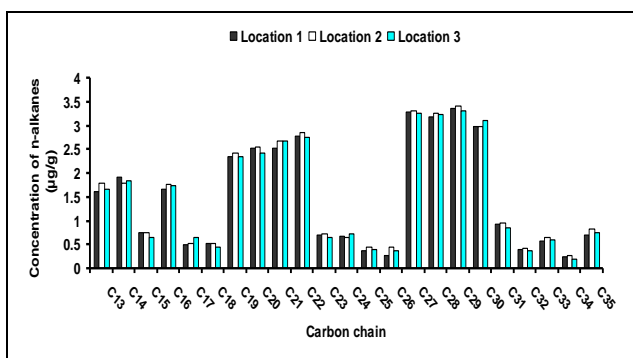
| n-alkane and hydrocarbon index | Location 1 | Location 2 | Location 3 |
|--------------------------------|------------|------------|------------|
| C <sub>13</sub>                | 0.10(±0.6) | 0.08(±0.8) | 0.09(±0.2) |
| C <sub>14</sub>                | 0.39(±0.3) | 0.34(±0.1) | 0.40(±0.6) |
| C <sub>15</sub>                | 0.72(±0.2) | 0.84(±0.8) | 0.76(±0.4) |
| C <sub>16</sub>                | 1.76(±0.8) | 1.67(±0.3) | 1.60(±0.6) |
| C <sub>17</sub>                | 2.41(±0.2) | 2.56(±0.7) | 2.46(±0.3) |
| C <sub>18</sub>                | 2.03(±0.1) | 2.23(±0.1) | 2.13(±0.4) |
| C <sub>19</sub>                | 1.93(±0.4) | 1.87(±0.6) | 1.82(±0.9) |
| C <sub>20</sub>                | 0.79(±0.6) | 0.66(±0.8) | 0.70(±0.1) |
| C <sub>21</sub>                | 2.28(±0.7) | 2.23(±0.3) | 2.38(±0.4) |
| C <sub>22</sub>                | 2.13(±0.3) | 2.27(±0.1) | 2.03(±0.7) |
| C <sub>23</sub>                | 0.73(±0.9) | 0.66(±0.5) | 0.71(±0.7) |
| C <sub>24</sub>                | 2.13(±0.5) | 2.17(±0.2) | 2.26(±0.3) |
| C <sub>25</sub>                | 2.25(±0.6) | 2.20(±0.7) | 2.34(±0.3) |
| C <sub>26</sub>                | 2.83(±0.7) | 2.77(±0.3) | 2.90(±0.8) |
| C <sub>27</sub>                | 3.61(±0.3) | 3.64(±0.9) | 3.58(±0.5) |
| C <sub>28</sub>                | 3.94(±0.2) | 3.88(±0.4) | 3.77(±0.4) |
| C <sub>29</sub>                | 3.58(±0.1) | 3.44(±0.2) | 3.51(±0.5) |
| C <sub>30</sub>                | 0.86(±0.4) | 0.76(±0.8) | 0.62(±0.1) |
| C <sub>31</sub>                | 0.97(±0.3) | 0.76(±0.7) | 0.84(±0.3) |
| C <sub>32</sub>                | 0.39(±0.6) | 0.33(±0.2) | 0.42(±0.4) |
| C <sub>33</sub>                | 0.67(±0.6) | 0.64(±0.1) | 0.54(±0.3) |
| C <sub>34</sub>                | ---        | ---        | ---        |
| C <sub>35</sub>                | ---        | ---        | ---        |
| Total                          | 36.50      | 36.00      | 35.86      |
| Odd                            | 19.25      | 18.92      | 19.03      |
| Even                           | 17.25      | 17.08      | 16.83      |
| CPI (Odd/Even)                 | 1.11       | 1.10       | 1.13       |



**Figure 2. Chromatographic distribution of carbon chain lengths of the n-alkanes concentrations (µg/g) of Shatt Al-Arab river bacteria.**

**Table 4. Concentrations of n-alkanes (µg/g dry weight ±SD), odd and even n-alkanes values, CPI values, and pristane in fungi of Shatt Al–Arab river.**

| n-alkane and hydrocarbon index | Location 1 | Location 2 | Location 3 |
|--------------------------------|------------|------------|------------|
| C <sub>13</sub>                | 1.63(±0.2) | 1.79(±0.1) | 1.66(±0.8) |
| C <sub>14</sub>                | 1.92(±0.6) | 1.81(±0.4) | 1.85(±0.6) |
| C <sub>15</sub>                | 0.77(±0.4) | 0.75(±0.1) | 0.67(±0.2) |
| C <sub>16</sub>                | 1.66(±0.6) | 1.78(±0.7) | 1.74(±0.4) |
| C <sub>17</sub>                | 0.51(±0.3) | 0.53(±0.3) | 0.66(±0.3) |
| C <sub>18</sub>                | 0.52(±0.4) | 0.53(±0.1) | 0.45(±0.8) |
| C <sub>19</sub>                | 2.36(±0.9) | 2.43(±0.4) | 2.36(±0.9) |
| C <sub>20</sub>                | 2.52(±0.1) | 2.55(±0.1) | 2.44(±0.6) |
| C <sub>21</sub>                | 2.53(±0.4) | 2.68(±0.5) | 2.69(±0.5) |
| C <sub>22</sub>                | 2.79(±0.7) | 2.87(±0.3) | 2.76(±0.2) |
| C <sub>23</sub>                | 0.71(±0.7) | 0.73(±0.6) | 0.66(±0.2) |
| C <sub>24</sub>                | 0.69(±0.3) | 0.67(±0.2) | 0.73(±0.7) |
| C <sub>25</sub>                | 0.38(±0.3) | 0.45(±0.6) | 0.41(±0.1) |
| C <sub>26</sub>                | 0.29(±0.8) | 0.45(±0.4) | 0.37(±0.5) |
| C <sub>27</sub>                | 3.30(±0.5) | 3.32(±0.5) | 3.26(±0.2) |
| C <sub>28</sub>                | 3.19(±0.4) | 3.26(±0.6) | 3.23(±0.3) |
| C <sub>29</sub>                | 3.37(±0.5) | 3.42(±0.9) | 3.32(±0.2) |
| C <sub>30</sub>                | 2.99(±0.1) | 3.00(±0.7) | 3.11(±0.1) |
| C <sub>31</sub>                | 0.93(±0.3) | 0.97(±0.3) | 0.87(±0.2) |
| C <sub>32</sub>                | 0.40(±0.4) | 0.44(±0.3) | 0.39(±0.5) |
| C <sub>33</sub>                | 0.59(±0.3) | 0.65(±0.3) | 0.60(±0.1) |
| C <sub>34</sub>                | 0.26(±0.2) | 0.28(±0.7) | 0.21(±0.2) |
| C <sub>35</sub>                | 0.70(±0.3) | 0.84(±0.8) | 0.75(±0.6) |
| Total                          | 35.01      | 36.20      | 35.19      |
| Odd                            | 17.78      | 18.56      | 17.91      |
| Even                           | 17.23      | 17.64      | 17.28      |
| CPI (Odd/Even)                 | 1.03       | 1.05       | 1.03       |
| Pristane                       | 1.20(±0.6) | 1.35(±0.2) | 1.41(±0.4) |



**Figure 3. Chromatographic distributions of carbon chain lengths of the n-alkanes concentrations (µg/g) of Shatt Al–Arab river fungi.**

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