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# BIODIESEL PRODUCTION USING BACTERIAL FATTY ACIDS EXTRACTS AS A FEEDSTOCK

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## Abstract

Biodiesel is another alternative energy source and could be a substitute for petroleum diesel fuel. Biodiesel production and consumption have been globally increased as a substitute for mineral diesel. In the present study, fifty bact and species were isolated from different sources. Screening of fatty acids producing bacteria using 2, 3, 5 Triphenyl Tetrazolium Chloride (TTC) and colorimetric methods was performed. About 21 bacterial strains were positive to the TTC test and their total lipids were estimated. The most potential bacterial species isolate number 39 was characterized and identified as *Streptomyces tunisiensis*. Lipids were extracted and injected into gas chromatography (GC) to show the level of unsaturated fatty acids which is considered a promising way for biodiesel production. The unsaturated palmitoleic acid methyl ester was the most abundant fatty acid followed by Palmitic acid methyl ester. In conclusion, the present study represent that *Streptomyces* sp. could be an attractive alternative renewable feedstock for biodiesel.

***Key words:*** Biodiesel, TTC, lipid production, Gas chromatography, *Streptomyces tunisiensis*.

## Introduction

The continuous growth of th,human population and industrialization increased energy demands all over the world. The current consumption of petroleum is at least 105 times faster than nature can create (Abomohra *et al*., 2017). If the development of the world energy consumption continues, the world will be threatened with an energy crisis, as the worldwide fossil oil reserves will be exhausted in shorter than 30 years.

In addition to the fact of carbon dioxide ($CO_2$) emission from the transport, the sector is contributing a major part to environmental pollution and global warming. Also, the cost of crude oil will continue to rise due to diminishing supply, so the production of fuels from alternate sources will be needed in the future decades (Ihsanullah *et al*., 2015).

Biodiesel is considered as the main alternative fuel for fossil fuel. The main advantages of biodiesel, other than being a renewable energy source, is that its burning is cleaner than that of fossil fuel, sustainable, environmentally friendlier and it can be used in the present diesel engines without changes (El-Sheekh and Abomohra, 2016).

Biodiesel is produced from vegetable oils, yellow grease, used cooking oils, animal fats, or algae. The production process, called esterification, converts fatty acids into chemicals called long-chain mono alkyl esters by reaction with alcohol in the presence of a catalyst. When the alkyl chain alcohol is methanol (most common), these are called fatty acid methyl esters (FAME). When FAME is used as fuel, it is commonly called biodiesel. For example, 100 ml of oil or fat are reacted with 10 ml of alcohol (as methanol) in the presence of a catalyst (as sodium hydroxide or sulfuric acid) to form 100 ml of biodiesel and 10 ml of glycerin. Glycerin, a co-product, is a sugar commonly used in the manufacture of pharmaceuticals and cosmetics (Janben and Steinbüchel, 2014).

Biodiesel production through microbial systems is, therefore, receiving increasing attention as a cost-effective, sustainable alternative biofuel. Recent research

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confirmed that microalgae are promising biodiesel feedstocks (El-Sheekh *et al*., 2013; Abomohra *et al*., 2014, Abomohra *et al*., 2016; El-Sheekh and Abomohra, 2016). Some microbes have been engineered to convert simple sugars into several types of biofuels (Lee *et al*., 2008; Alper and Stephanopoulos, 2009; Zhang *et al*., 2011). The first microbial biodiesel fatty acid ethyl esters (FAEE)-producing recombinant *E. coli* was created in 2006 and the first example of direct FAEE production by *E. coli* from xylan was reported in 2010 (Kosa and Ragauskas, 2011). More cellulases would be further engineered into the strains to produce FAEE directly from both cellulose and hemicel.lose for further reducing the cost of cellulosic biodiesel (Steen *et al*., 2010). Also, the biodiesel production utilizing a bacterial fatty acid methyltransferase (FAMT) and describe the identification of a bacterial FAMT and the engineering of *E. coli* to produce FAMEs and 3-hydroxy fatty acid methyl esters (3-OH-FAMEs) by expressing FAMT and novel bacterial FATs that exhibit distinct specificities was reported (Nawabi *et al*., 2011).

Some species of bacteria produce fatty acids and triacylglycerols (TAGs) in high levels that can be used as precursors of biodiesel production. However, few genera of the class actinomycetes can accumulate TAGs to high levels, as in the case of *Acinetobacter* (Nelson *et al*., 1996), *Mycobacterium* (Kaieda *et al*., 1999) and *Streptomyces* (Claude, 1999). So, it is necessary to look for becterial species that are lipid-rich as promising candidates for biodiesel production (El-Sheekh *et al*., 2017).

*Streptomyces* sp. S161 could produce fatty acid methyl esters (FAME) directly from starch (Lu *et al*., 2012). The ability of some novel bacterial isolate to produce fatty acids and lipids for their potential use as biodiesel as a cost-effective, sustainable and renewable biofuel feedstock was reported (El-Sheekh *et al*., 2017). The characterization of *S. lienomycini* and its potential application in biodiesel production was documented (Sen *et al.,* 2016). *Streptomyces* sp. CS326 could be used in biodiesel production according to Cho *et al*., (2012) reports. Scientists' reports (Sarac *et al*., 2016) show the activity of *Streptomyces* sp. AU-1 in biodiesel production. The efficiency of *Microbacterium* sp. as a good source for biodiesel production also recorded (Tripathi *et al*., 2014). The actinobacteria are well known for their capacity to produce a wide variety of metabolites, including the polyketides. The biosynthesis of some of these compounds uses the same set of precursor molecules as fatty acid biosynthesis (Arabolaza *et al*., 2010). The actinobacteria contain diverse polyketide synthases genes in their genomes and elevated lipid acc.ulation in some actinobacteria is well studied (Kosa and Ragauskas, 2011).

Thus, this study aims to isolate and study the fatty acids content of several bacterial species as a potential source for biodiesel production.

## Materials and Methods

### Isolation of bacterial isolates

Bacterial species were isolated from different soils and marine waters collected from Egypt, which were inoculated separately on both nutrient agar and starch nitrate medium plates. After purification, the stock cultures of these isolates were preserved on slants of nutrient and starch nitrate agar and stored at 4ºC. Monthly or bimonthly subcultures were made on new slants (El-Sheekh *et al.,* 2017).

### Screening of FAs Production

Colorimetric screening for fatty acid production is detected as follows: 0.1% w/v of the dye 2, 3, 5 Triphenyl Tetrazolium chloride (TTC) was added to 10 ml of the bacterial isolates broth. Then, the tubes were incubated at 25°C for one hour. The formation of red color was considered to be a positive result (Ryan *et al*., 2010).

### Preparation of bacterial culture

The cultures were prepared by dispensing 48 ml of both nutrient broth and starch nitrate broth in 250 ml flasks and sterilized. Each flask was inoculated by 2 ml of 24 hours old culture, from all bacterial isolates. The flasks were then incubated at 30°C for actinobacteria and 37°C for true bacteria in a shaker incubator (El-Sheekh *et al.,* 2017).

### Lipid extraction

Bacterial cells were harvested by centrifugation at 4000 g for 15 minutes. The pellet was rinsed three times and resuspended in sterilized distilled water to remove traces of growth medium (Rogers *et al*., 1994). The total lipids for bacteria were extracted following the modified methods of Folch *et al*., (1957). The cells were homogenized with chloroform/methanol (2:1) to a final volume 20 times of the sample volume. After dispersion, the mixture was agitated for 15-20 minutes in shaker at room temperature. The homogenate was either filtrated or centrifuged to recover the liquid phase. The solvent was washed with 0.2 volume of 0.9% NaCl solution. After overtaxing, the mixture was centrifuged at low speed (2000 rpm) to separate the two phases. The upper phase was removed by siphoning, the lower phase containing lipids was evaporated under vacuum in a rotary evaporator. The most producing organism is identified

according to the amount of lipid g/100 g dry wt. of bacterial culture.

### Characterization and Identification of most potent isolate

Identification of the potential isolate was performed using phenotypic method and identification was confirmed by molecular characterization using 16S RNA (Lebeda *et al*., 2012). Morphological (macroscopic and microscopic), biochemical and physiological characterization of potential species was tested following the standard a protocol of the International *Streptomyces* Project (ISP) (Shirling and Gottlieb, 1966) and identified with the help of keys of Bergy's Manual of Systematic Bacteriology (Williams *et al*., 1989).

### Preparation of fatty acid methyl esters (FAMEs) for analysis using gas chromatography (GC)

About 50 mg f lipids were transesterified by the addition of 4 ml benzene and 20 ml 1% sulfuric acid (as a catalyst) in absolute methanol. The mixture was left refluxed at 90°C for 90 minutes, then 20 ml distilled water was added and the resulted esters were extracted with 10 ml benzene upon separation of the layers. The benzene layer was dried using anhydrous sodium sulfate. The solvent was evaporated using a rotary evaporator. The composition of FAMEs was quantified and identified using GC-MS (Radwan, 1978).

**Table 1:** Screening of bacterial strains for producing FAs using TTC techniques and total Lipid content of the isolated bacterial species.

| Isolate no. | TTC | Total lipids (mg/g DW) | Isolate no. | TTC | Total lipids (mg/g DW) |
|---|---|---|---|---|---|
| 1 | +ve | 2.5 ± 0.4 | 26 | +ve | 5.1 ± 0.4 |
| 2 | +ve | 6.1 ± 0.4 | 27 | -ve | - |
| 3 | -ve | - | 28 | -ve | - |
| 4 | +ve | 4 ± 0.3 | 29 | +ve | 4 ± 0.5 |
| 5 | -ve | - | 30 | -ve | - |
| 6 | +ve | 4.5 ± 0.5 | 31 | +ve | 6 ± 0.6 |
| 7 | +ve | 6 ± 0.6 | 32 | -ve | - |
| 8 | +ve | 6.1 ± 0.3 | 33 | +ve | 4.1 ± 0.3 |
| 9 | -ve | - | 34 | +ve | 6.5 ± 0.4 |
| 10 | -ve | - | 35 | -ve | - |
| 11 | +ve | 6 ± 0.6 | 36 | +ve | 7 ± 0.4 |
| 12 | -ve | - | 37 | -ve | - |
| 13 | -ve | - | 38 | -ve | - |
| 14 | +ve | 3 ± 0.3 | 39 | +ve | 14 ± 0.6 |
| 15 | +ve | 7.5 ± 0.5 | 40 | -ve | - |
| 16 | -ve | - | 41 | +ve | 2.3 ± 0.3 |
| 17 | +ve | 5.5 ± 0.5 | 42 | -ve | - |
| 18 | -ve | - | 43 | -ve | - |
| 19 | -ve | - | 44 | -ve | - |
| 20 | -ve | - | 45 | -ve | - |
| 21 | -ve | - | 46 | +ve | 5 ± 0.4 |
| 22 | -ve | - | 47 | +ve | 4 ± 0.7 |
| 23 | -ve | - | 48 | -ve | - |
| 24 | +ve | 5 ± 0.8 | 49 | -ve | - |
| 25 | -ve | - | 50 | -ve | - |

+ ve describes the presence of FAs and -ve denotes non-producer of FAs. Values are the mean of three replicates ± Standard Deviation.

**Table 2:** Biochemical characteristics of the selected isolate.

| No. | Test | Results |
|---|---|---|
| 1 | Tyrosine degradation | -ve |
| 2 | Urea degradation | -ve |
| 3 | Pectin degradation | -ve |
| 4 | Esculin degradation | +ve |
| 5 | Lecithin degradation | -ve |
| 6 | Lipid hydrolysis | -ve |
| 7 | Starch hydrolysis | +ve |
| 8 | Gelatin hydrolysis | -ve |
| 9 | Casein hydrolysis | +ve |
| 10 | Catalase | -ve |
| 11 | Oxidase | +ve |
| 12 | Motility test | -ve |
| 13 | $H_2S$ production | -ve |
| 14 | Citrate utilization | +ve |

### GC analysis of fatty acid methyl esters

The total FA composition of lipids was analyzed as methyl esters with a gas chromatograph mass-spectrometer (7890A GC system, USA). Chromatographic conditions were: carrier gas, helium, flow rate, 1.5 ml/min, sample input temperature, 290°C, initial temperature, 90°C for 1 minute, programmed to 300°C, capillary column, HP-5MS, length 30m, diameter 0.25 mm. FAs were identified by mass spectra and were compared for retention times with those of standards ("Sigma", USA) (Rattanapoltee and Kaewkannetra, 2014).

## Results

### Isolation and cultivation of microorganisms

A total of 50 samples of (*Streptomyces* and bacteria)

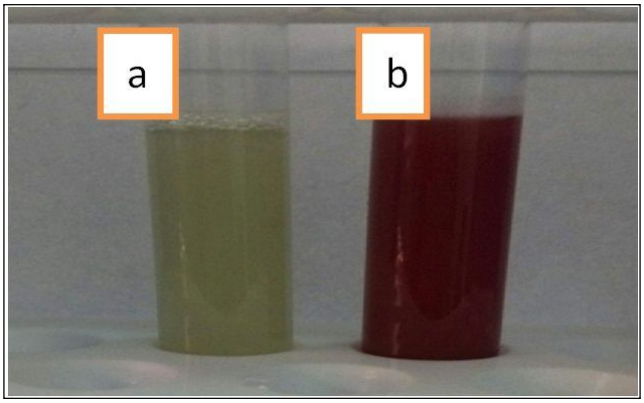


**Fig. 1:** TTC test: (a) negative test, (b) positive test

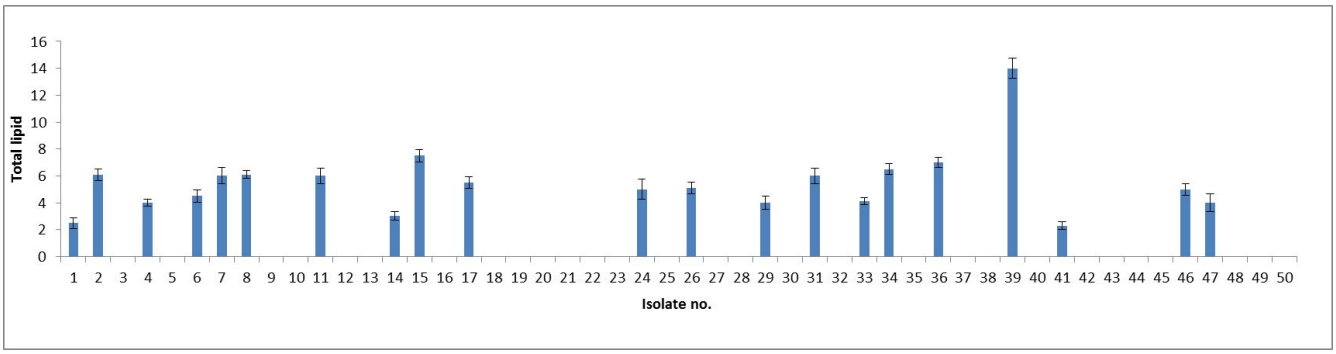


**Fig. 2:** Total Lipids content of the various isolated bacterial species.

were isolated from different sources. The isolates were inoculated separately on both nutrient agar and starch nitrate medium. After purification and identificat and the stock cultures of these isolates were preserved and stored at 4°C for further tests.

**Screening using colourimetric assay**

The reaction of 2, 3, 5-Triphenyl Tetrazolium chloride (TTC) from a colorless to red triphenyl formazan was a convenient indication of the formatio,of fatty acids. TTC techniques indicated that about (21) isolate could produce fatty acids as represented in table 1 and fig. 1.

**Determination of total lipid for selected isolates**

Selected isolates were tested for their lipids production ability and quantitative estimation of total lipids was recorded in table 1 and fig. 2. Total lipids production varied from 2.5 mg/g dry wt. (Isolate number 1) to 14 mg/g dry wt. (Isolate number 39) which showed the highest activity in lipid production which indicated it may be promising in our study. Therefore it was selected to study its fatty acids profile. As shown in fig. 2, the total lipid of the selected isolate was the highest value.

**Characterization and identification of the most potent lipid producer isolate**

According to ISP methods (Shirling and Gottlieb, 1966) and (Lebeda *et al*., 2012), the chosen isolate was characterized and identified. Light microscopic micrograph showing that after 14 days growth, recti-flexible type spore chain morphology with smooth spores appeared and the isolate has distinguishable sporangia with smooth and almond-shaped spores fig. 3.

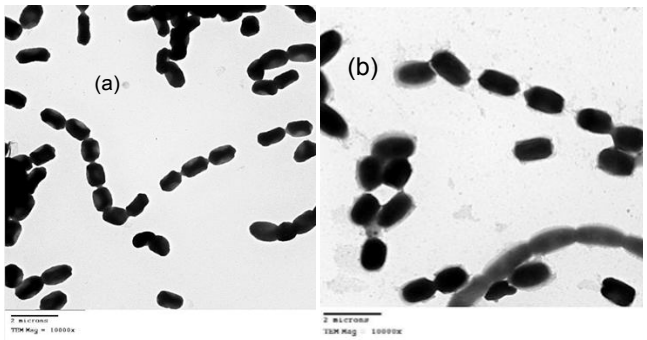


**Fig. 3:** (a) Transmission microscope micrograph showing recti-flexible shape sporophore. (b) smooth and almond-shaped spores.

Biochemical characteristics explain the behavior of the selected isolate toward different substrates (Table 2) where it could hydrolyze starch and casein but couldn't hydrolyze lipid and gelatin. It is also non-motile and could degrade esculin.

The isolate was found to grow well on almost all tested media including ISP 2, ISP 3, ISP 4 and ISP 5. The isolate showed moderate growth on ISP 7 and ISP 1 and no growth presented on ISP 6. Diffusible pigments observed to be pinkish grey to yellow on ISP 2, ISP 3, ISP 4, ISP 5 and ISP 7 (Table 3).

The aerial mycelium was observed to be white to light grey depending on the medium, the color of substrate mycelium was observed to be greyish-yellow to light grey depending on the medium.

The isolate was grown on a wide range of sugars and amino acids added to the medium as a sole carbon and nitrogen source. The growth was inhibited by phenol,

**Table 3:** Culture characteristics of the selected isolate grown on different ISP-media.

| Types of media | Growth | Color of aerial mycelium | Color of substrate mycelium | Color of diffusible pigments |
|---|---|---|---|---|
| ISP-1 | Weak | White | Grayish yellow | None |
| ISP-2 | Good | Gray | Pale Greyish | Pinkish gray |
| ISP-3 | Good | Light gray | Light Gray | Slightly purple |
| ISP-4 | Good | Light gray | Slightly purple | Slightly purple |
| ISP-5 | Good | White | Light Gray | Slightly purple |
| ISP-6 | -ve | -ve | -ve | -ve |
| ISP-7 | moderate | Light gray | yellow | yellow brown |

**Table 4:** Physiological characteristics of the selected isolate.

| Characteristics | Results |
|---|---|
| **Utilization of carbon sources** | |
| Fructose | +ve |
| Starch | +ve |
| Cellulose | -ve |
| sucrose | +ve |
| D- Glucose | +ve |
| Arabinose | +ve |
| L-Rhamnose | -ve |
| **Utilization of amino acids** | |
| L-Valine | -ve |
| L-Asparagine | +ve |
| L-Cysteine | -ve |
| L-Phenylalanine | +ve |
| L-Arginine | +ve |
| L-Methionine | +ve |
| L-Histidine | -ve |
| **Tolerance to toxic substances** | |
| Sodium azide 0.01 % (w/v) | -ve |
| Sodium azide 0.01 % (w/v) | -ve |
| Crystal violet 0.001% (w/v) | -ve |
| Phenol 0.1 % (w/v) | -ve |

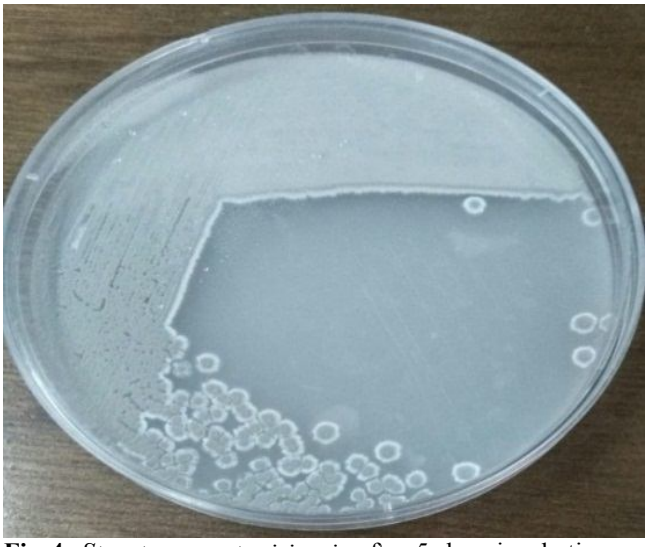

**Fig. 4:** *Streptomyces tunisiensis* after 5 days incubation on starch-nitrate media.

**Fig. 6:** FAMEs extracted from *Streptomyces tunisiensis*.

sodium azide and crystal violet. The optimum growth was observed on pH 7 and at 30°C under aerobic conditions.

The partial 16s rDNA sequence of the isolate was determined and deposited in GenBank under accession number (MN209228). This sequence was aligned with those of *Streptomyces* reference species available in the GenBank database, which confirmed the identification of isolate number 39 at the genus level. The selected isolate showed high sequence similarity with *Streptomyces tunisiensis* strain CN-207.

### GC analysis of fatty acids produced by *Streptomyces tunisiensis*

The lipids and fatty acid production were characterized by GC and the results confirmed a high variation in the fatty acids chain length ranged between C6 and C24 with variably saturated and unsaturated fatty acids. Fig. 5 and table 5, showed the detailed fatty acids composition which producing the highest total lipids concentration (14mg/g dry wt).

Among the detected fatty acids, the unsaturated palmitoleic acid (C16:1) (25.81% of total FAME) was the most abundant fatty acid. Followed by the unsaturated fatty acid palmitic acid (C16:0) (8.05% of total FAME) and the lowest amount were recorded for the saturated undeconoic acid (C11:0) (0.59% of total FAME).

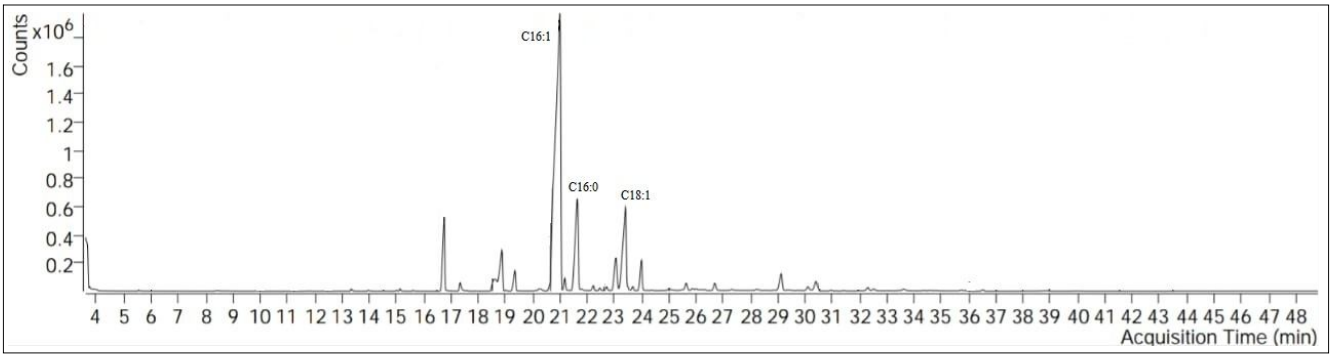


**Fig. 5**: Gas chromatography-mass spectrometry (GC-MS) runs of the fatty acid methyl esters from *Streptomyces tunisiensis.*

**Table 5:** Fatty acid profile of *Streptomyces tunisiensis.*

| Fatty acid methyl ester | Fatty acid chain | Fatty acid conc. (% of total FAME) |
|---|---|---|
| Caproic acid methyl ester | (C6:0) | nd |
| Caprylic acid methyl ester | (C8:0) | 5.87 |
| Capric acid methyl ester | (C10:0) | 0.65 |
| Undecanoic acid methyl ester | (C11:0) | 0.59 |
| Lauric acid methyl ester | (C12:0) | 0.68 |
| Tridecanoic acid methyl ester | (C13:0) | 0.82 |
| Myristoleic acid methyl ester | (C14:1) | nd |
| Myristic acid methyl ester | (C14:0) | 1.39 |
| cis-10-Pentadecenoic acid methyl ester | (C15:1) | 1.97 |
| Pentadecanoic acid methyl ester | (C15:0) | 3.73 |
| Palmitoleic acid methyl ester | (C16:1) | 25.81 |
| Palmitic acid methyl ester | (C16:0) | 8.05 |
| cis-10-Heptadecenoic acid methyl ester | (C17:1) | 2.43 |
| Heptadecanoic acid methyl ester | (C17:0) | 2.64 |
| γ-Linolenic Acid methyl ester | (C18:3) | nd |
| Linolenic acid methyl ester | (C18:3) | 3.43 |
| Oleic acid methyl ester | (C18:1) | 7.53 |
| Elaidic acid methyl ester | (C18:1) | nd |
| Stearic acid methyl ester | (C18:0) | 2.71 |
| Arachidonic acid methyl ester | (C20:4) | nd |
| cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester | (C20:5) | 3.88 |
| cis-8,11,14-Eicosatrienoic acid methyl ester | (C20:3) | 4.33 |
| cis-11,14-Eicosadienoic acid methyl ester | (C20:2) | nd |
| cis-11-Eicosenoic acid methyl ester | (C20:3) | nd |
| cis-11,14,17-Eicosatrienoic acid methyl ester | (C20:3) | 4 |
| Arachidic acid methyl ester | (C20:0) | 2.18 |
| Heneicosanoic acid methyl ester | (C21:0) | nd |
| cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester | (C22:6) | 4.30 |
| cis-13,16-Docosadienoic acid methyl ester | (C22:2) | nd |
| Erucic acid methyl ester | (C22:1) | nd |
| Behenoic acid methyl ester | (C22:0) | 5.27 |
| Tricosanoic acid methyl ester | (C23:0) | 3.36 |
| Nervonic acid methyl ester | (C24:1) | 4.38 |
| Lignoceric acid methyl ester | (C24:0) | nd |

The ratio of saturated and unsaturated fatty acids was summarized in table 5 and the obtained data showed that the *Streptomyces tunisiensis* was a good parameter for biodiesel production fig. 5.

## Discussion

The production of low-cost biofuels using microorganisms is of great interest due to the increase in world use of energy and the shortage of fossil oils. Biodiesel is a renewable fuel that can be produced by microorganisms such as algae and bacteria. Fatty acids methyl esters (FAME) are a common component of biodiesel and can be synthesized from either triacylglycerol or free fatty acids (Nawabi *et al*., 2011).

In the present study, 50 bacterial strains were isolated from different sources. All the collected isolates were screened preliminarily for their fatty acid production using colorimetric TTC techniques. A direct association between the ability to grow on broth containing TTC and to produce fatty acids has been found (Ryan *et al*., 2010). Researchers (Ryan *et al*., 2010) hypothesized that the ability to reduce colorless TTC to red-colored TF is due to the effect of Δ5-desaturase, an enzyme required in the metabolic biosynthesis of FAs. The TTC method is a fast and easy method of screening PUFA producers as it reduced the screening time. That correlates well with the findings of other scientists (Zhu *et al*., 2004) who recorded that the development of the red color confirms the reduction of colorless TTC to red triphenyl formazan (TF). All the negative TTC results can be excluded.

Total lipids of all positive isolates on the TTC test were estimated. Isolate number (39) was the most potent strain in lipid production. The tested bacterial isolate was characterized among the actinomycetes due to the presence of LL-diaminopimelic acid (LL-DAP) in their cell wall (Lecheralier and Lechevalier, 1976). It exhibited typical characteristics of genus *Streptomyces* (Williams *et al*., 1989) for the following reasons: the inability of vegetative mycelia to be fragmented into bacillary or coccoid forms (Grantcharova *et al*., 2005) the presence of large spore chains (Manteca *et al*., 2010) the isolate was aerobic, excessive branching and aerial mycelia (Claessen *et al*., 2006). Phylogenetic analysis of the 16S rRNA gene sequence of isolate number 39 revealed that it is confirmed as *Streptomyces tunisiensis* under accession number (MN209228) (Lebeda *et al*., 2012).

One of the major objectives of the present study was to evaluate the suitability of actinomycetes lipids as a reliable feedstock of biodiesel. The present results confirmed the suitability of *Streptomyces tunisiensis* lipids as feedback of biodiesel. This finding was supported by previous similar studies (Knothe, 2005; Basha and Jebaraj,

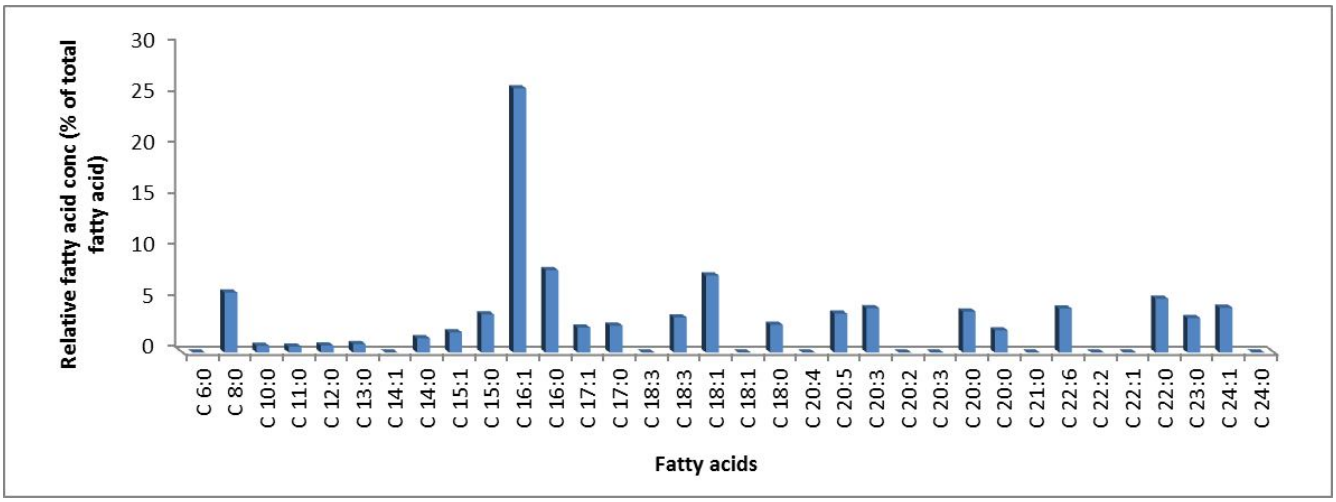


**Fig. 7:** Fatty acid composition (relative abundance of fatty acids) of *Streptomyces tunisiensis.*

2009; Ramachandra *et al*., 2009). These experimental outcomes alert for pursuing a lab-scale batch trail to convert the lipid fraction of *Streptomyces* to biodiesel. The analysis of its fatty acid profile revealed that the amount of unsaturated fatty acids was higher than saturated fatty acids which will be on important biodiesel quality determinant.

Extensive studies (Matsumoto *et al*., 2009; Meng *et al*., 2009) indicated unsaturated fatty acids mainly palmitoleic (C16:1) Oleic (C18:1) and linoleic (C18:2) acids in addition to the saturated fatty acids mainly palmitic (C16:0) and stearic (C18:0) acids could have a vital role in biodiesel production. The level of unsaturation affected biodiesel properties as that fuels with a higher level of unsaturation of the acyl chain have a higher cloud point, which was desirable. Some reports of Song *et al*., (2013) and Ma *et al*., (2014) concluded that the most common feedstock suitable for biodiesel production is enriched in the C16-C18 fatty acids. Our results showed that *Streptomyces tunisiensis* contained considerable amounts of C16 and C18 fatty acids. A reasonable balance for fuel could be achieved with oil containing high levels of monounsaturated fatty acids like octadecenoic acid (18:1) (Staurnas *et al*., 1995) and (Hoekman *et al*., 2012).

## Conclusion

The present results show that 2, 3, 5, Triphenyl Tetrazolium Chloride methods reduced the time, effort and cost involved in the screening and isolation of fatty acids from bacterial strains. These results may add rigid support of the suitability of *Streptomyces tunisiensis* lipid as a feedstock of biodiesel due to the high content of long-chain fatty acids with a carbon atom and a high ratio of unsaturated fatty acids, which may be considered as good characteristics for biodiesel properties. Further studies are underway to optimize the esterification process to maximize the conversion of the fatty acids into biodiesel**.**

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