Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, Egypt
28(3), 797-808, 2020
Website: http://ajs.journals.ekb.eg

ISOLATION AND IDENTIFICATION OF ANTIBIOTIC PRODUCING *PSEUDOMONAS FLUORESCENS* NBRC-14160 FROM DELTA SOIL IN EGYPT

[58]

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Received 9 January, 2020 Accepted 30 March, 2020

ABSTRACT

Pseudomonas fluorescens is one of plant Growth Promoting Rhizobacteria (PGPR) members which has a major role in the biological control of bacterial and fungal pathogens. A research was conducted at the Laboratory of Microbiology, Faculty of Agriculture, Ain Shams University, Qalubyia governorate intended for isolating *Pseudomonas fluorescens* isolate, efficient in antibiotic production. For isolation, soil samples, collected from Faculty of agriculture farm soil at depth of 10 cm, were screened for *Ps. fluorescens* isolates by cultivating the samples on King's medium. Out of 30 isolates obtained, one was selected based on its high lipid content, because of the lipid's correlation to antibiotic production and inhibitory activity. The selected isolate was characterized by morphological, physiological, biochemical tests then confirmed its identity by 16S rRNA gene sequence analysis and named *Pseudomonas fluorescens* NBRC-14160 16srRNA. Morphological features showed that *Ps. fluorescens* NBRC-14160 has large colonies, with irregular surface, opaque, producing green fluorescent pigments. Cells are short rods, Gram negative. Physiological features indicated that *Ps. fluorescens* NBRC-14160 is capable of producing several exoenzymes including lipase, phospholipase, protease and chitinase and incapable of producing amylase and cellulase. Carbohydrate fermentation tests were positive for fructose, glucose, D-glucose, and galactose, forming acids after 24 hrs. of incubation at 30˚C. However, it loses the ability to ferment inositol, mannose, xylose, mannitol, raffinose and rhamnose sugars. It's lipid content was 607 mg/g. Inhibitory activity was studied by the method of disc diffusion test against nine pathogenic bacterial and fungal strains. *Staphylococcus aureus* was the most sensitive bacterial pathogen towards *P. fluorescens* NBRC-14160 with a 4.5 cm zone of inhibition, while *Serratia marscens* had 0.95 cm zone. *Aspergillus niger* and *Alternaria solani* were the most sensitive fungal pathogens towards *P. fluorescens* NBRC-14160 with 2.55 and 2.5 cm of inhibition zone, respectively. However, the most resistant fungus was *Fusarium oxysporum* with 1.3 cm inhibition zone.

Keywords: *Pseudomonas fluorescens*, physiological characteristics, exo-enzymes, production, Antimicrobial activity, 16s rRNA.

INTRODUCTION

Pseudomonas fluorescens is a soil and water microorganism, could grow well on routine microbiological culture media at 37ºC. *Pseudomonas* spp. cells are thin, rod-shaped, non-spore formulating, gram-negative bacilli, motile by means of one flagella (e.g. *P. oryzihabitans, P. stutzeri, P aeruginosa*) or more (e.g. *P. fluorescens, P. putida*). *Pseudomonas* spp. are facultative anaerobes in the presence of nitrate source **(Filiatrault et al 2006)**.

Some *Pseudomonas* spp., such as *P. fluorescens,* are capable to grow and adapt to low temperatures as 4ºC but most grow between 28ºC and 42ºC, showing visible growth within 24 to 48 hrs. It may also produce pyorubin toxin (red), pyocyanin

toxin (blue) or pyomelanin pigment (black). *Pseudomonas fluorescens* strains are indophenol producers and oxidase positive microorganisms. Also, they represent an important source of single cell oils as a source of unsaturated fatty acids, thus, poly unsaturated fatty acids such as docosahexaenoic acid (DHA) can be used as nutritional supplementation **(Ochsenreither et al 2016, Hou and Ray 2019).**

As a biocontrol agent, it is considered as one of the most important rhizosphere microorganisms involved in control the growth of pathogenic plant fungi **(Ayers and Papavizas, 1963 and Ownley et al 2003)**. Its physiological features play an important role in determining the inhibition potential level of plant pathogens. *Pseudomonas fluorescens* strains are antibiotic producers which having a wide spectrum of antimicrobial activity against pathogenic bacteria such as *Salmonella typhimurium* **(Laine et al 1996)**, *Proteus vulgaris, Candida albicans*, *Bacillus subtilis* **(Trujillo et al 2007)**, *Staphylococcus aureus, Aeromonas hydrophila* and *Escherichia coli* **(Vachée et al 1997)**. Some species of *Pseudomonas fluorescens* had a high efficiency to control disease caused by *Gaeumannomyces graminis var. tritici* **(Raaijmaker and Weller, 1998)**. Studies also showed that *P. fluorescens* have a significant inhibitory activity against the newly formed microsclerotia of *Verticillium dahliae* and could suppress its wilt disease on *Arabidopsis thaliana* and eggplant. **Soesanto and Mugiastuti, (2011)** reported that *Pseudomonas* spp. could also suppress 92% of sclerotial germination of *Sclerotium rolfsii*. Also, it is found that 92% of stem rot disease intensity could be suppressed when soil is inoculated with *Pseudomonas fluorescens.*

The antagonistic effect of *pseudomonas* spp could also decrease the disease severity of some plant pathogens such as *Fusarium* wilt on shallot **(Akter et al 2016)** and gladiol.

Therefore, the aim of this work was to isolate antibiotic producing *P. fluorescens* testing its antimicrobial activity against bacterial and fungal pathogens of human and plants.

MATERIALS AND METHODS

Micro-organisms

All plant and human pathogens were obtained from Cairo Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Media used

Medium (1): Nutrient agar and broth medium **(APHA, 1992)** were used for stock cultures maintenance. It also acted as a base for enzymatic detections and carbohydrate tests. Its composition (g/l): meat extract 3 g, peptone 5 g, agar 20 g, and pH 7.0±0.2.

Medium (2): King's Medium was used for the isolation of *Pseudomonas fluorescens* (King, 1954). It has the following composition (g/l)): proteose peptone 20 g, K_2HPO_4 1.5 g, $MgSo_4.7H_2O$ 1.5 g, glycerol 10 ml, agar 15 g and pH 7.2±0.2.

Medium (3): (Mueller Hinton Agar), (Sigma-Aldrich) was used for testing the inhibitory activity of the selected isolate against different microbial pathogens **(Thornsberry, 1983)**. It was prepared by suspending 38.0 g in 1l of distilled water then boiled to dissolve the medium completely.

Medium (4): Glucose Phosphate Broth Medium was used for Voges-Proskauer Test **(Voges and Proskauer, 1898)**. Its formula is (g/l): peptone 7 g, phosphate buffer 5 g, glucose 5 g and pH 6.9 ± 0.2

Medium (5): Koser citrate Medium (Koser, 1923) was used for testing the utilization of citrate as a sole carbon source for *Pseudomonas fluorescens.* It is composed of (g/L of distilled water): (NH_4) $NaHPO_4$ 1.5 g, KH_2PO_4 1 g, $MgSO_4$ 0.2 g, $Na_2C_6H_6O_7$ 3 g and pH 6.7±0.2.

Medium (6): Tryptone Broth **(Baird et al 2012)** was used for testing the indole production of *Pseudomonas fluorescens* which contained (g/l): tryptone 10g, sodium chloride 5g, and a final pH of 7.5±0.2

Medium (7): Glucose Broth was used in the Methyl Red Test **(Clark and Lubs, 1915)**. It contained (g/l): tryptone 10 g, glucose 5 g, NaCl 5 g and a final pH of 7.3±0.2.

Medium (8): Tryptic soy agar supplemented with egg yolk (Sigma). It is used for phospholipase detection. It is composed of tryptone 10, soybean meal 15, agar 20 (g/l) and egg yolk 2% (v/v). It was prepared by suspending 40.0 g in 1L of distilled water and then boiled to dissolve the medium completely. Egg yolk was added separately during pouring plates.

Medium (9): Skim milk agar **(APHA, 1992)** was used for proteases detection according to coagulation and proteolysis of casein. It has the following composition (g/l of distilled water): meat extract 3, peptone 5, skim milk 100, agar 20, and pH 7.0.

Standard inoculum

Standard inoculum was prepared by inoculating 50 ml of nutrient broth in 250 ml conical flasks with a loop of tested culture. The inoculated flask was incubated on a rotary shaking incubator (Lab-line Inc. Ltd.) at the rate of 120 rpm for 24 h at 37˚C and considered as the standard inoculum (1 ml contained 6.0 – 7.0 x 10^5 viable cells) for shake flasks.

Soil sampling and isolation of *Pseudomonas fluorescens* isolates

For isolation of *Pseudomonas fluorescens*, soil samples were collected from Faculty of Agriculture Ain Shams University, located in Qalubyia governorate. Soil samples were collected at depth of 10 cm and packed in sterilized glass jars, transported to the lab and stored at 4°C for further studies. Isolation of *Pseudomonas fluorescens* was carried out by inoculating plate with King's medium with soil samples and incubating the plates at 30ºC for 24 hrs **(Houghtby et al 1992)**. Colonies with greenish fluorescent pigment were picked up and streaked on King's medium agar slants. They were stored at 4°C and sub-cultured at monthly intervals.

Morphological characteristics of the selected isolate

For the morphological features of *Pseudomonas fluorescens* isolates, colony size, surface, opacity, color, cell shape, cell size, lipid content and Gram's staining were examined microscopically.

All morphological characteristic tests were done in triplicates. lipid content was examined by using Sudan black B stain (Sigma) according to **(Hartman, 1940)**.

Total lipid determination

To estimate the accumulated total lipids by *Pseudomonas* isolates, King's medium was inoculated with a single colony of the tested culture and incubated at 30°C for 24 hrs to extract total lipids, 10ml of the grown culture was withdrawn and centrifuged at 10000 rpm for 15 min. Cell pellets were obtained and washed with distilled water twice. Total lipids content was estimated according to **(Politz et al 2013).**

Enzymatic activities

Protease production was performed according to the method described by **(Nassar et al 2015)**. where, skim milk agar plates were inoculated by placing a filter paper disc (9 mm in diameter) saturated with the selected culture's inoculum at the center of each plate and incubated at 30ºC for 24 hrs. Protease production was detected by flooding plates with freshly prepared 1% HCL solution. Clear zone around the filter paper disc indicated the production of protease.

Lipase production. To detect the lipolytic activity of selected isolate **(Veerapagu et al 2013)**, nutrient agar medium was prepared and autoclaved at 121ºC for 30 min. Olive oil, pre sterilized at 180°C for 2h, was added during pouring to give 2% (v/v) ratio. Oil agar plates were inoculated by disc method as previously described and incubated at 30°C for 24 hrs. Lipase production was detected by flooding the plates with 0.01% methyl orange acid base indicator and formation of pink zones around the disc indicated positive result of olive oil hydrolysis.

Amylase production. Detection of amylase production by the selected isolate was performed according to **(Alariya et al 2019)**. Nutrient agar medium, supplemented with 2% (w/v) starch, was autoclaved at 121ºC for 30 min. Starch agar plates were inoculated and incubated as previously described. For amylase detection, Iodine solution was added to the plates as an indicator. The formation of clear zones around the disc indicated hydrolysis of starch, thus production of amylase.

Cellulase production. Detection of cellulase production by the selected isolate was done according to the method described by **(Jarallah, Eman and Abdul-kadhim, 2013)**. Nutrient agar medium, supplemented with 2% (w/v) carboxymethyl cellulose (CMC) and sterilized at 121ºC for 30 min. CMC agar plates were inoculated and incubated as previously described. After incubation, the plates were immersed with iodine solution and formation of clear zones indicated degradation of CMC, therefore production of cellulase.

Chitinase production. The production of chitinase by the selected isolate was tested according to **(Neiendam and Sørensen, 1999)**. Nutrient agar medium, supplemented with 0.2% (w/v) colloidal chitin. were sterilized at 121°C for 30 min. Chitin agar plates were inoculated and incubated as previously described. The presence of clear zone around the paper disc indicated the production of chitinase.

Phospholipase production. To detect the phospholipase activity of the selected isolate, tryptic soy agar was prepared and sterilized at 121°C for 30 min. Egg yolk was added during pouring agar plates to give final concentration of 2%. TSA plates were inoculated and incubated as previously described. Formation of clear zones around indicated the hydrolysis of phospholipases **(Jae et al 2005)**.

Carbohydrate fermentation tests

To test carbohydrate fermentation by the selected isolate, carbohydrate fermentation test was performed according to **(Liu, 1952)**, where glucose broth was used as a basal medium and glucose was replaced by one of the following 10 carbon sources: glucose, Dglucose, fructose, lactose, lactose, inositol, mannose, xylose, mannitol, rhamnose, and raffinose maintaining the same carbon ratio in the glucose. All media were inoculated with a full loop of the selected isolate's culture and incubated at 30°C for 24 hrs. Fermentation of carbohydrates was observed by change in color from yellow to red and gas formation in Durham tube.

Biochemical tests

Voges–Proskauer Test: Glucose phosphate broth was prepared and autoclaved at 121 ºC for 30 min. Medium was inoculated with a loop of the selected isolate's culture, then incubated at 37ºC for 24-48 hrs. After incubation, 1 ml of NaOH (1N) and 2-3 drops of Alpha-naphthol were added as an indicator solution. Formation of reddish-brown ring at the surface of the test tube after 15 to 20 min indicated the production of acetoin.

Citrate Test: Citrate medium was prepared and autoclaved at 121ºC for 30min. Medium was inoculated with a loop of the selected isolate's culture, then incubated at 37ºC for 24-48 hrs. The presence of turbidity indicated the consumption of the citric acid as a sole carbon source.

Indole Test: Tryptone broth was prepared with fixing a filter paper moistened with oxalic acid at the cotton plug then, autoclaved at 121ºC for 30min. Medium was inoculated with a loop of the selected isolate's culture, then incubated at 37ºC for 24-48 hrs. All inoculated media were incubated at 37ºC for 24 - 48 hrs. The change in the color of the filter paper from white to pink indicated indole gas elevation due to tryptone consumption.

Methyl Red Test: glucose broth was prepared and autoclaved at 121 ºC for 30min. Medium was inoculated with a loop the selected isolate's culture, then incubated at 37ºC for 24-48 hrs. After incubation, methyl red was added as an indicator. The remaining red color indicated the acidity of the medium as a result of fermenting the glucose and production of acids.

Inhibitory activity of *Pseudomonas* fluorescens against various human and plant pathogens

Antimicrobial susceptibility of plant and human pathogens against different antimicrobial agents produced by the selected isolate was tested using Muller Hinton Agar **(Muller Hinton et al 1941)** against four bacterial pathogens (*Staphylococcus aureus*, *E. coli*, *Serattia marcescens*, *Bacillus subtilis*) and five fungal plant pathogens (*Fusarium oxysporum*, *Rhizopus nigricans*, *Aspergillus niger*, *Alternaria solani* and *Rhizoctonia solani*). Muller Hinton agar plates were inoculated with bacterial and fungal cultures using the Kirby-Bauer agar disc diffusion method **(Alfred et al 1959)**. The plates were incubated at 30ºC for 24h and clear zones around the discs were measured in cm.

Identification of the selected isolate using 16srRNA

The selected isolate was further identified using phylogenetic analysis of 16SrRNA gene sequences. Isolation of cellular DNA was completed as described by **(Fredrick, 1999)** and amplification of 16SrRNA was done according to **(Lane et al 1991)** using the two universal primers (F1: 5, AGAGTTT (G/C) ATCCTGGCTCAG 3, and R1 5, ACGG (A/C) TACCTTGTTACGACTT 3,). The sequence reads were edited and assembled using BioEditversion 7.0.4 and cluster W version 4.5.1. BLAST searches were done using the NCBI server According to **(Vinnere et al 2002)**.

RESULTS AND DISCUSSION

Isolation of *Pseudomonas fluorescens* isolates from soil sample

This experiment was carried out to isolate *Pseudomonas fluorescens* on King's medium from Delta soil in Egypt. Thirty isolates were obtained and single colonies with greenish yellow (fluorescent) pigment were picked up and streaked onto king's medium petri dishes then, incubated at 30°C. All the

isolates were maintained at 4°C and sub-cultured at monthly intervals.

Morphological characteristics of *Pseudomonas fluorescens* isolates

Morphological characteristics including colony size, surface, opacity, color, cell size and shape were tested as a primary step for *Pseudomonas fluorescens* identification. Gram's staining and lipid content were performed on the thirty *pseudomonads* isolates. The results showed that all the isolates had identical morphological characteristics showing large colonies with irregular surface, opaque and produce green fluorescent pigments. Cells are short rod, small in size, capable of producing lipid and negative to gram stain. These results were similar to **(Labhasetwar et al 2019)** who found that *Pseudomonas fluorescens* have the same characteristics on the other hand (Meyer and Abdallah, 1978) reported that *Pseudomonas fluorescens* from soil have yellow fluorescent pigment.

Total lipid content of Pseudomonas *sp.* isolates

This test was carried out to select the *Pseudomonas* isolate with the highest lipid content. Based on quantitative determination of total lipids production by the thirty isolates, five pseudomonas isolates were selected for having high lipid accumulation. Out of the five lipid producers, one isolate (Ps.15) was selected due to having the highest lipid production (607 mg/g) as shown in **Fig 1.** As reported by **(Yoon et al 2018)**, some fatty acids and monoglycerides showed to be promising antibacterial agents that destabilize bacterial cell membranes, causing a wide range of direct and indirect inhibitory effects.

Enzymatic activities

Pseudomonas isolate (Ps. 15) was tested for lipase, amylase, cellulase, protease and chitinase production activity. **Table 1** proved that *Pseudomonas* (Ps.15) could hydrolyze lipids, proteins, phospholipids and chitin due to the secretion of lipases, proteases and phospholipases, respectively rather than cellulose and starch due to its inability to produce cellulases and amylases.

As noticed by **(Veerapagu et al 2013)** *Pseudomonas* spp. could produce various exo-enzymes such as lipases, proteases, amylases, chitinases, cellulases and phospholipases. Also, he reported that Pseudomonads are the major lipase producers in soil and could be found in vegetable oil processing factories, dairy plants and soil contaminated with oils. **(Neethu et al 2015)** reported that pseudomonads lipases could be used in the biodegradation of petrochemicals and their proteases could be applied in the biodegradation of biofilms.

3.5. Biochemical tests

IMViC test was carried out to test *Pseudomonas* isolate (Ps. 15) ability for production of Indole acetic acid, acid formation, methyl acetyl carbinol and using citrate as a sole carbon source. Data represented in **Table 1** indicates that *Pseudomonas* Ps.15 was positive to VP, Citrate, Indole and Methyl red tests. These results were similar to (Rhodes, 1959) who characterized *P. fluorescence* as positive for IMViC tests.

3.6. Carbohydrate fermentation tests

The ability of *Pseudomonas* Ps. 15 isolate was examined by testing the fermentation of different carbon sources and production of acids and gas. **Table 1** showed that *Pseudomonas fluorescens* Ps.15 is able to ferment (Glucose, D-glucose, Fructose, Galactose), producing acid after 24h of incubation at 30°C, while unable to ferment Inositol, Mannose, Xylose, Mannitol, Raffinose and Rhamnose. As identified by **(Cowan 1974)**, *Pseudomonas fluoresces* Ps.15 could ferment glucose, galactose and fructose as sole sources of carbon to produce acids.

3.7. Inhibitory activities of *Pseudomonas* Ps.15 isolate against plant and human pathogens

Root rot caused by *Fusarium oxysporum, Rhizopus nigricans, Aspergillus niger, Alternaria solani* and *Rhizoctonia solani* are widely distributed in many countries also the treatment by fungicides causes health and environmental hazards. On the other hand, bacterial resistance against current antibiotics increases all over the world. So, it's indeed needed to search for biocontrol agents for pathogenic fungi and alternative antibacterial agents.

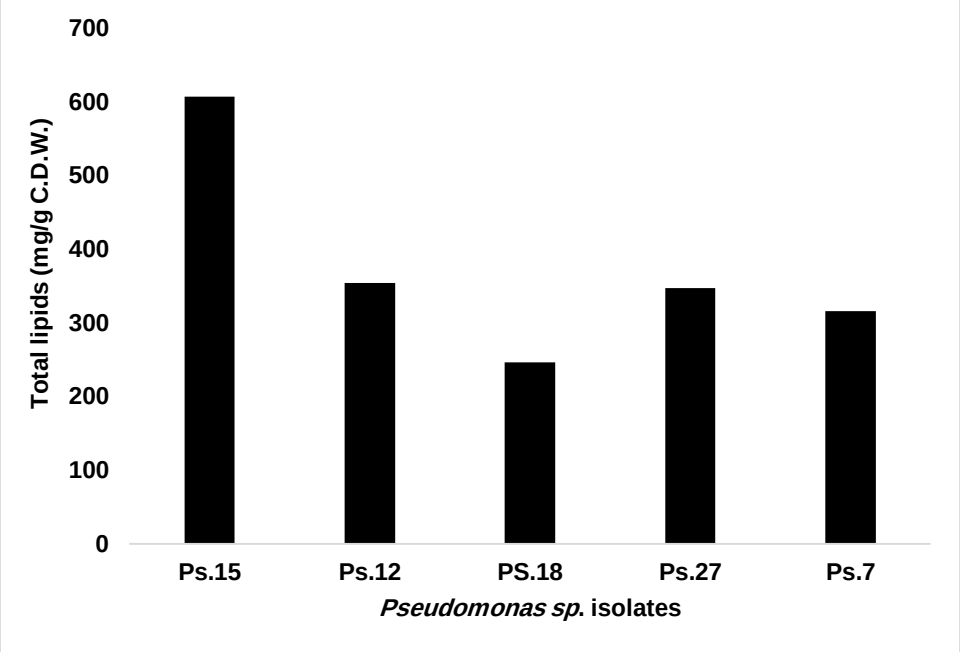

Fig 1. Total lipid content produced by five *Pseudomonas* isolates, obtained from Delta soil in Egypt

Table 1. Morphological and physiological features of the selected isolate

***Pseudomonas fluoroscens* Ps.15**	**Physiological tests**	**Characterization reactions**
Biochemical Tests	Lipases	Positive
	Amylases	Negative
	Cellulases	Negative
	Proteases	Positive
	Chitinase	Positive
	phospholipase	Positive
	IAA	Positive
	M.R.	Positive
	V.P.	Positive
	Citrate	Positive
Carbohydrate fermentation tests	Glucose	Positive
	D-glucose	Positive
	Fructose	Positive
	Galactose	Positive
	Inositol	Negative
	Mannose	Negative
	Xylose	Negative
	Mannitol	Negative
	Raffinose	Negative
	Rhamnose	Negative

Inhibitory effect of *Pseudomonas* Ps.15 isolate was tested against four bacterial pathogens (*Staphylococcus aureus, E. coli, Serattia marcescens, Bacillus subtilis*) and five fungal pathogens (*Fusarium oxysporum, Rhizopus nigricans, Aspergillus niger, Alternaria solani and Rhizoctonia solani*). **Fig 2** revealed that *Staphylococcus aureus* was the most sensitive microbe with 4.5cm zone, whereas *Serattia marcescens* was the least sensitive microbe with 0.95 ± 0.05 cm zone and was recorded for *Alternaria solani, Rhizoctonia solani and Rhizopus nigricans* with zone diameters reached 2.5, 2.4 and 1.95 cm, respectively. Inhibitory effect of *Pseudomonas fluorescens* showed no significant difference against *Fusarium oxysporum and Serattia marcescens* with diameter zones reached 1.5 and 0.95 cm, respectively from the data shown in Table (4 *Pseudomonas* PS.15isolate could be used as an antagonistic agent to inhibit both human and plant diseases.

Similar results were shown by **(Rekha et al 2010),** who found that *Pseudomonas fluorescens* presented a significant inhibitory value against *Salmonella typhi, Streptococcus mutans, Bacillus subtilis, Salmonella sonnei.* Contrarily, they found no inhibitory activity against *Staphylococcus aureus*, *Escherichia coli* and *Serratia marcescens* Theses results indicated the ability of *Pseudomonas fluoresens* to produce high level of toxic HCN as well as producing a wide variety of growth hormones like auxins, gibberellins, cytokinins and anti-microbial metabolites like siderophores (iron-sequestering compounds) and nutrition or site competition **(Erdogan and Benlioglu, 2010)**.

3.8. Molecular identification of Pseudomonas PS.15isolate using 16S rRNA genetic sequencing

As illustrated in **Fig 3** Upon the amplification of 16S r RNA sequence, using universal primer, an amplified product of 1465 bp, the sequence was obtained and compared with the Gen Bank databases using BLASTN software by the Finch-TV. (http://www.geospiza.com/Products/finchtv.sht ml). Similarity percentage is shown in **Table 2**, where 16SrRNA sequence of the isolate *Pseudomonas* PS.15revealed a close relatedness to *Pseudomonas fluorescens* with 99.97% similarity. As shown in **Fig 4**, the phylogenetic analysis of nucleotide sequences based on 16S rRNA revealed closely to *Pseudomonas fluorescens*. Hence, the strain was confirmed as *Pseudomonas fluorescens* NBRC14160 strain.

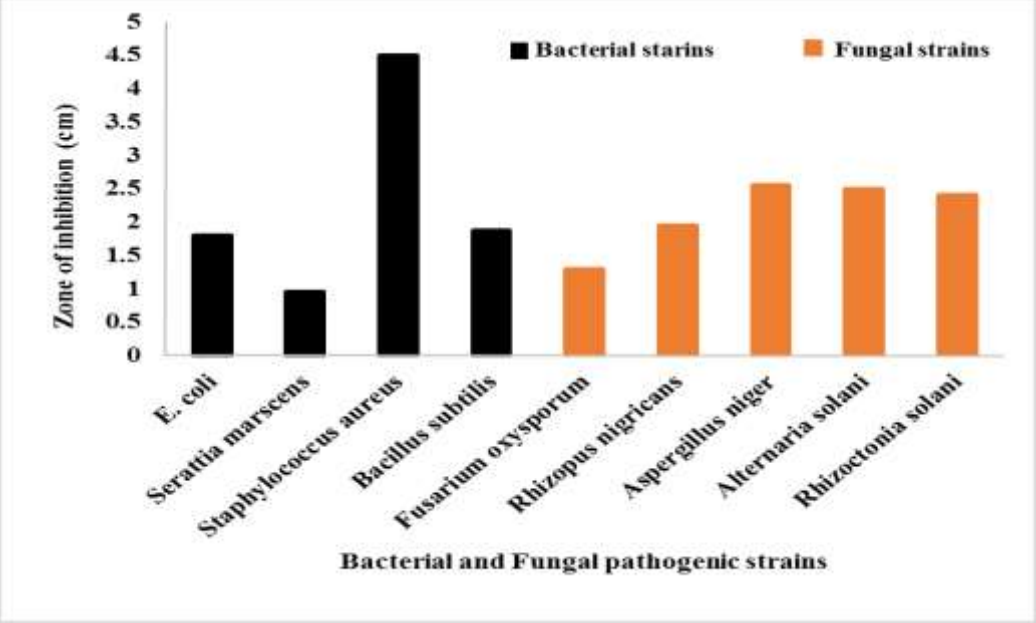

Fig 2. Inhibitory activities of *Pseudomonas* PS.15 isolate against plant and human pathogen using disc diffusion method.

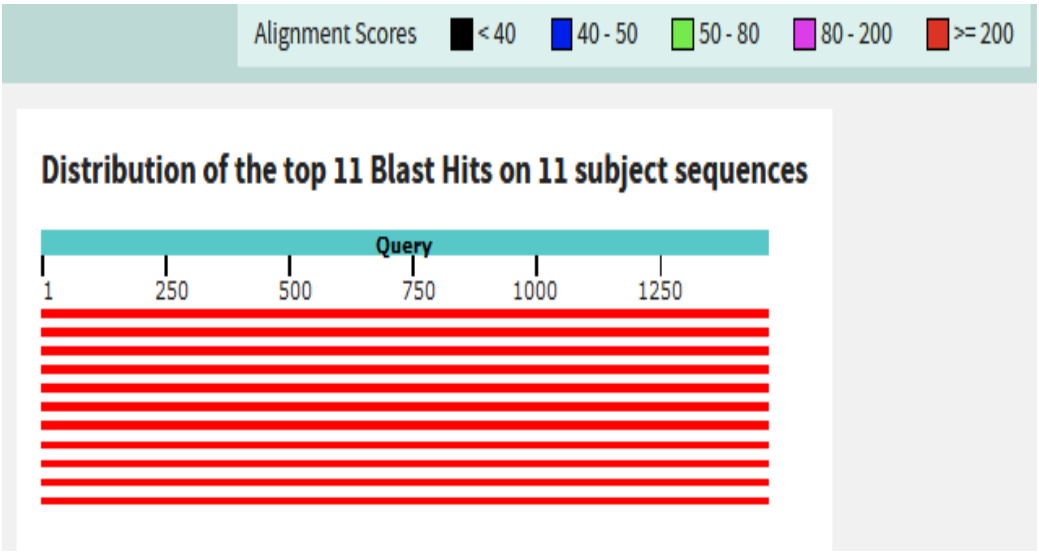

Fig 3. Color key for alignment scores of partial sequences of 16SrRNA of *Pseudomonas fluorescens NBRC14160* starin.

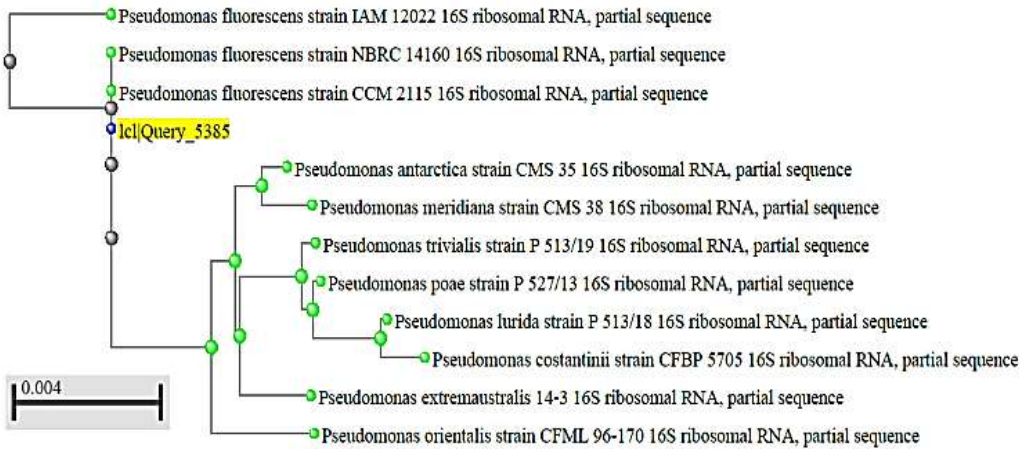

Fig 4. Phylogenetic tree of partial sequence of 16S rRNA of *Pseudomonas fluorescens NBRC14160* starin compared to eleven *Pseudomonas* strains recorded in GenBank.

Table 2. Sequences producing significant alignments of partial sequence of 16SrRNA of *Pseudomonas* isolate PS.15with E-value (0.0).

Description	Per. Identities (%)	Accession
Pseudomonas fluorescens strain NBRC 14160 16S ribosomal RNA, partial sequence	99.97%	NR_113647.1
Pseudomonas fluorescens strain CCM 2115 16S ribosomal RNA, partial sequence	99.85%	NR_115715.1
Pseudomonas antarctica strain CMS 35 16S ribosomal RNA, partial sequence	99.52%	NR_025586.1
Pseudomonas trivialis strain P 513/19 16S ribosomal RNA, partial sequence	99.45%	NR_028987.1
Pseudomonas extremaustralis 14-3 16S ribosomal RNA, partial sequence	99.45%	NR_114911.1
Pseudomonas meridiana strain CMS 38 16S ribosomal RNA, partial sequence	99.45%	NR_025587.1
Pseudomonas orientalis strain CFML 96-170 16S ribosomal RNA, partial sequence	99.45%	NR_024909.1
Pseudomonas fluorescens strain IAM 12022 16S ribosomal RNA, partial sequence	99.45%	NR_043420.1
Pseudomonas poae strain P 527/13 16S ribosomal RNA, partial sequence	99.38%	NR_028986.1
Pseudomonas lurida strain P 513/18 16S ribosomal RNA, partial sequence	99.32%	NR_042199.1
Pseudomonas costantinii strain CFBP 5705 16S ribosomal RNA, partial sequence	99.32%	NR_025164.1

CONCLUSION

In this study, *Pseudomonas fluorescens* NBRC-14160 showed ability to ferment glucose, fructose and galactose and produce lipase, phospholipase, protease, and chitinase. *Pseudomonas fluorescens* NBRC-14160 is an efficient strain for producing total lipids which indicates wide inhibitory activities against the human pathogens, *Staphylococcus aureus, E. coli* and *Bacillus subtilis* as well as against the plant pathogenic fungi*, Rhizobus nigricans, Aspergillus niger, Alternaria solani* and *Rhizoctonia solani*.

ACKNOWLEDGEMNT

The authors would like to express their deepest appreciation for their committee chair, Prof. Dr. Ahmed Galal, Dean of Fac. Agric. Ain Shams Univ., for his continually and unlimited support for the Biotechnology program. Without his guidance and persistent help, this research would not have been possible.

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مجلة اتحاد الجامعات العربية للعلوم الزراعية، جامعة عين شمس، القاهرة، مصر
مجلد(28)، عدد(3)، 797-808، 2020
Website: http://ajs.journals.ekb.eg

عزل وتعريف بكتيريا السيدوموناس فلوروسنس NBRC14160 المنتجة للمضادات الحيوية من أرض الدلتا بمصر

[58]

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Received 9 January, 2020 Accepted 30 March, 2020

الموجـــــز

أجري هذا البحث بهدف عزل بكتيريا *Pseudomonas fluorescens* من التربة واختبار كفائتها في إنتاج المضادات الحيوية. أظهرت النتائج أنه من أصل 30 عزلة تم إختيار بكتيريا *Pseudomonas fluorescens Ps.15* من عينات أراضي الدلتا بالقاهرة لإرتفاع محتواها من الليبيدات والذي يرتبط بكفاءة قدرتها علي تثبيط الميكروبات المرضية. تم دراسة صفات العزلة المختارة المزرعية والشكلية والفسيولوجية ثم تعريفها بواسطة تحليل الجينات 16srRNA. وأظهرت النتائج ان العزلة كانت لها مستعمرات كبيرة الحجم ذات سطح غير منتظم غير شفافة وتكون صبغات فلوروسنتية خضراء. اما عن الشكل الميكروسكوبي للخلايا، فكانت عصويات قصيرة سالبة لجرام مخزنة للدهون. وعن صفاتها الفسيولوجية تم إجراء بعض الاختبارات الانزيمية وأشارت النتائج بقدرة العزلة علي افراز انزيمات البروتييز والفوسفوليبيز والليبيز والكيتينيز وعدم قدرتها علي إنتاج كل من الأميليز والسليوليز. تم اجراء اختبارات IMViC علي العزلة واثبتت العزلة ايجابيتها للاربع اختبارات. تم اختبار قدرة العزلة علي تخمير عشر مصادرمن الكربوهيدرات لانتاج الحامض والغاز وأثبتت النتائج قدرتها علي تحليل كل من الفركتوز والجلوكوز والجالاكتوز كذلك تم دراسة قدرة عزلة *Pseudomonas* fluorescens Ps.15 في تثبيط الميكروبات الممرضة للانسان والحيوان قد تم اختبار تأثيرها علي نموأربع سلالات بكتيرية ممرضة للإنسان وخمس سلالات فطرية ممرضة للنبات وأظهرت النتائج قدرتها الفائقة علي تثبيط نمو كل من البكتيريا الممرضة للإنسان *Staphylococcus aureus* ليصل قطر هالة التثبيط الي 4.5 سم وكان التأثير ضعيف في حالة بكتيريا *Serratia* marscens حيث كانت هالة التثبيط 0.95 سم. وفي حالة الفطريات الممرضة للنبات فكان أعلي تثبيط تم اكتشافه في حالة فطر *Aspergillus niger* و*Rhizopus nigricans*. وعند تعريف عزلة *Pseudomonas fluorescens* علي المستوي الجيني باستخدام تقنية 16s rRNA أثبتت النتائج انها تتبع جنس *Pseudomonas fluorescens* NBRC-141605 بنسبة تشابه 99.7%.