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Stenotrophomonas rhizophila a Novel Plant-Associated Bacterium With Distinguished PGPRs Properties

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Abstract: *Stenotrophomonas* sp. is a plant-associated rhizospheric bacteria. It has the capability to enhance plant productivity. It acts as a mineral dissolution and growth promoter for plants under salt-stress conditions. The selected isolates revealed the general morphological and biochemical characteristics of *Stenotrophomonas* sp. All the isolates exhibited the highest growth density after 15 hr. at 30ºC, resistance to different levels of salinity, different pH levels, and different temperature degrees. Also, the isolates revealed their capabilities to solubilize phosphorus and potassium and to produce different phytohormones. The isolate X.M9 exhibited the highest growth performance in the form of 1.376,1.326,1.292,1.053 and 1.059 OD in most salts' concentrations compared to all tested isolates respectively. As well it (X.M9) exhibited the highest resistance to low temperatures 10 and 15ºC being 0.917 and 1.354 OD respectively. The isolate X.M9 recorded the highest IAA of 83.73 mg/100ml compared to all the tested isolates. The isolate X.M9 were identified using 16 SRNA sequencing and submitted to the GenBank database under accession number No: OP050187 as *Stenotrophomonas rhizophila.*

1 Introduction

Stenotrophomonas sp. is a genus of bacteria that is commonly known as plant-associated microorganisms. It is present in many soils and roots of various crops.

The genus *Stenotrophomonas* sp. is classified phylogenetically as gamma proteobacteria. The genus' name is intended to emphasize the bacterium's limited food spectrum as it derives from the Greek words 'stenos', meaning narrow, 'trophus', which means one who feeds, and 'monas', that meaning unity. However, several studies showed that it has high metabolic versatility and intraspecific heterogeneity (Nesme et al 1995, Berg et al 1999).

Stenotrophomonas rhizophila is a model of rhizospheric bacteria that, is often found in association with plants, and it can be isolated from roots particularly from the rhizosphere or from internal root tissues of vascular plants (Berg et al 2002). *S. rhizophila* also can enhance plant productivity through several mechanisms, including the production of the plant growth hormone (Suckstorff and Berg 2003) particularly under salt stress conditions, nitrogen fixation, and elemental sulfur oxidation, which in turn supplies plants with sulfur, and mineral dissolution to facilitates min-

erals in the soil in addition to controlling a wide range of plant pathogens through the production of antifungal products (Alavi et al 2013, Egamberdieva et al 2016, Singh and Jha 2017, Alexander et al 2020).

Moreover, *S. rhizophila* has an indirect effect on promoting plant growth by influencing the microbial community in the rhizosphere (Pinski et al 2020). Also, it has promising applications in bioremediation as it can metabolize a wide range of organic matter such as plant root exudates, including phenol, polycyclic aromatic hydrocarbons, selenium compounds, benzene, toluene, ethylbenzene (Lee et al 2002). These metabolic properties are widespread and can provide protection to plants from the toxic effects of these compounds.

The present work aims to characterize and molecularly identify *Stenotrophomonas* sp. isolated from saline soils.

2 Material and Methods

2.1 Samples collections

Representative four saline soil samples were collected from two different locations and were used to isolate specific groups of PGPRs. Three samples were obtained from saline soil in the Research Station - Desert Research Center, located in Ras Sidr, –South Sinai Governorate, which was previously cultivated by barley. These soil samples showed salt concentrations of 20, 18.75, and 14.5 dS/m. The fourth soil sample was collected from the rhizosphere of sugar beet plants grown in the saline experimental unit, Fac. Agric., Ain Shams Univ. Cairo Governorate which showed a salt concentration of 31.25 dS/m.

2.2 Isolation of *Stenotrophomonas* sp.

Ten grams of soil samples were suspended in 90 ml sterilized water in conical flasks (250 ml) and one gram of the rhizosphere soil was suspended in 9 ml sterilized water in a test tube, then both of them were thoroughly shaken for 10 min., and serial dilution up to 10^{-5} were prepared. Modified Aleksandrov's agar medium Media was prepared in four salt concentrations using sodium chloride (NaCl) to achieve a salt ratio of 20, 18.75, 14.5 and 31.25 dS/m, **Table 1**. In triplicate, one milliliter of each dilution was plated and filled with each medium concentration. Plates were incubated at 30±1ºC for 3-5 days. Individual colonies with *Stenotrophomonas* sp. characteristics were picked up for purification. The purified isolates were maintained on the specific agar media amended with NaCl salt for each one at 4±1ºC. Subculturing of the purified isolates was done monthly.

Table 1. The EC of the prepared medium for isolation, purification, and maintenance of *Stenotrophomonas* sp.

Microbiological Media	EC dS/m	Code
Modified Aleksandrov`s Media	31.25	X.S20
	20	X.E15
	18.75	X.K12
	14.5	X.M9

2.3 Morphological and biochemical characterization of the selected isolates

The morphologies of the isolates were characterized using standard techniques [colony morphology on nutrient agar medium, Gram staining, cell shape, (color, shape, elevation, and margin)]. The biochemical tests such as catalase, gelatinase and amylase tests were carried out based on Bergey's Manual of Systematic Bacteriology (Garrity et al 2005).

2.4 The growth pattern of the selected isolates.

Four selected bacterial isolates were cultured separately in their specific medium, each supplemented with sodium chloride (NaCl) concentration and incubated at 30ºC. The growth of the tested isolates was measured at 600 nm on a spectrophotometer (Unicum) as a function of time every 3 hours for 30 hours. A standard curve was prepared for each isolate in the form of OD as a function of time.

2.5 The growth performance of the tested isolates on different levels of salinity

Each of the selected tested bacterial isolates was cultivated on its specific medium containing the same concentration of salinity used in the isolation medium of 5, 10, 15, 20, 25, and 30 dS/m (cells density were adjusted at 35×10^{7} CFU /ml for all the tested parameters). The growth of each isolate was measured by measuring the optical density (OD) of the isolates.

2.6 The growth performance of the tested isolates on different levels of PH

Each of the selected isolates was cultivated on its specific medium (containing the same concentration of salinity used in the isolation medium) with different levels of pH ranging from pH 4 to pH10 by adding 1N

HCl or 1N NaOH. The growth of each isolate was measured by measuring the optical density (OD) of the isolates.

2.7 Effect of different temperatures on the growth performance of the tested isolates

The tested isolates were cultivated on their specific medium separately contain the same concentration of salinity used in the isolation medium) and incubated at different temperature degrees (10,15, 20, 25, 30, 35, 40, 45 and 50ºC). The growth of each isolate was measured by measuring the optical density (OD) of the isolates.

2.8 Production of plant growth promoters (PGPRs)

The selected isolates were tested for their ability to produce some phytohormones such as Cytokinins, Gibberellic acid, Indole acetic acid, and Abscisic acid by using High-performance liquid chromatography (HPLC) in the following steps: Ethyl acetate extraction was conducted as described by (Tien et al 1979). The ethyl acetate extract was evaporated to dryness and the residue was dissolved in one ml of ethanol. For identification, 30 µl samples were filtered through a 0.45 µm filter, then injected into the column. The filtrate was subjected to separation by high-performance liquid chromatography (HPLC) using methanol: acetic acid: water (30: 0.5: 70) as mobile phase at the rate of 1.5 ml/min. Agilent 1100 series (Waldborn, Germany), quaternary pump (G1311A), Degasser (G1322A), Thermostat Autosampler (G1329A), variable wavelength detector (G1314A); and column: Zorb ax 300SB C18 column (4.5 X 250 mm) (Agilent Technologies, USA). The injection was carried out at wavelengths of 252 nm for separation. The growth hormones were identified based on the retention time of the standard cytokinin, indole acetic acid and gibberellic acid by using UV detector at 252 nm. The concentration of each product was calculated based on the peak area.

2.9 Efficiency of the tested isolates in solubilizing phosphorus and potassium mobilization

Phosphate solubilizing and Potassium mobilization were examined according to Jackson (1973).

2.10 Identification of the selected isolate using 16S rRNA gene sequencing

2.10.1 Genomic RNA extraction

An axenic colony was collected from the culture and placed in Eppendorf with 40 µl of NaOH (0.20 M) (the genetic material was extracted by alkaline lysis). The solution was subjected to a microwave of 700 W for one minute and immediately cooled on ice for 5 min. The lysed cells were subjected to 10,000 gravities for 10 min and the supernatant was collected.

2.10.2 Amplification and sequencing of 16S rRNA:

25 µl of the PCR mixture containing 1 µl of the crude extract containing DNA, 1 µl (10 µM) of the universal primers 27F (5'- AGAGTTTGATCCT GGCTCAG-3') and 1492R (5'-GGTTACCTTGTT ACGACTT-) were used (Criollo et al 2012), which allows obtaining amplicons of around 1500 bp, and GoTaq® Green Master Mix (Promega Corporation, USA). Amplification products were subjected to 1% agarose gel electrophoresis. The purified products were performed by the Sanger method at the Macrogen® Company (Republic of Korea).

The 16S rRNA sequence was aligned on the database, of the National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov). The BLAST program (http://www.ncbi.nlm.nih.gov/BLAST) on the NCBI database was used to compare the obtained sequence with the sequence of the reference strain stored in the public database. The computer software package Clusta lW was used for sequence alignment.

3 Results and Discussions

3.1 Morphological and biochemical characterization of the selected isolates

The examined isolates revealed clear morphological and biochemical properties for the members of the genus *Stenotrophomonas* sp. **Table 2**. Colonies presented light yellowish, flat, translucent, mucous apperance with smooth edges, which are similar to those described by Bergey's Manual of Systematic Bacteriology (Garrity et al 2005). The vegetative cells were gram-negative, non sporulated, straight or slightly curved rods (bacilli or cocobacilli) which are coincided with the proposed morphological specifications for the genus *Stenotrophomonas* sp. Biochemical characterization of the isolates revealed that all of the 4 isolates were catalase positive, amylase negative and

gelatinase negative which are consistent with to those described by Bergey's Manual of Systematic Bacteriology (Garrity et al 2005).

Table 2. Biochemical characteristics of the selected isolates

Isolates code	X.S20	X. E15	X. K12	X. M9
Amylase test	-	-	-	-
Gelatinase liquification test	-	-	-	-
Catalase test	+	+	+	+

3.2 The growth pattern of the selected isolates

Data presented in **Fig 1** reveals the growth pattern of the selected isolates (X.S20, X.E15, X.K12 and X.M9) in the form of microbial optical density as a function of time during 30hr. incubation period at 30ºC. The maximum microbial growth of 0.243, 0.146, 0.277 and 0.264 OD respectively was recorded within all isolates after 15hr. The growth of the tested isolates exhibited increasing in the growth rate, from zero time and no lag phase appeared in a similar manner to those reported by Su et al (2021).

3.3 The growth performance of the tested isolates on different levels of salinity

Data illustrated in **Fig 2** reveals the optical density of the tested isolates grown on Modified Aleksandrov`s medium supplemented with different NaCl concentrations. Generally, all the isolates revealed clear growth in all tested salt concentrations. The isolate X.M9 exhibited the highest growth performance in the form of OD in most salts' concentrations compared to all tested isolates (1.376, 1.326, 1.292, 1.053 and 1.059 OD respectively).

These results revealed new isolates have high resistance to NaCl higher than those reported by Wang et al (2022) who stated the tolerance of *Stenotrophomonas* sp. to NaCl was up to 15% and Suzina et al (2018) who stated the tolerance of *Stenotrophomonas* sp. to NaCl was up to 1.0%. However, Most *S. rhizophila* isolates were able to tolerate NaCl up to 5% (Wolf et al 2002). Tolerance to high salinities is an important factor for selecting isolates as PGPRs for adaptation to the microenvironments of the rhizosphere (Miller and Wood 1996).

3.4 Effect of the different levels of pH on the growth performance of isolates

The Effect of different pH levels from 4 to 10 pH on the growth performance of tested isolates is illustrated in **Fig 3**. Generally, all the isolates revealed clear growth in all pH levels. The isolates (X.E15, X.K12 and X.M9) revealed increasing in the growth by increasing the pH up to 7 and exhibited a decrease in the growth by increasing the pH of the medium from 7 up to 10. The isolate (X.E15) revealed the highest growth in all the pH levels of 2.546,3.168.3.704,4.33,4.013,5.04 and 5.514 OD in respective order. These results revealed that the tested isolates are more resistant to high pH than those reported by Suzina et al (2018) and Wang et al (2022) who stated that *Stenotrophomonas* sp. strains have the capability to grow in a pH range between 5–10 pH and the optimum growth ranges between pH 9-10.

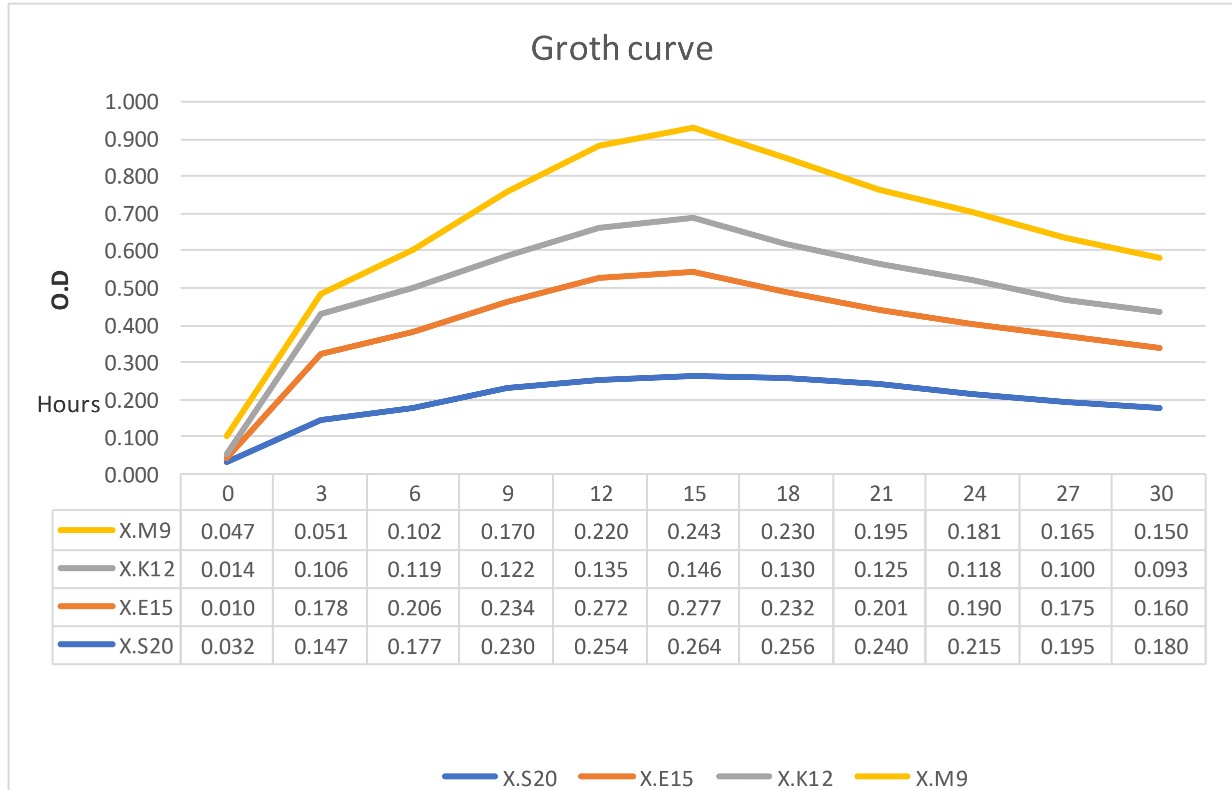
3.5 Effect of different temperatures on the growth performance of the tested isolates

Data presented in **Fig 4** shows the growth performance of the tested isolates subjected to different temperatures. The optimal growth of the tested isolates (X.S20, X.E15 and X.M9) is 30ºC. While the optimum temperature of the isolate (X.K12) is 35ºC. The isolate (X. K12) exhibited the highest resistance to elevated temperatures of 45 and 50ºC of 1.395 and 1.353 OD respectively. While isolate (X.M9) exhibited the highest resistance to low temperatures 10 and 15ºC being 0.917 and 1.354 OD respectively.

In this investigation, the highest growth occurred at a range of 15–45 °C, and the optimum growth at 30 °C which is in line with those reported by Wolf et al (2002), Suzina et al (2018), and Wang et al (2022).

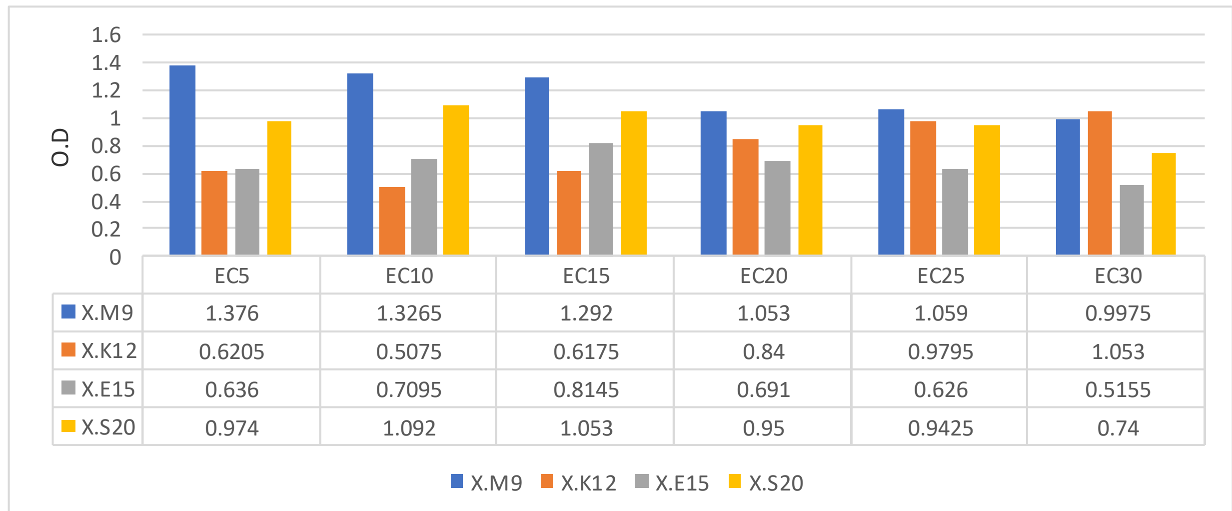
3.6 The efficiency of isolates in producing plant growth promoters (PGPRs)

Fig 5 represents the capability of the selected isolates to produce plant growth regulators (PGPRs). Generally, all tested isolates revealed the capability to produce PGPRs in the form of (IAA, ABA, Ziaten, Kinten and GA3). The isolate (X.S20) recorded the highest ABA, Ziaten and GA3 of 23.59,240.79 and 1.353 mg/100ml respectively. While the isolate (X.E15) recorded the highest Kinten content of 101.05 mg/100ml. While the isolate (X.M9) recorded the highest IAA of 83.73 mg/100ml compared to the tested isolates.



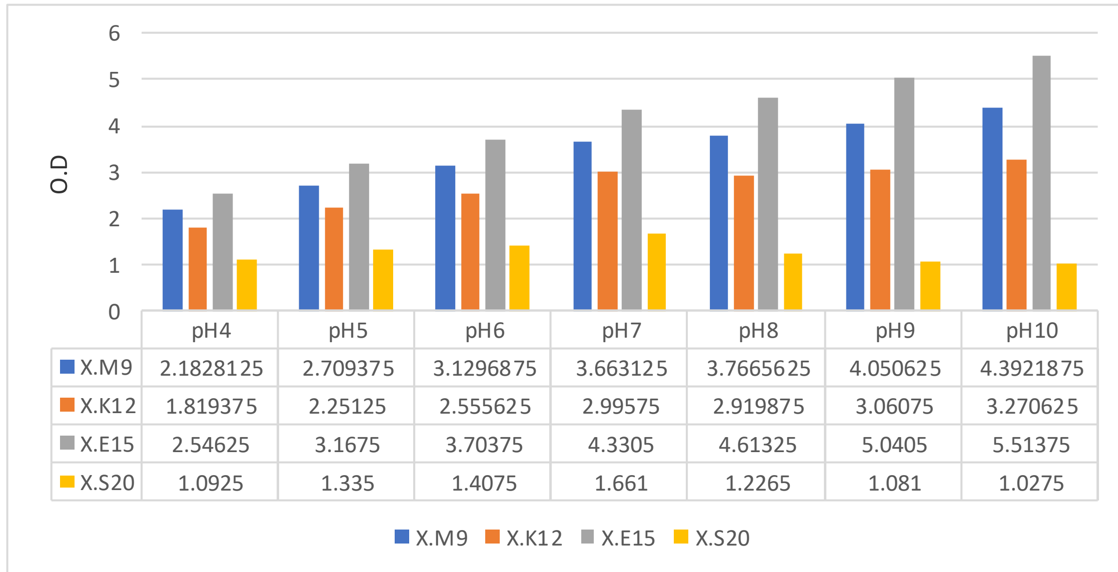
	0	3	6	9	12	15	18	21	24	27	30
X.M9	0.047	0.051	0.102	0.170	0.220	0.243	0.230	0.195	0.181	0.165	0.150
X.K12	0.014	0.106	0.119	0.122	0.135	0.146	0.130	0.125	0.118	0.100	0.093
X.E15	0.010	0.178	0.206	0.234	0.272	0.277	0.232	0.201	0.190	0.175	0.160
X.S20	0.032	0.147	0.177	0.230	0.254	0.264	0.256	0.240	0.215	0.195	0.180

Fig 1. The growth performance of the selected isolates



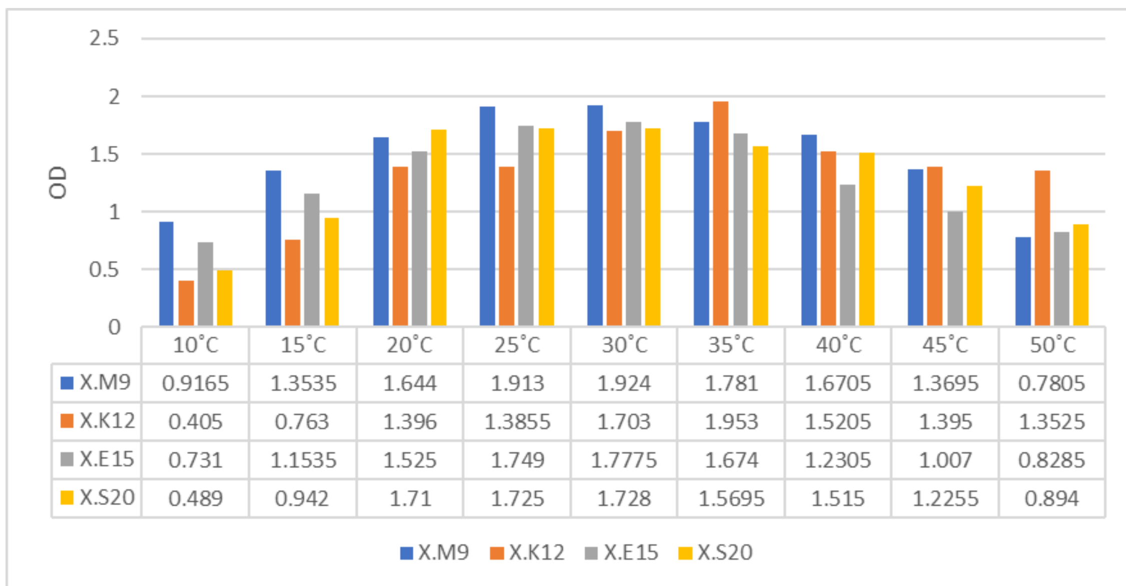
	EC5	EC10	EC15	EC20	EC25	EC30
X.M9	1.376	1.3265	1.292	1.053	1.059	0.9975
X.K12	0.6205	0.5075	0.6175	0.84	0.9795	1.053
X.E15	0.636	0.7095	0.8145	0.691	0.626	0.5155
X.S20	0.974	1.092	1.053	0.95	0.9425	0.74

Fig 2. Effect of different levels of salinity on the growth performance of the isolates



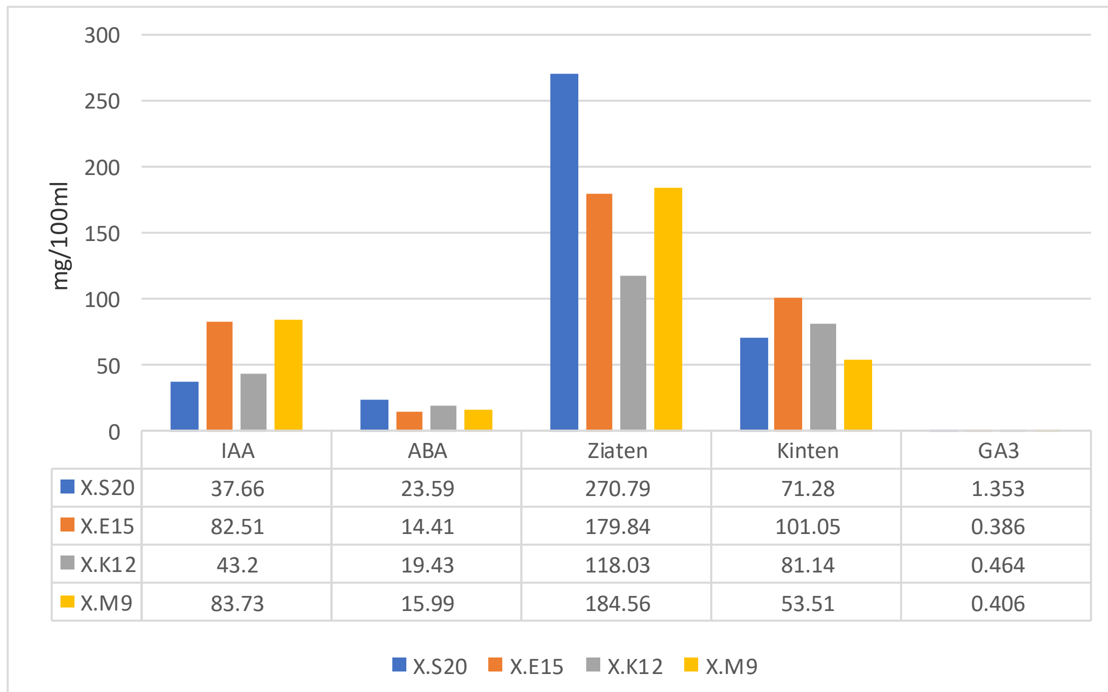
	pH4	pH5	pH6	pH7	pH8	pH9	pH10
X.M9	2.1828125	2.709375	3.1296875	3.663125	3.7665625	4.050625	4.3921875
X.K12	1.819375	2.25125	2.555625	2.99575	2.919875	3.06075	3.270625
X.E15	2.54625	3.1675	3.70375	4.3305	4.61325	5.0405	5.51375
X.S20	1.0925	1.335	1.4075	1.661	1.2265	1.081	1.0275

Fig 3. Effect of different temperatures on the growth performance of the isolates



	10˚C	15˚C	20˚C	25˚C	30˚C	35˚C	40˚C	45˚C	50˚C
X.M9	0.9165	1.3535	1.644	1.913	1.924	1.781	1.6705	1.3695	0.7805
X.K12	0.405	0.763	1.396	1.3855	1.703	1.953	1.5205	1.395	1.3525
X.E15	0.731	1.1535	1.525	1.749	1.7775	1.674	1.2305	1.007	0.8285
X.S20	0.489	0.942	1.71	1.725	1.728	1.5695	1.515	1.2255	0.894

Fig 4. Effect of different levels of pH on the growth performance of the isolates



	IAA	ABA	Ziaten	Kinten	GA3
X.S20	37.66	23.59	270.79	71.28	1.353
X.E15	82.51	14.41	179.84	101.05	0.386
X.K12	43.2	19.43	118.03	81.14	0.464
X.M9	83.73	15.99	184.56	53.51	0.406

Fig 5. Efficiency of isolates in producing plant growth promoters (PGPRs)

Many previous studies reported that endophytic bacteria belonging to the genus *Stenotrophomonas* sp. demonstrated plant growth promotion traits (Silambarasan et al 2020). Peralta et al (2012) and Patel and Saraf (2017) reported the capability of *Stenotrophomonas* sp. to produce Indole-3-acetic acid, Gibberellic acid, trans-Zeatin and Abscisic acid in the media.

3.7 Efficiency of isolates in solubilizing phosphorus and potassium

Data presented in **Fig 6** reveals the ability of the selected isolates to solubilize potassium and phosphorus. All the tested isolates revealed a high capability to solubilize of potassium and phosphorus. The isolate (X.K12) revealed the highest capability of solubilization 1236.25 ppm for potassium and 196.225 ppm for phosphorus.

Many prior studies stated that endophytic *Stenotrophomonas* sp. showed high potentiality for phosphate-potassium solubilizing activity (Xiao et al 2009).

3.8 Identification of the selected isolate using 16S rRNA gene sequencing and analysis of the Genome Sequence

According to the previous data, the isolated (X.M9) was selected for identification as it revealed the highest properties.

The Blast analysis of the 16S rRNA gene for the selected isolate (X.M9) amplified product revealed 96.6% similarity to *Stenotrophomonas rhizophila* strain and the partial 16S rRNA sequence was submitted to the GenBank database (http://www.ncbi.nlm.nih.gov/GenBank) under accession number No OP050187. The phylogenetic tree which was constructed using the MEGA 7 tool and by retrieving the sequences obtained in the BLAST search was reported in **Fig 7.**

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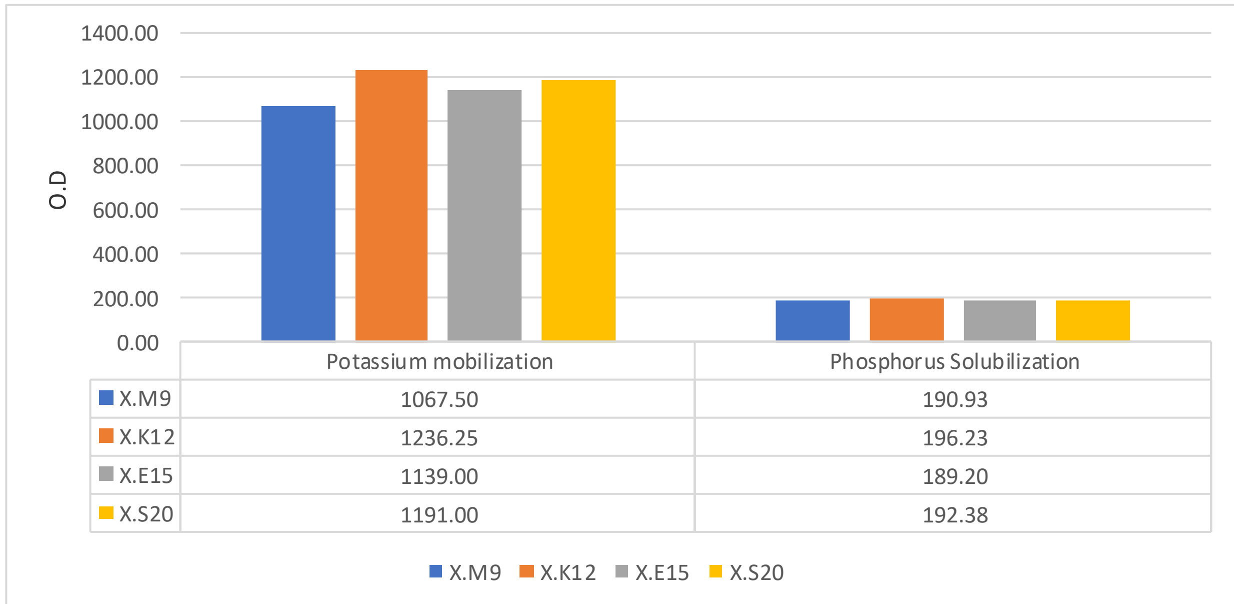

Fig 6. Efficiency of the isolates in solubilizing phosphate and potassium

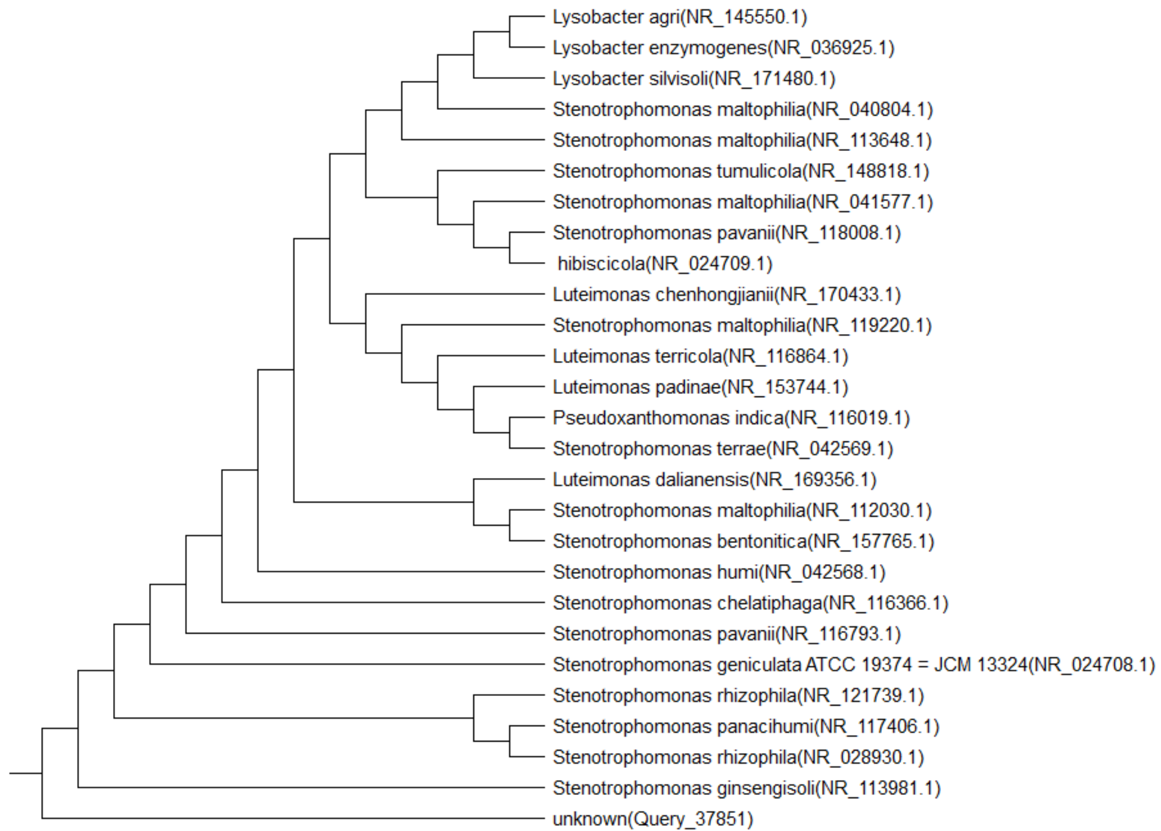

Fig 7. The phylogenetic tree of the *Stenotrophomonas rhizophila* strain

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