Env. Biodiv. Soil Security, **Vol.** 5, pp: 289 - 303(2021)

Environment, Biodiversity & Soil Security

http://jenvbs.journals.ekb.eg/

Activities Profile of Irradiated *Streptomyces Alfalfae* Strain XY25 *in Vitro*

Rashed A. Zaghloul[1], Yehia Galal Galal[2], Hussein A. Abdel Aziz[2], Hany M. Abdel-Rahman[1], Ahmed A. Salem[1], Abeer M. Mousa[2], Susan E Weesa[2]

[1]Microbiology Department, Faculty of Agriculture, Benha University, Egypt
[2]Nuclear Research Centre, Egyptian Atomic Energy Authority, Egypt

Different samples used for *Streptomyces* spp. isolation. Obtained isolates examined for their zinc solubilization efficiency % (ZSE), phosphate solubilization efficiency (PSE), siderophore production, indole acetic acid (IAA), cytokinin, and gibberellin production capacity. The most potent three isolates were genetically identified by 16S rRNA gene sequence analysis. The identified isolates were related to *Streptomyces alfalfae* strain XY25, *Streptomyces litmocidini* strain NRRL B-3635 and *Streptomyces hawaiiensis* strain ISP 5042. The spores of *S. alfalfae*, *S. litmocidini*, and *S. hawaiiensis* were exposed to increasable doses of 60Co ϒ-rays (5, 10, 15, and 20 kGy), according to the climatic changes, the effect of irradiation formed a stress condition, so that examination the effect of irradiation on the most efficacious three strains' activities. Amongst the four radiation doses, e.g., 5 kGy, 10 kGy, 15 kGy and 20kGy, the 15 kGy ϒ-rays show the most efficacious radiation dose on the ZSE, p-solubilizing ratecytokinin and gibberellin, especially for the 1st or 2nd generation activities of *S. alfalfae*. This is a new study that uses high radiation dose treatments to enhance the activity of *S. alfalfae*, *S. litmocidini* and *S. hawaiiensis*. Because as far as our knowledge, this is the first report on their activity.

Keywords: Plant growth-promoting (PGP); 16S rRNA gene sequence analysis; *Streptomyces alfalfae* strain XY25; *Streptomyces litmocidini* strain NRRL B-3635; *Streptomyces hawaiiensis* strain ISP 5042

1. Introduction

The members of the *Streptomycetes* family show a wealthy rate of the soil microflora. They are colonizers of plant roots, and they maintain unfavorable growth conditions by forming spores **(Barka *et al.*, 2016)**. The percentage of *Streptomycetes* accounts for 40% of soil bacteria **(Boone *et al.*, 2001)**. *Streptomyces* species have been isolated from various habitats, including insects, plants, freshwater, marine invertebrates, and marine sediments. The primary source of their isolation is soil **(Kanchanasin *et al.*, 2017)**. Members of the genus *Streptomyces* are described as filamentous, aerobic, and gram-positive bacteria with high GC DNA content, complex morphological differentiation, and immeasurable secondary metabolism **(Barka *et al.*, 2016)**.

In addition to being the most abundant and possibly the most important producers of biocontrol agents or bioactive compounds (such as extracellular enzymes and antibiotics), *Streptomyces* has recently been recognized as a biofertilizer. Thus, these genera show great potential for strengthening agriculture in the future. They directly promote nitrogen fixation,

*Corresponding author e-mail: weesasusane@yahoo.com
Received: 03/11/2021; Accepted: 21/12/2021
DOI: 10.21608/JENVBS.2021.104220.1151

plant growth via phytohormones (e.g., cytokinins), auxins, and gibberellins (Gas) production, and suppress plant stress by 1-aminocyclopropane-1-carboxylate (ACC) deaminase production. Also, produced siderophores scavenge ferric iron from the environment **(Verma *et al.*, 2011; Sadeghi *et al.*, 2012; Kunova *et al.*, 2016)**. The term biofertilizer or plant growth-promoting bacteria (PGPB) includes a wide range of soil microorganisms, in particular they have a variety of activities, such as atmospheric nitrogen fixation, solubilizing phosphate, releasing potassium, producing plant hormones and promoting plant growth **(Afify *et al.*, 2018, and Abdel-Rahman *et al.*, 2021).**

Westerhof *et al.* (2004) studied the effect of *Streptomyces griseus* as PGPB on the seeds of oats, carrots, and wheat. Among them, its influence depended on the production of streptomycin or streptomycin-like substances. The average grain yield, dry leaf weight, the number of tillers, and advanced head merger for oats and wheat were maximized. In the case of carrots, the marketable yield was increased.

Olanrewaju and Babalola (2019) summarized the plant growth-promoting mechanisms of *Streptomyces*. They can change the pH of the environment, produce secondary metabolites and volatile organic compounds that can regulate environmental conditions by signaling. These signals can regulate the expression of genes in the surrounding microflora. They act as activators or inhibitors (antifungal and antibacterial agents), especially when used with trimethylamine. **Sathya *et al.* (2017)** and **Abdel-Gawad and El-Howeity (2019)** investigated agricultural strategies for actinomycetes that promote plant growth and grain legumes. Actinomycetes include bacterial diversity, various enzymatic activities, plant growth induction, defense pathways, stress management strategies, and effective control of grain legume pests and pathogens while increasing their yield.

Streptomyces species, especially endophytes, are used in several cropping systems as bio-fertilizers and bio-control agents for sustainable agriculture. This activity is regulated by produced IAA, soluble P, and siderophores. These active agents promote root and shoot growth, seed germination and develop resistance against pathogens through antifungal and antibacterial activities **(Verma *et al.*, 2011; Sousa and Olivares, 2016; Viaene *et al.*, 2016; Vurukonda *et al.*, 2018)**.

Gamma rays are short-wavelength electromagnetic radiation, usually shorter than a few tenths of an angstrom (10^{-10} meters), and have the highest energy. It is produced by the decay of certain subatomic particles during the disintegration of radioactive atomic nuclei. The energy of gamma radiation is between several keV and several MeV (the energy of ɣ-rays photons is greater than the energy of tens of thousands of electron volts (eV)). Cobalt 60 (^{60}Co) used for irradiation of cultural heritage artifacts emits 1.17 and 1.33 MeV gamma radiation **(IAEA, 2017)**.

Gamma irradiation treatments in most *Streptomyces* species are used as an enhancement tool to activate the antimicrobial activities or for more efficient agro-industrial compound production **(Moussa *et al.*, 2005; Khaliq *et al.* 2009; Lazim *et al.*, 2010)** and further optimization of antibiotic production **(Petkovie *et al.* 2006)**. In comparison, **Sakr *et al.* (2013)** used gamma radiation as a sterilization treatment to eliminate the contamination of different *Streptomyces* species on traditional paintings without reducing the tensile strength of the paper or causing color changes. Therefore, the effect of the gamma irradiation treatment depends on the irradiation dose, treatment aim, and the *Streptomyces* species type of media and their carbon, nitrogen, and pH content after their growth during irradiation **(Abdel Aziz *et al.*, 2016)**. The ability of *Streptomyces* species to resist the effects of radiation doses on growing cells and their activity enhancement may be due to evolutionary development caused by environmental stress, mainly driven by drought and high temperature **(Mao *et al.*, 2007)**.

Also, **Abbas *et al.* (2020)** demonstrated the role of gamma radiation as a technique, especially under biotic/ abiotic stresses, not only on the soil microbial communities, but also on improving the yield and quality of plants such as canola.

This work aims to isolate, purify and study some plant growth-promoting activities of *Streptomyces* species. The most efficacious *Streptomyces* spp. was identified genetically. Finally, the resistance and their activity were reevaluated after ^{60}Co gamma radiation was studied *in vitro*.

2. Materials and Methods

Isolation and characterization of *Streptomyces* isolates.

Fresh cow dung samples and different soil samples at a depth of 0 – 30 cm were collected. The samples were air-dried at room temperature and sieved. 10g of the fine particles of the samples were transferred into 100 ml Erlenmeyer flasks containing 90 ml sterilized distilled water. The flasks were then heated at 60ºC in a water bath for 20 minutes and left to cool down and clarify (Abdul Malek *et al*., 2015). 1ml from the flasks were plated onto isolation media (malt extract yeast agar (ISP-2), oatmeal agar (ISP-3), and inorganic salt starch agar (ISP-4) and incubated at 28°C for 14 days (Shirling and Gottlieb, 1969). *Streptomyces*-like growth colonies were selected, identified by both morphological and physiological characteristics according to Shirling and Gottlieb (1969); Kämpfer (2006) and Kämpfer (2012), screened for its profile activities and confirmed by the 16S rRNA gene sequence method.

Determination of some biochemical activities of *Streptomyces* isolates *in Vitro*

Zinc solubilization efficiency (ZSE %)

A modified Bunt and Rovira agar medium (containing 0.1% $ZnCO_3$ as an insoluble source) were used to evaluate the ZSE % of the *Streptomyces* isolates by a triplicate plate assay. The plates were incubated at 28°C for 14 days. After incubation, the clear zone diameter and colony growth diameter were measured. The ZSE % was tested according to the equation (Chaiharn and Lumyong, 2011):

$$\text{Zn Solubilization efficiency (\%)} = \frac{\text{Zn solubilization diameter}}{\text{Diameter of colony growth}} \boldsymbol{X\ 100}$$

Quantitative of rock phosphate solubilization rate

The National Botanical Research Institute's phosphate growth Broth (NBRIP) with 5 g L^{-1} rock phosphate 30% P_2O_5, as a sole phosphate source, was carried out using Erlenmeyer flasks (100 ml), containing 50 ml of broth. Each flask was inoculated with 1 ml of spore suspension with approximately 1-2x10^6 CFU ml^{-1} in a triplicate (Farhat *et al*., 2015). Autoclaved un-inoculated medium was used as a control. The flasks were incubated for 20 days at 28 ºC (Biglari *et al*., 2016). The growth cells of *Streptomyces* isolates were harvested by centrifugation at 10,000 rpm for 10 minutes. The available water-soluble phosphorus in the supernatant was analyzed by the chlorostannous reduced molybdophosphoric acid blue method at a wavelength of 880 nm (Jackson 1973; Ngosong *et al*., 2014).

Calculation of available phosphorus releasing rate

The inorganic P-solubilizing and releasing rate η ($mgL^{-1}h^{-1}$) was calculated using the following formula: $\boldsymbol{\eta = \frac{(Cn - Co)}{T}}$

Where: Co is the initial soluble P concentration in the liquid medium, Cn is the soluble P concentration in the liquid medium after n hours, and T is the incubation time (Cao *et al*., 2018).

Quantitative siderophores estimation

A modified CAS-shuttle assay performed the quantitative estimation of siderophores in the modified ISP-4 supernatant medium (Alexander and Zuberer, 1991). In the modified ISP-4 broth medium, $FeSO_4.7H_2O$ was replaced by 1g L^{-1} yeast extract (Lee *et al*., 2012), then the medium was inoculated with one ml spore suspension in a triplicate and incubated at 28°C for 14 days. After the incubation time, the broth medium was centrifuged at 10,000 rpm for 15 minutes, and the cell-free supernatant was used to detect and estimate siderophores. A 0.5 ml of culture supernatant was mixed with 0.5 ml of CAS reagent, and absorbance was measured at 630 nm. The reference was composed of 0.5 ml of un-inoculated broth and 0.5 ml of CAS reagent. Siderophores content was calculated in an aliquot by using the following formula:

$$\text{siderophores (\%)} = \frac{\mathbf{Ar - AS}}{\mathbf{Ar}} \mathbf{X\ 100}$$

Where; Ar = absorbance of reference at 630 nm (CAS reagent) and

As = absorbance of the sample at 630 nm (Sayyed *et al*., 2005).

Determination of some phytohormones activities of *Streptomyces* isolates

Indole acetic acid (IAA) determination

The production of IAA by the isolates was determined according to Bano and Musarrat's (2003) method. One ml of 1-2 X 10^8 spore suspension inoculum was grown in Tryptone Yeast extract broth (ISP-1), containing 2 mg ml^{-1} Tryptophan as indole acetic acid precursors in a triplicate, then incubated at 28°C for 14 days. After the incubation time, the isolated cells were collected by centrifugation at

10,000 rpm for 15 minutes. 1 ml of the supernatant was mixed with 2 ml of the Salkowski reagent (12g/l $FeCl_3$, 7.9 M H_2SO_4) 1:1 (v/v) and incubated in the dark for 30 minutes (Glickmann and Dessaux, 1995). The pink color indicated IAA production. Optical density was read at 535 nm. The level of IAA production was estimated against the IAA standard (Chaiharn and Lumyong, 2011).

Cytokinin determination

Preparation of cultures for examination

One ml of 1-2 X 10^8 spore suspension inoculum was cultured in 250 ml Erlenmeyer flasks, each flask containing 50 ml of starch casein broth then incubated at 28°C for 14 days (Mansour *et al*., 1994; Patel *et al*., 2016). Cells were harvested by centrifugation at 10,000 rpm for 30 min, and the supernatant was used to analyze cytokinins according to the technique reported by Fletcher *et al*. (1982).

Bioassay of Cytokinins

The estimation is based on the technique of Fletcher and McCullagh (1971). The cytokinin content is determined by colorimetrically at 665 nm. The amount of cytokinin was calculated based on the cytokinin standard curve.

Gibberellin determination

One ml of 1-2 X 10^8 the spore suspension was inoculated in ISP-4 broth and then incubated for 14 days at 28 °C with shaking at 150 rpm. Gibberellins' production potential of isolates was determined by the spectrophotometer using a simplified fluorometric method in a broth after the incubation period with GA3 as a reference (Candau *et al*., 1991; Mali *et al*., 2011). About 0.2 ml aliquot of the culture medium was well mixed with 0.2 ml of ethanol (96%, v/v) and 2 ml of a cooled mixture of equal volumes of sulfuric acid and 96% ethanol. After the mixture was incubated at 48° C for 30 min, the fluorescence emission at 464 nm was measured by a spectrophotometer (Reyes *et al*., 1992).

Effect of Gamma rays on growth and activities of *Streptomyces* isolates

The most effective and active isolates obtained were grown on an ISP-4 agar medium for 15 days until the sporulation stage. The spore count before irradiation was recorded ≈ 1 X 10^6 CFU ml^{-1}. The agar plate method was used for the irradiation treatment (Shirling and Gottlieb, 1966). Triplicates of plates were then exposed to increased doses of ^{60}Co ɣ-rays (e.g., 5, 10, 15, and 20 Kgy) in the irradiation chamber of a gamma cell 220 equipment, Cyclotron project, Nuclear Research Center, Egyptian Atomic Energy Authority. The source of irradiation was cobalt-60 (^{60}Co MC20, Russia). The average dose rate of this gamma radiation source was 600.515 Gy/h at the time of the experiment (Choi *et al*., 2012; Sakr *et al*., 2013).

Molecular characterization of efficient *Streptomyces* isolates

The extracted DNA of the most efficacious *Streptomyces* isolates was identified by PCR, using 16S rRNA gene sequences, at Sigma Scientific Services Co., Giza, Egypt. The universal primers were F- (5'-AGA GTT TGA TCC TGG CTC AG-3'), R- (5'- GGT TAC CTT GTT ACG ACT T-3'). An automated thermal cycler (OmniGene thermocycler from Hybaid Ltd., Teddington, United Kingdom) was used. Amplification was performed with the following conditions: initial denaturation at 94 ºC for 6 min followed by 35 cycles at 94 ºC for 45s, 72 ºC for 1 min, and 5 min final extension at 72 ºC. The sequencing data was analyzed by OligoAnalyzer 3.1 (Integrated DNA Technologies). Related sequences were identified using BLASTN programs in the GenBank (on the website: http://blast.ncbi.nlm.nih.gov/Blast.cgi). Subsequently, the sequences were aligned online using BLASTN, version 2.5.1+ (on the website: http//blast.ncbi.nlm.nih.gov/Blast). The phylogenetic tree was built using the website: http://www.phylogeny.fr/simple_phylogeny.cgi, based on the Neighbor-Joining method.

3. Results and discussion

Isolation and characterization of *Streptomyces* isolates

Among the 250 isolates of *Streptomyces* obtained from different soil types and different governorates in Egypt, only 40 isolates had different growth rates, colors, and growth colony sizes, as shown in **Table 1**.

Table1. Numbers of *Streptomyces* isolates from different locations in Egypt

Sample number	Type of Sample	Collected from	Number of isolates	Aerial mass color of growth on ISP-4 medium	Colony Diameter*	Isolate code
1	Soil	Cold lakelet bottom, Siwa Oasis	3	grey and pinkish	Small and Medium	1A, 1B, 1C
2	Soil	Wahed well, Siwa Oasis	22	White, grey, Whitish grey, deep grey, pale yellow, and yellow	Most colonies were small, to medium and only one was large	2A,2B, 2C, 2D, 2E, 2F, 2G, 2H, 2I, 2J, 2K, 2L, 2M, 2N, 2O, 2P, 2Q, 2R, 2S, 2T, 2U, 2V
3	Clay bean's rhizosphere	Qalyubia Governorate	2	grey-green	Medium	3A, 3B
4	Clay pea's rhizosphere	Sharqiya Governorate	5	White	Small to medium	4A, 4B, 4C, 4D, 4E
5	Clay bean's rhizosphere	Monifia Governorate	4	yellowish brown and grey	Medium	5A, 5B, 5C, 5D
6	Cow dung	Sharqiya Governorate	4	Orange yellow, light purple and violet	Small to medium	6A, 6B, 6C, 6D

*Small less than 0.5 cm diameter, medium; between 0.5 to 1 cm diameter and Larger than 1 cm diameter.

Biochemical and phytohormones activity of *Streptomyces* isolates before the irradiation

The activities of ZSE%, P-solubilization, siderophores production%, IAA, cytokinin, and gibberellin of the obtained 40 isolates were studied, as shown in **Table 2**. The results of the ZSE % showed that 12 isolates grew slowly on the medium used without a clear zone around (2A, 2O, 2Q, 2R, 2U, 4A, 4B, 4C, 5A, 6A, 6C, and 6D). Also, about six isolates grew effectively on the medium used (1B, 2B, 2E, 2M, 2N, and 5B). However, there was no clear zinc solubilization area around these isolates. An observed transparent area surrounded the 22 isolates. The highest zinc solubilization efficiency (ZSE %) of isolate 2L was 203.2 % and isolates 2S, 2C, 2D, 4E, 2V and 3A were 183.8%, 181.5%, 178.6%, 162.96%, 157.1 % and 152.4 %, respectively. The lowest values of ZSE (%) were 120.5%, 116.0%, and 114.1% of isolates 2P, 5C and 2H, respectively. Although the isolates grew on the medium, they did not solubilize $ZnCO_3$, leading to negative results. **El Sayed *et al.* (2011)** studied the relationship between *Streptomyces* spp. and zinc concentration, showing that *Streptomyces* spp. has zinc solubilization ability in the medium and are the most tolerant species to high zinc concentrations. For example, *S. aureofacienes* can absorb zinc with a concentration of up to 734.8 µg g^{-1} biomass. **Choi *et al.* (2017)** showed that high zinc conditions directly impact the transcription of the *zitB* gene that controls the utilization of Zn by *Streptomyces*. **Verma *et al.* (2021)** and **Kushwaha *et al.* (2021)** discussed the role of microorganisms during post-harvest processing. They found that microorganisms mediate the modeling of micronutrient transporters and the disintegration of micronutrients from crops through one or more previous mechanisms.

The phosphate solubility and release rate results show variation in the solubilization capacity. Consequently, the isolates were divided into two groups. The first group contains 20 isolates and shows the lowest phosphate solubility, ranging from 0.06 (isolates 2L and 2K) to 0.98 (isolate 2J). The second group contains 20 other phosphate soluble isolates, ranging from 1.01 with isolate 2C and maximum 7.34 mg L^{-1} h^{-1} with isolate 2F, 6.97 mg L^{-1} h^{-1} with isolate 4B, 5.45 mg L^{-1} h^{-1} with isolate 6D, 5.43 mg L^{-1} h^{-1} with isolate 4E and 4.73 mg L^{-1} h^{-1} with isolate 6A. According to **Farhat *et al.* (2015)**, the main explanation for the ability of certain *Streptomyces* species to solubilize rock phosphate is due to their ability to secrete gluconic acid (GA) and the subsequent drop in pH. In addition, **Faried *et al.* (2018)** and **Chaiharn *et al*. (2018)** isolated species of *Streptomyces* genus capable of solubilizing inorganic phosphate on pikovskaya's modified agar medium. The mechanism of solubilization by organic acids is produced from the fermentation of sugars.

On the other hand, ectomycorrhizal (ECM) fungi can also provide the available phosphorous indirectly. It absorbs iron and prevents the formation of iron and phosphorous complexes in the soil, therefore both nutrients would be available to plant in soil.

Table 2. The profile activities of the forty *Streptomyces* isolates.

Isolates code	zinc solubilization efficiency (ZSE)%	P-solubilizing releasing rate (mg $L^{-1}h^{-1}$)	Siderophores%	Indole acetic acid (IAA) µg ml^{-1}	Cytokinin by µg ml^{-1}	Gibberellins by µg ml^{-1}
1A	115.7	0.85	68	1.598	2.519	ND
1B	ND	0.95	77.3	1.176	2.734	ND
1C	144.4	1.95	74	1.266	2.001	ND
2A	ND	0.47	77.5	1.902	2.582	ND
2B	ND	0.35	52.2	1.06	2.064	ND
2C	181.5	1.01	74.8	1.566	2.09	ND
2D	178.6	0.66	60.4	1.227	2.325	ND
2E	ND	1.70	68.1	1.565	1.702	ND
2F	137.5	7.34	81.1	2.166	3.441	63.538
2G	123.5	1.42	70.2	1.622	2.191	ND
2H	114.1	1.42	68.5	1.158	1.782	ND
2I	141.9	0.54	74.6	1.307	1.277	ND
2J	147.5	0.98	66	1.163	2.216	ND
2K	136.2	0.06	77.5	1.558	1.521	47.023
2L	203.2	0.06	55	1.5	2.174	ND
2M	ND	0.22	77.2	1.16	2.149	46.361
2N	ND	0.63	64.3	1.323	2.241	47.098
2O	ND	2.14	75.5	1.166	1.555	48.311
2P	120.5	0.35	69.1	1.293	1.185	ND
2Q	ND	2.81	67.6	1.037	1.976	ND
2R	ND	0.13	46	1.189	2.262	ND
2S	183.8	1.07	71.2	1.711	2.081	47.932
2T	125.0	0.16	70.2	1.451	1.176	ND
2U	ND	0.57	72.5	1.105	1.841	54.77
2V	157.1	1.36	64.9	1.479	1.963	ND
3A	152.4	0.22	76.7	1.242	0.991	48.689
3B	128.6	0.95	69.1	1.292	2.136	ND
4A	ND	1.39	30.9	1.507	1.745	48.689
4B	ND	6.97	76	1.273	2.031	ND
4C	ND	2.52	75.7	1.192	2.384	ND
4D	122.2	3.31	65	1.359	2.729	ND
4E	162.96	5.43	73.3	1.83	2.978	43.917
5A	ND	0.13	72.6	1.262	0.911	62.598
5B	ND	1.80	72.3	1.151	1.698	ND
5C	116.0	0.79	64.3	1.281	2.317	ND
5D	127.8	0.43	65.9	1.134	2.485	ND
6A	ND	4.73	76.7	1.188	1.812	ND
6B	149.21	2.36	80	1.92	2.854	45.886
6C	ND	1.13	82.2	1.143	2.443	ND
6D	ND	5.45	88.4	1.099	2.149	ND

ND: Clear zone not detected

This ECM adsorbs iron to prevent iron toxicity, especially in high-concentration Fe soils. These mechanisms include acidification, reduction of ferric to ferrous, and secretion of iron-chelating molecules (such as siderophores and melanin) **(de Souza *et al.*, 2021)**.

The siderophores production % results showed that all 40 *Streptomyces* isolates could grow on broth medium with different production levels, where the maximum activity was 88.4 % with isolate 6D, followed by 82.2%, 81.1%, and 80% with isolates 6C, 2F, and 6B, respectively. The lowest activity of isolate 4A was 30.9 %. In the liquid or solid media, the ability of numerous microorganisms to produce siderophores as secondary metabolites has been fully established. According to **Dimkpa (2016)**, different siderophores classes have been identified, such as catecholate, hydroxamate, and mixed ligand siderophores. The aim of the production is to transfer iron to microorganisms under iron-deficient conditions.

Furthermore, this study also discussed the effect of pH on the type of siderophores produced by *Streptomyces* spp. For example, in acidic soil, the hydroxamate siderophores are mainly produced by fungi and *Streptomyces*, forming ferric complexes. Moreover, both hydroxamate and catecholate siderophores are

mainly produced in neutral to alkaline soils. Similarly, **Kunova *et al.* (2016)** and **Vurukonda *et al.* (2018)** summarized the role of siderophores produced by actinobacteria and other enzymes in nutrient mineralization and biocontrol activities. **Amaresan *et al.* (2018)** pointed out that *Streptomyces* species such as *S. rochei, S. carpinensis*, and *S. thermolilacinus* produce siderophores as potential plant growth promoters and disease suppressors by limiting the iron content of the rhizosphere.

The highest IAA concentration is 2.166 µg ml^{-1} for isolate 2F, 1.92 µg ml^{-1} for isolate 6B, 1.902 µg ml^{-1} for isolate 2A, 1.83 µg ml^{-1} for isolate 4E. In contrast, the lowest IAA concentration is 1.037 µg ml^{-1} for isolate 2Q. The results are consistent with those of **Vijayabharathi *et al.* (2016),** who reported a high production capacity of IAA by endophytic *S. corroborates*, which ranged between 2 and 12 µg ml^{-1}. In the study of **Shutsrirung *et al.* (2013),** researchers reported that the average IAA yields of endophytes' six are as follows: *Nocardia* genera (3.36 µg ml^{-1}), *Nocardiopsis* (140.38 µg ml^{-1}), *Streptomyces* (13.34 µg ml^{-1}), *Spirillospora* (12.55 µg ml^{-1}), *Micromonospora* (6.19 µg ml^{-1}) and *Microbispora* (1.40 µg ml^{-1}). Also, the range of IAA production in *Nocardia* genera was found to be from 1.4 to 140.3 µg ml^{-1}.

Streptomyces isolates were found to potentially produce cytokinins in tremendous amounts except for 5A and 3A isolates that produced cytokinin with concentrations of 0.911 and 0.991 µg ml^{-1}, respectively. The general range of cytokinin secretion is 1.176 to 3.441 µg ml^{-1}. The highest cytokinin production was found in isolate 2F (3.441 µg ml^{-1}), followed by isolate 4E (2.978 µg ml^{-1}). Although the ecological role of bacterial cytokinins is still unclear, the study by **Yang *et al.* (2006)** indicated that the presence of cytokinins in the medium of *Streptomyces* improved its antibiotic production and increased the inhibition capacity against plant pathogens. Likewise, the study by **Olanrewaju et al. (2017)** and **Olanrewaju and Babalola (2019)** proved that the production of antibiotics, volatile compounds, auxins, gibberellins, cytokinins, ethylene, and enzymes are parts of the mechanism of action of *Streptomyces* species in the rhizosphere of plants.

The gibberellin results revealed that of the 40 *Streptomyces* isolates, only 12 isolates could produce gibberellins, and the remaining 28 isolates have not been detected to produce. The highest gibberellin content recorded in isolate 2F was 63.538 µg ml^{-1}, followed by isolates 5A and 2U, whose concentrations were 62.598 and 54.770, µg ml^{-1} respectively. On the other hand, isolate 4E had the lowest gibberellin production (43.917 µg ml^{-1}). These results are consistent with the results of **Patil and Patil (2012)** and **Gusmiaty *et al.* (2019),** who showed that the gibberellin production capacity of some *Streptomyces* species in the growth medium does not have a significant average difference between the maximum and minimum values.

Selecting the most effective isolates

The high or moderate biochemical and phytohormone production activities of *Streptomyces* isolates indicated that only isolates 2F, 4E, and 6B were associated with the highest outcomes **(Table 3)**. The isolate 2F has a superior P-solubilizing capacity (7.34 mg $L^{-1}h^{-1}$), siderophores production (81.1%), IAA, cytokinin, and gibberellin production of 2.166, 3.441, and 63.538 µg ml^{-1}, respectively. At the same time, the isolate 4E can solubilize zinc and phosphate at a moderate value. The isolate 6B has a good production activity of IAA, gibberellin, and cytokinin.

Finally, the most effective isolates in studying activities are 2F, 4E, and 6B isolates used for genetic identification.

Table 3. Biochemical and phytohormones activity of the selected three *Streptomyces* isolates

Isolates code	Biochemical Activities			Phytohormones (µg ml^{-1})		
	ZSE (%)	P-solubilizing releasing rate (mg $L^{-1}h^{-1}$)	Siderophores (%)	IAA	cytokinin	gibberellin
2F	137.5	7.34	81.1	2.166	3.441	63.538
4E	162.96	5.43	73.3	1.83	2.978	43.917
6B	149.21	2.36	80	1.92	2.854	45.886

Molecular identification of *Streptomyces* isolates

The Molecular characteristics of the three most effective isolates were investigated by the 16S rRNA gene sequence analysis to ascertain their taxonomic positions. The sequences were analyzed by OligoAnalyzer 3.1 and identified using BLASTN programs in the GenBank (on the website: http://blast.ncbi.nlm.nih.gov/Blast.cgi). The phyloge-

netic tree was constructed based on the neighborhood-joining method and built using the website http://www.phylogeny.fr/simple_phylogeny.cgi.

The 16S rRNA gene sequence of our *Streptomyces* species was obtained by BLASTN search; however, the strain of *Streptomyces* was selected based on high identity (%) with a good E value. The result shows that query sequences of 2F isolate were the best pairwise aligned with 16S rRNA gene sequence of *Streptomyces alfalfae* strain XY25 16S ribosomal RNA gene, partial sequence NR 147713.1, sequence similarity (86.16%), and E value of 0 **(Fig. 1)**. The query sequences of 4E isolate were the best pairwise aligned with 16S rRNA gene sequence of *Streptomyces litmocidini* strain NRRL B-3635 16S ribosomal RNA, partial sequence NR 116096.1, sequence similarity (98.54%), and E value of 0 **(Fig. 2)**. The query sequences of 6B isolate were best pairwise aligned with 16S rRNA gene sequence of *Streptomyces hawaiiensis* strain ISP 5042 16S ribosomal RNA, complete sequence NR 114824.2, sequence similarity (97.66%), and E value of 0 **(Fig. 3)**.

Effect of Gamma rays on biochemical and phytohormones activities of the three *Streptomyces* strains in *Vitro*

The resistance of the three selected *Streptomyces* strains: *S. alfalfae*, *S. hawaiiensis*, and *S. litmocidini* to 5, 10, 15, and 20 KGy ɣ-rays have been investigated. All biochemical activities such as zinc solubilization efficiency (%) (ZSE %), rock phosphate solubilizing efficiency (%), releasing rate, and quantitative estimation of siderophores were assessed. The phytohormone production activities (IAA, cytokinin, and gibberellin) were estimated for each irradiation dose of the three strains of first and second generations *Streptomyces* **(Table 4)**.

Regarding ZSE%, *S. alfalfae* gives the highest value at all irradiation doses during the first and second generations. Also, compared to native strain, the ZSE% value of *S. alfalfae* was increased 1.94 times for 15KGy treatment in both first and second generations. As to *S. litmocidini*, ZSE% shows different effects by irradiation doses. In the first generation, the ZSE% increased by 1.84 times for 10 KGy and 1.9 times at 20 KGy treatments. However, it decreased in the second generation.

On the other hand, *S. hawaiiensis* ZSE% was inhibited by 5KGy treatment in the first and second generations. Although the ZSE% value of the first generation was maximized by 1.63 times under 20 KGy treatment, other doses showed stable activity within average in the native strain. **El Sayed *et al.* (2011)** could explain the ability of some *Streptomyces* species to solubilize zinc complexes in the plate diffusion method, which is due to the resistance of zinc as heavy metal and their ability to be uptaken into the viable cells. **Kallifidas (2010)** and **Choi *et al.* (2017)** mentioned that *Streptomyces* utilize Zn dominantly according to genetic status, especially direct transcription of the *zitB* gene by controlling gene expression at Zn high concentrations.

Compared with the native strains, the phosphate solubilization rates of the first and second generations of the three *Streptomyces* strains were significantly reduced after irradiation with two exceptions of the *S. alfalfae* first generation for 15 KGy and *S. litmocidini* for 5 KGy. After irradiation, the highest P-solubilization rate of *S. alfalfae* under 15 KGy was 11.81 mg $L^{-1}h^{-1}$ in the 1st generation, and it was severely decreased by 294.6% in the 2nd generation. Also, 5 KGy maximized the P-solubilization activity of *S. litmocidini* by 23% in the 1st generation. However, it then decreased by 59.9% in the 2nd generation with the same treatment. According to **Aallam *et al.* (2021)**, actinomycetes species are microorganisms that convert insoluble phosphate into a soluble form to be accessible to plants. Likewise, it might be considered a novel kind of fertilizer beneficial for plant nutrition and more environmentally friendly than chemical fertilizers in current use. For example, **Chouyia *et al.* (2020)** found that *Streptomyces roseocinereus* MS1B15 can increase soluble phosphate concentration in the liquid medium as the pH value decreases. The siderophores % production was decreased by all irradiation treatments for the first and second generations of *S. hawaiiensis* and *S. litmocidini* strains.

However, *S. hawaiiensis* with 5KGy for the second generation showed some stability. On the other hand, an increase in siderophores production was observed in the *S. alfalfae* first generation after 5KGy and 10KGy irradiation to be 97.6 % and 83.2%, respectively. Nevertheless, it decreased to around 72% for the 2nd generation with the 10KGy and 15KGy. Siderophores and other actinobacteria enzymes function in nutrient mineralization and biocontrol activities **(Vurukonda *et al.*, 2018)**. Therefore, the effect of ɣ-rays on the production of siderophores depends on the microorganism species, the type of medium,

and its carbon, nitrogen, and pH content after the growth during the irradiation treatment **(Abdel Aziz *et al*. 2016)**.

Table 4. Effect of Gamma rays on biochemical and phytohormones activities of the three *Streptomyces* strains in *vitro*.

The activities	Native Strain XY25	Irradiated *S. alfalfae* strain XY25							
		First-generation				Second generation			
		5KGy	10KGy	15KGy	20KGy	5KGy	10KGy	15KGy	20KGy
ZSE %	137.5	179.4d	173.2e	266.7a	138.6f	184.4c	181.1c	266.7a	211.1b
p-solubilizing rate ($mgL^{-1}h^{-1}$)	7.3	1.08n	6.14g	11.81a	1.80mn	0.21n	1.10fg	1.86b	0.43klm
Siderophores %	81.1b	97.6a	83.2b	37d	2.0f	70.9c	72.4c	72.3c	32.4e
IAA (µg ml^{-1})	2.2	1.83f	3.10b	2.3c	3.15b	2.24d	1.72h	1.51j	1.63i
Cytokinin (µg ml^{-1})	3.4	2.10g	2.64e	4.96a	2.43f	2.43f	2.64e	4.96a	3.44b
Gibberellin (µg ml^{-1})	63.5	11.69n	28.93l	50.38e	53.48d	68.96b	55.03c	73.82a	73.49a
		Irradiated *S. litmocidini* strain NRRL B-3635							
ZSE %	162.96d	0.0f	300.0b	0.0f	309.1a	0.0f	144.4e	0.0f	166.7c
p-solubilizing rate ($mgL^{-1}h^{-1}$)	2.43	2.99de	1.64hi	0.75lm	1.25jk	1.52c	1.23ef	0.47kl	0.82h
Siderophores %	73.3a	42.7d	14.9f	48.9c	39.2e	48.3c	56.7b	56.2b	56.4b
IAA (µg ml^{-1})	1.8	1.78g	3.58a	1.88e	1.36l	1.11o	0.95r	1.03p	1.04o
Cytokinin (µg ml^{-1})	2.98	1.16f	1.35de	1.29e	1.37d	1.48c	1.37d	1.32de	1.54c
Gibberellin (µg ml^{-1})	43.9	10.66o	24.75m	44.98gh	32.63j	42.1i	42.33i	28.69l	45.43gh
		Irradiated *S. hawaiiensis* strain ISP 5042							
ZSE %	149.21c	0.0g	147.4cd	111.0f	242.86a	0.0g	155.6b	141.7e	144.4d
p-solubilizing rate ($mgL^{-1}h^{-1}$)	2.36	0.30lm	0.85fg	0.63h	1.04cd	0.62ij	1.15fg	1.04g	1.54c
Siderophores %	80.0a	6.6g	4.2h	52.4d	43.3e	79.3a	40.1f	56.0c	64.4b
IAA (µg ml^{-1})	1.92	1.50k	1.613ij	1.071p	1.29m	0.97q	0.87s	1.16n	1.05o
Cytokinin (µg ml^{-1})	2.85	1.55f	1.53f	1.97c	1.42g	1.42g	1.53f	1.97c	1.79de
Gibberellin (µg ml^{-1})	45.89	42.71i	43.61h	49.75f	53.01d	35.13i	30.74k	50.43e	46.871g

A column followed by the same letters is not significantly different at p= 0.05 when compared by the Duncan test.

The effect of the irradiation doses on IAA production (µg ml^{-1}) was assessed on three selected *Streptomyces* strains. The strain most affected by irradiation is *S. hawaiiensis*. In the 1st generation, IAA production decline ranged from 16 to 44%. However, in the 2nd generation, the decline ranged from 39.6% to 54.7%. On the other hand, the 10KGy for *S. litmocidini* showed duplication of IAA production (3.580 µg ml^{-1}). In 10, 15, and 20KGy for *S. alfalfae*, the production was increased by 43.21%, 6%m, and 45.2%, respectively, compared with the native strains. The IAA production of the 2nd generation showed a significant decrease. However, for *S. alfalfae* with 5KGy treatment, the production increased by 1.02 times. The ability of *Streptomyces* species to resist the effects of radiation doses on growing cells and their activity enhancement may be due to evolutionary development caused by environmental stress such as drought and high temperature **(Mao *et al*., 2007)**. Many studies have been conducted on the IAA production capacity of several *Streptomyces* species such as *S. olivaceoviridis, S. rimosus, S. rochei, S. griseoviridis*, and *S. lydi* **(El-Tarabily, 2008** and **Anwar *et al*., 2016)**. In fact, most actinobacteria produce IAA as secondary metabolites and sporulation substances, which increase the number of adventitious roots and root exudates is in the rhizosphere **(Glick, 2012, Duca *et al*., 2014**, **Nafis *et al*., 2019)**.

The ability of three strains of *Streptomyces* to produce cytokinin is summarized as follows: after 15KG irradiation, the cytokinin production of *S. alfalfae* increased by 44.14% (from 3.441 to 4.960 µg ml^{-1}) in the first and second generations. After the same strain was treated with 20KG, the production of cytokinin at the first generation decreased. However, the native production value was restored at the second generation. Although *S. hawaiiensis* and *S. litmocidini* strains are severely negatively affected by all radiation doses, their activity is lower than the native strain. Cytokinin production in the *Streptomyces* genus was affected directly by ɣ-rays treatment because the production occurs during the hyphal extensions. **Sousa and Olivares (2016)** studied the genes controlling cytokinin production, cell membrane proteins, and septal peptidoglycan in *Streptomyces* species. It was found that the direct effect of irradiation treatment on the *Streptomyces* division, growth, hyphal division involves the extension of the tip and sub-apical branches of the hyphae.

The radiation doses' data showed an adverse effect on the gibberellin production capacity of the 3 *Streptomyces* strains. Therefore, the increasing rate shows low values ranging from 3.5% to 16.25%, in general. The optimal gibberellin production is associated with 5, 15, and 20 KGy treatment of *S. alfalfae* 2nd generation, 15KGy treatment of *S. litmocidini*, and *S. hawaiiensis* 1st generations, 20KGy treatment of *S. litmocidini* 2nd generation, and 15KGy treatment of *S. hawaiiensis.* Compared with the native strain, the maximum positive effect of irradiation on gibberellin production was observed in the 2nd generation of *S. alfalfae* with 15KGy and 20Kgy treatments. These differences in the effect of irradiation on the gibberellins' productivity in microorganisms can be explained by several factors: radiation exposure rate and time, direct radiation exposure, and the resistance of the microorganism, which depend on the type of microorganism and the temperature **(Silva Aquino 2012)**.

Eventually, it can be concluded that the *S. alfalfae* strain XY25 shows a decent phytohormones production level, such as native growth and irradiated strain, besides its resistance for radiation, where it can grow after irradiation and showed stability or increasing in its biological activities. The irradiation dose of 15KGy significantly improved the activity of the first generation and sometimes the second generation. Some studies explain the mode of action of ɣ rays. The resistance of *Streptomyces alfalfae* strain XY25 to high radiation doses is due to its ability to grow in the presence of 0-10% NaCl (**She *et al.*, 2016)**. For example, in *S. albaduncus* and *S. erythogresius,* ɣ-irradiation treatment increases cellular RNA, some doses increase cellular DNA, and others reduce cellular DNA **(Moussa *et al.*, 2005)**. **El Shobaky *et al.* (2014)** and **Ibrahim *et al.* (2017)** studied the effects of higher doses of radiation on enhancing antimicrobial activity, pigmentation, melanin production, utilization of carbon sources in both first and second generations. The impact of such high doses was shown to be positive effects. As well, *Streptomyces* spp. exhibits a high degree of genetic stability to gamma rays. However, more studies are needed to understand the behavior of *Streptomyces* and the effect of ɣ rays on it.

In conclusion, the *Streptomyces* species can be considered as plant growth-promoting bacteria. They can effectively solubilize zinc and rock phosphate and have siderophores, IAA, cytokinin, and gibberellin production capacity at high levels. The identification results showed that the strains obtained were: *Streptomyces alfalfae* strain XY25, *Streptomyces litmocidini* strain NRRL B-3635 and *Streptomyces hawaiiensis* strain ISP 5042. The first obtained strain, *Streptomyces alfalfae* strain XY25, was found to have the highest P-solubilization efficiency, siderophores production, IAA, cytokinin, and gibberellin production. In comparison, *Streptomyces litmocidini* strain NRRL B-3635 was found to have the highest ZSE% and high production of other PGP parameters. Finally, *Streptomyces hawaiiensis* strain ISP 5042 showed moderate PGP activities. The high-energy Cobalt 60 (^{60}Co) electromagnetic ɣ-rays on the strains spores made the *S. alfalfae* strain XY25 more resistant to the irradiation treatments for first and second generations than the other two strains. Also, *S. litmocidini* strain NRRL B-3635 showed resistance to low irradiation doses for the first generation. The sensitive strain to irradiation is *S. hawaiiensis* strain ISP 5042. Finally, the *S. alfalfae* strain XY25, either irradiated or non-irradiated, is recommended as plant growth-promoting biofertilizers.

4. Acknowledgments

Greetings and thanks to the soul of late Dr. Osama A. Abdel Aziz for his guidance and education in all work steps. Thanks to all members of the Soil and Water Research Department, Nuclear Research Center, Egyptian Atomic Energy Authority (EAEA), and Microbiology Department, Faculty of Agriculture, Benha University, for supporting this work.

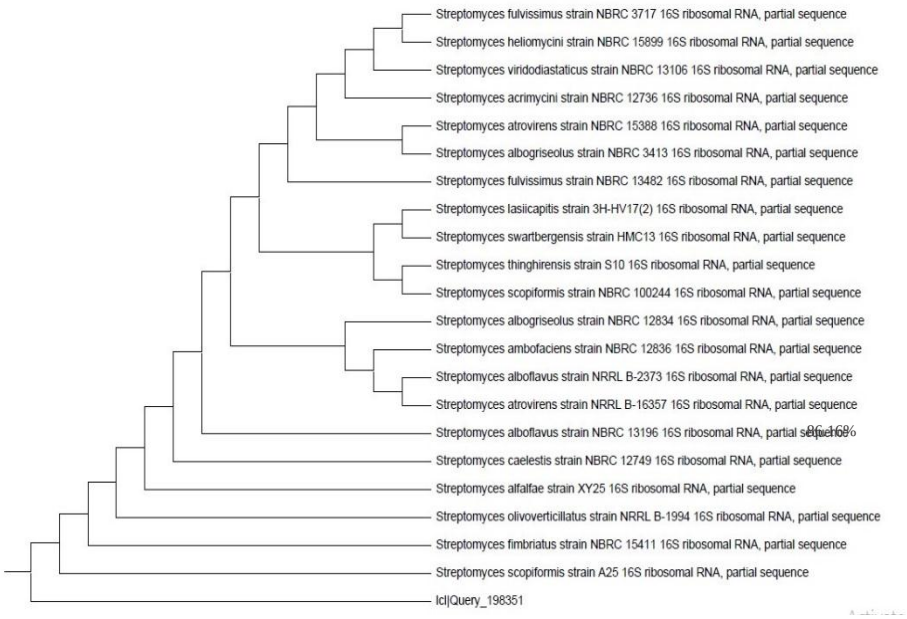

Fig. 1. Un-rooted neighbor-joining phylogenetic tree inferred from 16S rRNA gene sequences, showing the phylogenetic position of *Streptomyces alfalfae* strain XY25 and the type strains of related taxa. The minimum-evolution and maximum-parsimony methods also recovered the branches. Supported values from neighbor-joining bootstrapping are shown down the branches if. 50 %. Bar, 0.002 expected substitutions per site.

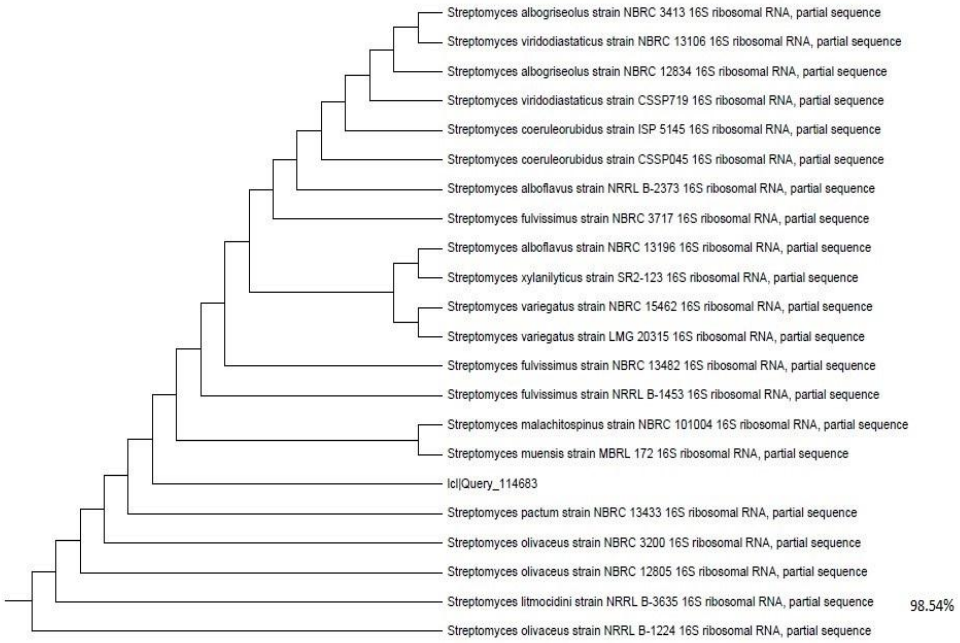

Fig. 2. Un-rooted neighbor-joining phylogenetic tree inferred from 16S rRNA gene sequences, showing the phylogenetic position of *Streptomyces litmocidini* strain NRRL B-3635 and the type strains of related taxa. The minimum-evolution and maximum-parsimony methods also recovered the branches. Supported values from neighbor-joining bootstrapping are shown down the branches if. 50 %. Bar, 0.002 expected substitutions per site.

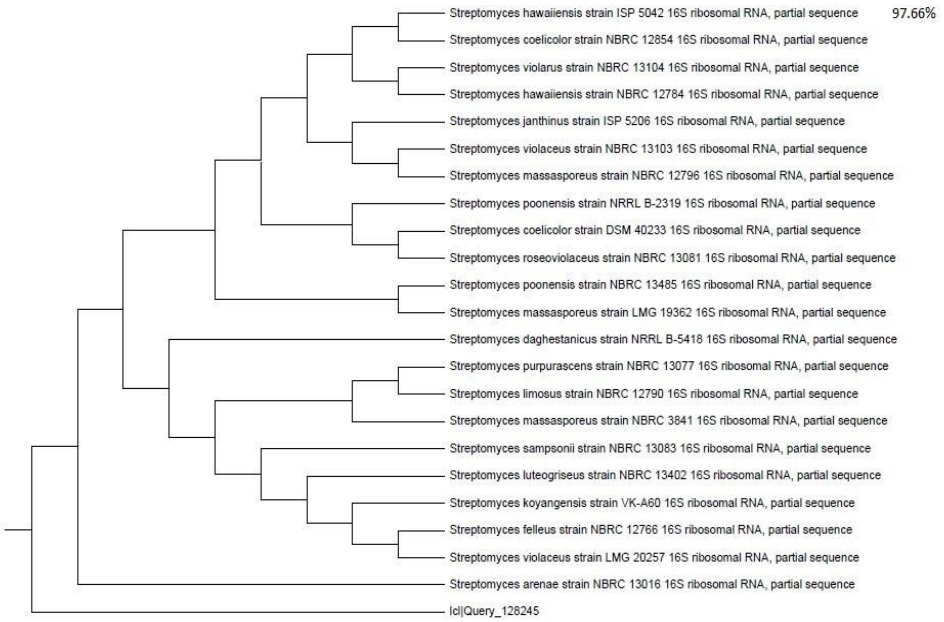

Fig. 3. Un-rooted neighbor-joining phylogenetic tree inferred from 16S rRNA gene sequences, showing the phylogenetic position of Streptomyces hawaiiensis strain ISP 5042 and the type strains of related taxa. The minimum-evolution and maximum-parsimony methods also recovered the branches. Supported values from neighbor-joining bootstrapping are shown down the branches if. 50 %. Bar, 0.002 expected substitutions per site.

5. References

Aallam Y, Dhiba D, Lemriss S, Souiri A, Karray F, El Rasafi T, Saïdi N, Haddioui A, El Kabbaj S, Virolle MJ, Hamdali H (2021). Isolation and characterization of phosphate solubilizing *Streptomyces* sp. endemic from sugar beet fields of the Beni-Mellal region in Morocco. Microorganisms 2021, 9, 914. https://doi.org/10.3390/microorganisms9050914.

Abbas HH, Soliman SM, Farid IM, Galal YGM, Abbas MHH, Mohamed I, Moursy AA, Moslhy SH (2020). Oil Yield and Nutrients Uptake by Irradiated Canola (Brassica napus L.) in Response to Different Nitrogen and Irrigation Water Sources. Env. Biodiv. Soil Security 4, 181 - 199 (2020). DOI: 10.21608/jenvbs.2020.32736.1099.

Abdel Aziz OA, Helal GA, Galal YGM, Rofaida AS (2016). Fungal siderophores production in vitro as affected by some abiotic factors. Int. J. Curr. Microbiol. App. Sci 5(6), 210-222. http://dx.doi.org/10.20546/ijcmas.2016. 506.025.

Abdel-Gawad ShA, El-Howeity MA (2019). Effect of Microbial Inoculation and Mineral Amendments on Improving Compost Quality. Env. Biodiv. Soil Security 3, 97 -107 (2019). DOI: 10.21608/jenvbs.2019.12225.1059.

Abdelrahman H M, Zaghloul RA, Hassan Enas A, El-Zehery HRA, Salem AA (2021). New strains of plant growth-promoting rhizobacteria in combinations with humic acid to enhance squash growth under saline stress. Egyptian Journal of Soil Science. 61(1): 81-90.

Abdul Malek N, Zainuddin Z, Chowdhury AJK, Zainal Abidin ZA (2015). Diversity and antimicrobial activity of mangrove soil actinomycetes isolated from tanjung lumpur, kuantan. . jurnal teknologi (sciences & engineering) 77(25), 37–43.

Afify AH, Hauka FIA, El-Sawah AM (2018). Plant Growth-Promoting Rhizobacteria Enhance Onion (Allium cepa L.) Productivity and Minimize Requisite Chemical Fertilization. Env. Biodiv. Soil Security 2, 119-129 (2018). DOI: 10.21608/JENVBS.2019.6002.1036.

Alexander DB, Zuberer DA (1991). Use of chrome azurol S reagents to evaluate Siderophores production by rhizosphere bacteria. Biol Fertil Soils, 12, 39-45.

Amaresan N, Kumar K, Naik JH, Bapatla KG, Mishra RK (2018). *Streptomyces* in plant growth promotion: mechanisms and role. *Actinobacteria*: diversity and biotechnological applications. https://doi.org/10.1016/B978-0-444-63994-3.00008-4.

Anwar S, Ali B, Sajid I (2016). Screening of Rhizospheric Actinomycetes for Various In-vitro and In-vivo Plant Growth-promoting (PGP) Traits and for Agroactive Compounds. Front. Microbiol, 7, 1334-1345. doi: 10.3389/fmicb.2016.01334.

Bano N, Musarrat J (2003). Characterization of a New *Pseudomonas aeruginosa* Strain NJ-15 as a potential biocontrol agent. Current Microbiol, 46, 324–328. doi: 10.1007/s00284-002-3857-8.

Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk H-P, Clément C, Ouhdouch Y, van

Wezel GP (2016). Taxonomy, physiology, and natural products of *Actinobacteria*. Microbiol. Mol. Biol. Rev. 80, 1– 43. doi:10.1128/MMBR.00019-15.

Biglari N, Hassan HM, Amini J (2016). The Ability of *Streptomyces* spp. Isolated from Iranian soil to solubilize rock phosphate. Advances in Bioscience & Clinical Medicine ISSN: 2203-1413, 04 (03). doi: 10.7575/aiac.abcmed.16.04.03.03.

Boone R, Castenholtz R, Garrity G (2001). Bergey's manual of systematic bacteriology'. SpringerVerlag. New York, Berlin Heidelberg. 1, 163-164.

Candau R, Ávalos J, Olmedo EC (1991). Gibberellins and carotenoids in wild type and mutants of *Gibberella fujikuroi*. Appl Environ Microbiol 57 (11), 3378-3382.

Cao Y, Fu D, Liu T, Guo G, Hu Z (2018). Phosphorus Solubilizing and Releasing Bacteria Screening from the Rhizosphere in a Natural Wetland. Water J, 10, 195. doi: 10.3390/w10020195.

Chaiharn M, Aree WP, Sujada N, Lumyong S (2018). Characterization of phosphate solubilizing *Streptomyces* as a biofertilizer. Chiang Mai J. Sci., 45(2), 701-716.

Chaiharn M, Lumyong S (2011). Screening and optimization of indole-3-acetic acid production and phosphate solubilization from rhizobacteria aimed at improving plant growth. Curr. Microbiol. 62, 173-181. Doi 10.1007/s00284-010-9674-6.

Choi J, Chung YJ, Kang D, Lee KSh, Lee JW (2012). Effect of radiation on disinfection and mechanical properties of Korean traditional paper, Hanji. Radiation physics and chemistry. 81, 1051-1054.

Choi SH, Lee KL, Shin JH, Cho YB, Cha SSh, Roe JH (2017). Zinc-dependent regulation of zinc import and export genes by Zur. Nature communications 8:15812. doi: 10.1038/ncomms15812.

Chouyia EF, Romano I, Fechtali T, Fagnano M, Fiorentino N, Visconti D, Idbella M, Ventorino V, Pepe O (2020). P-solubilizing *Streptomyces roseocinereus* MS1B15 with multiple plant growth-promoting traits enhance barley development and regulate rhizosphere microbial population. Front. Plant Sci. 11,1137. doi: 10.3389/fpls.2020.01137.

de Souza SB, Bertolazi AA, Eutrópio FJ, Dutra AM, Brandão AB, Lopes JVSR, do Nascimento MM, Cruz C, Ramos AC (2021). Iron toxicity and its relation to nitrogen and phosphorus availability in ectomycorrhizal fungi. Soil nitrogen ecology Ch23. Soil biology 62. (eBook). Doi.org/10.1007/978-3-030-71206-8_23.

Dimkpa Ch (2016). Microbial siderophores: Production, detection and application in agriculture and environment. Endocytobiosis and Cell Research, 27(2): 7-16.

Duca D, Lorv J, Patten CL, Rose D, Glick BR (2014). Indole-3-acetic acid in plant–microbe interactions. Antonie Van Leeuwenhoek, 106, 85–125. doi 10.1007/s10482-013-0095-y.

El Sayed OH, Refaat HM, Swellam MA, Amer MM, Attwa AI, El Awady ME (2011). Bioremediation of zinc by *Streptomyces aureofacienes*. J. Applied Sci., 11(5), 873-877.

El Shobaky A, Keyba HM, Moussa LA (2014). Gamma ray induced effects on new species of *Streptomyces* (Aefo2) (Hm775973.1gi:302495616) isolated from Egyptian soil. Int. J. Bioassays, 3 (12), 3586-3593.

El-Tarabily KA, Nassar AH, Sivasithamparam K (2008). Promotion of growth of bean (*Phaseolus vulgaris* L.) in a calcareous soil by a phosphate-solubilizing, rhizosphere-competent isolate of *Micromonospora endolithica*. Appl. Soil Ecol., 39, 161–171.

Farhat M, Boukhris I, Chouayekh H (2015). Mineral phosphate solubilization by *Streptomyces* sp. CTM396 involves the excretion of gluconic acid and is stimulated by humic acids. FEMS Microbiology Letters, 362 (5). doi: 10.1093/femsle/fnv008.

Faried Al-ShM, Mohamed HM, El-Dsouky MM, El-Rewainy HM (2018). Isolation and characterization of phosphate solubilizing Actinomycetes from rhizosphere. Assiut J. Agric. Sci., 49 (4), 125-137. Doi: 10.21608/AJAS.2018.28381.

Fletcher RA, Kallidumbil V, Steele P (1982). An improved bioassay for cytokinins using cucumber cotyledons. Plant Physiol. 69 (3), 675-677. http://www.jstor.org/stable/4267274.

Fletcher RA, McCullagh D (1971). Cytokinin-induced chlorophyll formation in cucumber cotyledons. planta (Berl.), 101, (1), 88-90 .doi: 10.1007/BF00387693.

Glick BR (2012). Plant Growth-Promoting Bacteria: Mechanisms and Applications. Scientifica, Vol. 2012, 1–15. http://dx.doi.org/10.6064/2012/963401.

Glickmann E, Dessaux Y (1995). A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl. Environ. Microbiol., 61 (2), 793-796.

Gusmiaty MR, Bachtiar B, Larekeng Sh (2019). Gibberellin and IAA production by Rhizobacteria from Various Private Forest. Conf. Ser.: Earth Environ. Sci. 270 012018. doi:10.1088/1755-1315/270/1/012018.

IAEA (International Atomic Energy Agency) (2017). Uses of ionizing radiation for tangible cultural heritage conservation. IAEA Radiation Technology Series (6).

Ibrahim AA, Aboshanab KM, Yassien MA, Hassouna NA (2017). Improvement of paromomycin production by *Streptomyces rimosus* subsp *paromomycinus* NRRL 2455 using gamma irradiation mutagenesis. Arch Pharm Sci., 1(2), 26-30.

Jackson ML (1973). Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd., New Delhi, 498.

Kallifidas DB, Pascoe B, Owen GA, Damerell CMS, Hong HJ, Paget MSB (2010). The Zinc-Responsive Regulator Zur Controls Expression of the Coelibactin Gene Cluster in *Streptomyces coelicolor*. J. of Bacterio. 192(2), 608–611. doi:10.1128/JB.01022-09.

Kämpfer P (2006). The family *Streptomycetaceae*, Part I, Taxonomy. The Prokaryotes - A Handbook on the Biology of Bacteria. Archeae. Bacteria: Firmicutes, Acti-

nomycetes. 3, 3rd Ed., (Eds: Dworkin, M., *et. al*), Springer-Verlag, Berlin: 538-604. doi: 10.1007/0-387-30743-5_22.

Kämpfer P (2012). Order XIV. *Streptomycetales* ord. nov. Bergey's manual of systematic bacteriology. eBook, Vol.5 The Actinobacteria, Part A and B, 2nd Ed. doi 10.1007/978-0-387-68233-4.

Kanchanasin P, Moonmangmee D, Phongsopitanun W, Tanasupawat S, Moonmangmee S (2017). *Streptomyces cerasinus* sp. nov., isolated from soil in Thailand. Int J. Systematic Evol Microbiol.; 67 (10), 3854–3859. doi: 10.1099/ijsem.0.002212.

Khaliq S, Akhtar K, Ghauri MA, Iqbal R, Khalid AM, Muddassar M (2009). Change in colony morphology and kinetics of tylosin production after UV and gamma irradiation mutagenesis of *Streptomyces fradiae* NRRL-2702. Microbiol Res 164,469–477.

Kunova A, Bonaldi M, Saracchi M, Pizzatti C, Chen X, Cortesi P (2016). Selection of *Streptomyces* against soil borne fungal pathogens by a standardized dual culture assay and evaluation of their effects on seed germination and plant growth. BMC Microbiol, 16: 272. doi 10.1186/s12866-016-0886-1.

Kushwaha P, Srivastava R, Pandiyan K, Singh A, Chakdar H, Kashyap P, Bhardwaj AK, Murugan K, Karthikeyan N, Bagul SY, Srivastava AK, Saxena AK (2021). Enhancement in plant growth and zinc biofortification of chickpea (*Cicer arietinum* L.) by *Bacillus altitudinis*. J. Soil Sci. Plant Nutr. 21,922–935. https://doi.org/10.1007/s42729-021-00411-5.

Lazim H, Slama N, Mankai H, Barkallah I, Limam F (2010). Enhancement of oxytetracycline production after gamma irradiation-induced mutagenesis of *Streptomyces rimosus* CN08 strain. World J. Microbiol Biotechnol 26:1317–1322.

Lee J, Postmaster A, Soon HP, Keast D, Carson KC (2012). Siderophore production by actinomycetes isolates from two soil sites in Western Australia. Biometals, 25, 285 – 296. Doi: 10.1007/s10534-011-9503-9.

Mali GV, Patil RC, Bodhankar MG (2011). Antifungal and phytohormone production potential of *Azotobacter Chroococcum* isolates from groundnut (*Arachis Hypogea* L.) rhizosphere and their effect on nodulation and dry mass, alongwith native rhizobia in pot culture experiment. Res. J. Chem. Environ., 15 (2), 1 – 7.

Mansour FA, Aldesuquy HS, Hamedo HA (1994). Studies on plant growth regulators and enzymes production by some bacteria. Qatar Univ. Sci. j., 14 (2): 281-288.

Mao J, Tang Q, Zhang Z, Wang W, Wei D, Huang Y, Liu Z, Shi Y, Goodfellow M (2007). *Streptomyces radiopugnans* sp. nov., a radiation-resistant actinomycetes isolated from radiation-polluted soil in China. Int. J. of Systematic and Evolutionary Microbiology, 57, 2578–2582. Doi 10.1099/ijs.0.65027-0.

Moussa LAA, Mansour FA, Serag MS, Abou El-Nour SAM (2005). Effect of gamma radiation on the physiological properties and genetic materials of *Streptomyces albaduncus* and *S. erythogresius*. Int. J. Agri. Biol., 7 (2), 197-202.

Nafis A, Raklami A, Bechtaoui N, El Khalloufi F, El Alaoui A, Glick BR, Hafidi M, Kouisni L, Ouhdouch Y, Hassani L (2019). Actinobacteria from Extreme Niches in Morocco and Their Plant Growth-Promoting Potentials. Diversity, 11, 139,1-15. doi: 10.3390/d11080139.

Ngosong Ch, Gabriel E, Ruess L (2014). Collembola grazing on arbuscular mycorrhiza fungi modulates nutrient allocation in plants. Pedobiologia, 57, 171–179. https://doi.org/10.1016/j.pedobi.2014.03.002.

Olanrewaju OS, Babalola OO (2019). *Streptomyces*: implications and interactions in plant growth promotion. Appl. Microbiol, and Biotechnology 103(3), 1179–1188. https://doi.org/10.1007/s00253-018-09577-y.

Olanrewaju OS, Glick BR, Babalola OO (2017). Mechanisms of action of plant growth promoting bacteria. World J Microbiol Biotechnol 33(11):197. https://doi.org/10.1007/s11274-017-2364-9.

Patel K, Goswami D, Dhandhukia P, Thakker J (2016). Techniques to study microbial phytohormones. DK. Maheshwari (Ed.), Bacterial Metabolites in Sustainable Agroecosystem, Sustainable Development and Biodiversity 12, doi 10.1007/978-3-319-24654-3_1.

Patil NG, Patil VR (2012). Isolation and characterization of Actinomycetes producing gibberellic acid. Int. J. Sci and Research (IJSR). (3)9, 1400-1403. ISSN (Online): 2319-7064.

Petkovie H, Cullum J, Hranueli D, Hunter IS, Perić-Concha N, Pigac J, Thamchaipenet A, Vujaklija D, Long PF (2006). Genetics of *Streptomyces rimosus*, the oxytetracycline producer. Microbiol. Mol. Biol. Rev 70(3), 704 -728. doi: 10.1128/MMBR.00004-06.

Reyes C, Avalos J, Olmedo EC (1992). Regulation of gibberellin biosynthesis in *Gibberella fujikuroi*. Plant Physiol. 100, 1184- 1188. doi: 10.1104/pp.100.3.1184.

Sadeghi A, Karimi E, Dahaji PA, Javid MG, Dalvand Y, Askari H (2012). Plant growth-promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil conditions. World J. Microbiol. Biotechnol. 28,1503–1509. https://doi.org/10.1007/s11274-011-0952-7.

Sakr AA, Ghaly MF, Ali MF (2013). The use of gamma irradiation in the sterilization of *Streptomyces* colonizing the Tempra paintings in ancient Egyptian Tombs. Int J Conserv Sci 4, 3, 283-294.

Sathya A, Vijayabharathi R, Gopalakrishnan S (2017). Plant growth-promoting actinobacteria: a new strategy for enhancing sustainable production and protection of grain legumes 3 Biotech 7:102. doi 10.1007/s13205-017-0736-3.

Sayyed RZ, Badgujar MD, Sonawane HM, Mhaske MM, Chincholkar SB (2005). Production of microbial iron chelators (siderophores) by fluorescent *Psedomonads*. Indian J of Biotechn., 4, 484-490.

She W, Zhongfeng S, Lei Y, Shumiao Z, Yunxiang L (2016). *Streptomyces alfalfae* sp. nov. and comparisons with its closest taxa *Streptomyces silaceus*, *Streptomyces flavofungini* and *Streptomyces Intermedius*. Int J. of

Systematic and Evolutionary Microbiol., 66, 44-49. Doi: 10.1099/ijsem.0.000671.

Shirling EB, Gottlieb D (1966). Methods for Characterization of *Streptomyces* Species, Int. J. of Systematic Bacteriol., 16(3), 313-340.

Shirling EB, Gottlieb D (1969). Cooperative Description of Type Cultures of *Streptomyces*. Iv. Species Descriptions from the Second, Third and Fourth Studies. Int J of Systematic Bacteriol., 19(4), 391-512.

Shutsrirung A, Chromkaew Y, Pathom-Aree W, Choonluchanon S, Boonkerd N (2013). Diversity of endophytic Actinomycetes in mandarin grown in northern Thailand, their phytohormone production potential and plant growth-promoting activity. Soil Science and Plant Nutrition, 59, 322-330. https://doi.org/10.1080/00380768.2013.776935.

Silva Aquino KAda (2012). *Sterilization by Gamma Irradiation. Gamma Radiation.* Gamma Radiation Edited by Prof. Feriz Adrovic Ch 9. (eBook), ISBN 978-953-51-0316-5. doi:10.5772/34901.

Sousa JAJ, Olivares FL (2016). Plant growth promotion by *Streptomycetes*: ecophysiology, mechanisms and applications. Chem. Biol. Technol. Agric. (2016) 3, 24. doi 10.1186/s40538-016-0073-5.

Verma Sh, Chakar H, Kumar M, Varma A, Saxena AK (2021). Microoranisms as a sustainable alternative to traditional biofortification of iron and zinc: status and prospect to combat hidden hunger. J. Soil Sci. Plant Nutr. 21, 1700-1717. Doi.or/10.1007/s42729-021-00473-5.

Verma V, Singh S, Prakash S (2011). Bio-control and plant growth promotion potential of siderophore producing endophytic *Streptomyces* from Azadirachta indica A. Juss. J. Basic. Microbiol., 51(5), 550–556.

Viaene T, Langendries S, Beirinckx S, Maes M, Goormachtig S (2016). Streptomyces as a plant's best friend?. FEMS Microbiology Ecology,2016, 92(8), fiw119. doi: 10.1093/femsec/fiw119.

Vijayabharathi R, Sathya A, Gopalakrishnan S (2016). A renaissance in plant growth-promoting and biocontrol agents by endophytes. In: Singh D., Singh H., Prabha R. (Eds) Microbial Inoculants in Sustainable Agricultural Productivity. Springer, New Delhi. https://doi.org/10.1007/978-81-322-2647-5_3.

Vurukonda SShKP, Giovanardi D, Stefani E (2018). Plant Growth-promoting and Biocontrol Activities of *Streptomyces* spp. as Endophytes. Int. J. Mol. Sci., 19, 952: doi: 10.3390/ijms19040952.

Westerhof W, Relyveld GN, Kingswijk MM, de Man P, Menke HE (2004). Propionibacterium acnes and the pathogenesis of progressive macular hypomelanosis. Arch Dermatol 140, 210–214. http://dx.doi.org/10.1001/archderm.140.2.210.

Yang YY, Zhao XQ, Jin YY, Huh JH, Cheng JH, Singh D, Kwon HJ, Suh JW (2006). Novel function of cytokinin: a signaling molecule for promotion of antibiotic production in *Streptomycetes*. J. Microbiol. Biotechnol., 16(6), 896–900.