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First Report of Endophytic Bacteria Isolated from *Senecio glaucus* L., Egypt

Primer Informe de Bacterias Endofíticas Aisladas de *Senecio glaucus* L., Egipto

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Abstract

Microorganisms are naturally associated with plants in several ways. The study was conducted to isolate bacteria endophytes from the internal cells of roots, stems, leaves, and capitula of *Senecio glaucus* collected from 2 diverse (coastal and desert) habitats in Egypt. A total of 10 endophytic bacteria were obtained from the isolation; the highest diversity of bacterial endophytes was observed in desert samples roots and leaves. The isolates were recognized based on morphology, biochemical and 16S rRNA sequence genes. All isolates indicated the ability for enzyme production as amylase, cellulase, lipase, catalase, and protease in their biochemical descriptions; analyses also gave a significant indication of their potential to produce plant growth hormones, as their ability to dissolve Phosphate. In the world and Egypt, we are the first to report bacterial endophytes isolated from *Senecio glaucus*. This study could aid in determining the role of endophytic bacteria in severe habitats, as well as their potential applications in medicine, bioremediation, agriculture, and industry.

Keywords: Bacterial endophytes, Biochemical, 16S rRNA, Senecio, *Asteraceae*.

Resumen

Los microorganismos están naturalmente asociados con las plantas. El presente experimento se llevó a cabo para aislar bacterias endófitas de las células internas de raíces, tallos, hojas y Tejido capitulear de *Senecio glaucus* recolectadas en 2 hábitats diversos (costeros y desérticos) de Egipto. Del aislamiento se obtuvieron un total de 10 bacterias endófitas; la mayor diversidad de endófitos bacterianos se observó en raíces y hojas de muestras del desierto. Los aislamientos se reconocieron con base en la morfología, la bioquímica y los genes de la secuencia del ARNr 16S. Todos estos aislados indican la capacidad de producir enzimas como amilasa, celulasa, lipasa, catalasa y proteasa en sus descripciones bioquímicas; los análisis también mostraron una indicación significativa de su potencial para producir hormonas de crecimiento vegetal; como su capacidad para disolver el fosfato. En el mundo y en Egipto, somos los primeros en reportar endófitos bacterianos aislados de *Senecio glaucus*. Este estudio podría ayudar a determinar el papel de las bacterias endófitas en hábitats severos, así como sus posibles aplicaciones en medicina, biorremediación, agricultura e industria.

Palabras clave: Endófitos bacterianos, Bioquímica, 16S rRNA, Senecio, *Asteraceae*.

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1 Introduction

How to define an endophyte is a point of contention. It was suggested that bacteria that are isolated from the internal tissues of the plant and that do not cause any damage to their host are classified as endophytes. Other descriptions recommend that it is essential to establish that the bacterial occupation is of the inner tissues of the plant. Altruism, commensalisms, symbiosis, or passivity to pathogenicity have been used to describe this unique host endophyte interaction; so, on the specific relationships involved, internal plant colonization by bacteria constitutes a vast and, yet little mapped ecological niche (Kobayashi and Palumbo, 2000; Hallmann et al., 1997). The bacterial diversity that has been reported as endophytes spans a variety of important Gram-negative and -positive bacteria that contain genera of Alpha-, Beta- and Gamma-proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes (Bacon and Hinton, 2007; Lodewyckx et al., 2002).

Nearly 1250 Senecio species are widely distributed and comprise about 6 species that occur in Egypt including *S. glaucus, S. flavus, S. aegyptius, S. Vulgaris, S. hoggariensis*, and *S. belbeysius*. This genus is important due to its pharmacological, botanical, and toxicological properties (Singh et al., 2017a; Nori-Shargh et al., 2008). A survey on the phytochemical examination of Senecio extracts revealed antioxidant, antimicrobial, cytotoxic activity (Tundis et al., 2009), anti-inflammatory, insecticidal and antiviral properties (Sultan et al., 2022; El-Amier et al., 2014; Joshi et al., 2013; Kahriman et al., 2011).

Species of Senecio that inhabit sandy plains and desert wadies are used as a sedative of the central nervous system, diuretic, and emetic in Egypt (Eissa et al., 2014). *Senecio glaucus* L. (Morrar) is an annual herb that grows in Egypt and has two subspecies; *S. glaucus* subsp. *coronopifloius* (Maire) C. Alexander. Subsp. *coronopifloius* and *S. glaucus* L. subsp. *glaucus* grows in desert wadis, saline soils, coastal sandy, and cultivation edges and it is the most common in Egypt than subsp. *glaucus Boulos2002*.

Endophytes may benefit plants indirectly by improving the herbivore's infections or stress resistance, or by further unexplained processes (Schulz and Boyle, 2005). Endophytes have been found in several studies to be able to protect their plant hosts from drought (Clay and Schardl, 2002). Infected plants with endophytes showed salt and temperature tolerance, according to Waller et al. (2005). Endophytes function as a biological trigger to stimulate the stress response more quickly and robustly than non-symbiotic plants, promoting plant growth and protecting the plant to reduce diseases and insect pests, according to Redman et al. (2002).

Endophytic bacteria can solubilize phosphate and provide plants with assimilable nitrogen (Rosenblueth and Martínez-Romero, 2006). Furthermore, interactions between plants and endophytic bacteria may aid in ecosystem restoration processes, protecting plants from biotic and abiotic stress and promoting the production of important secondary metabolites (Mowafy et al., 2021; Cheng et al., 2019; Müller et al., 2015; Alavi et al., 2013).

The genetic background of plant host species, appropriateness, nutrients, and ecological niches (Jia et al., 2016); environmental circumstances, host genotypes, bacterial species (Chebotar et al., 2015); and host developmental stage and inoculum density (Dudeja and Giri, 2014), all have a significant impact on the endophytic bacteria population.

Some cold-resistant bacteria were discovered in the roots and leaves of *Senecio vulgaris* and defined as core bacterial operational taxonomic units and reported as having an apparent strong antibacterial effect and the ability to survive in extremely low temperatures, dry, and UV-contaminated settings (Gaspard and Rice, 1989; Koo et al., 2016; Vishnivetskaya et al., 2009).

Endophytes are advantageous to *S. vulgaris* (Cheng et al., 2019; Singh et al., 2016), and their application to rice resulted in a reduction of arsenic accumulation and generation of IAA, which aids in growth promotion; heavy metal resistance, particularly cadmium tolerance; and nitrogen fixation ability (Purchase et al., 1997); and maize and lettuce plant growth promotion (Gamel et al., 2017; Chabot et al., 1996).

Because of its long history of usage in traditional medicine and selection in a variety of climatic, edaphic, and biotic habitats in geographically different places, *S. glaucus* exhibits amazing diversity.

It was reported that 10^2 to 10^4 endophytic bacteria populations exist per plant tissue gram (Kobayashi and Palumbo, 2000). This study aims to assess the variety of bacterial endophytes communities isolated from *S. glaucus* in two different habitats in Egypt: Gamasa City (Mediterranean Coastal) and Wadi Araba (Eastern Desert).

2 Materials and Methods

2.1 Plant Material Collection

Healthy entire plants of *S. glaucus* in the flowering phases were randomly taken from two separate locations Wadi Araba (Eastern Desert, 29°4′23.72″N 32°25′38.49″E) and Gamesa City (Mediterranean coast, 31°26′58.78″N 31°28′36.14″E) for the isolation of bacterial endophytes. Samples were packed in clean plastic bags and transported to the Microbiology Laboratory for further testing as shown in Figure 1.

2.2 Isolation and Purification of Endophytes

The isolation and purification of endophytes were done according to the procedure by Bacon and Hinton (2002) using LB agar medium (1.25 gm yeast extract, 2.5 gm peptone, 2.5 gm sodium chloride, 3.75 agar, and 250 ml distilled water). The plant samples were first washed under tap water then separated into 4 parts including root, stem, leaf, and capitula, then surface sterilized resulting in the (Geris dos Santos et al., 2003) method.

Surface sterilization was achieved by rinsing the plant parts with 70% ethanol (C_2H_5OH) for 30 seconds, then 0.5 percent sodium hypochlorite (NaOCl) for 2-3 minutes, and finally sterile distilled water (Dil.H_2O) for 10 minutes (2-3 times). After that, the plant material was dried between the folds of sterile filter papers. With a flame sterilized scalpel, the cut ends of surface-sterilized segments were removed and placed in appropriate LB agar media, with the cut surface touching the agar. The maximum possible colonies of bacterial endophytes were determined by incubating plates at 35 °C for 48 hours.

2.3 Characterization of Endophytic Bacteria

2.3.1 Morphological characterization

Aneja et al. (2006); Cappuccino and Sherman (1992) used the standard gram staining procedure to determine cell shape, colony color, and texture were used to define the isolates to establish the morphology of the bacterial cells.

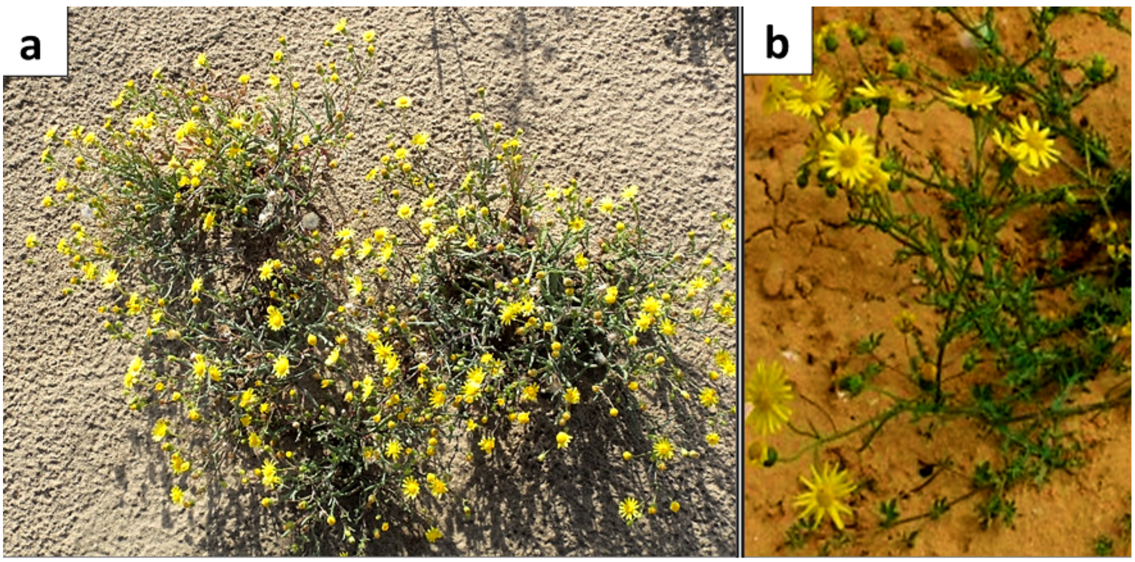

Figure 1. a) General views of *S. glaucus*, and b) Close-up views of *S. glaucus* in the study area.

2.3.2 16S rRNA gene sequencing

The isolated bacteria were molecularly identified using the MicroSeq® 500 16SrRNA Bacterial Identification Kits methodology. The sequencing reactions were carried out in the 9700 thermal cyclers with a total volume of 20 l (7 l purified PCR product and 13 l sequencing module) by setting the thermal cycler to 96 °C for 10 seconds, 50 °C for 5 seconds, and 60 °C for 4 seconds (25 cycles). The Dye ExTM 2.0 Spin Kit was then used to remove the excess dye terminators and primers from the cycle sequencing reaction (Qiagen PN 63204). Finch TV (version1.4.0) and MEGA-X (version10.2.5) software were used to analyze the sequences, and Seaview software was used to create phylogenetic trees using the closest published type of strain sequences. The sequences of the isolates obtained in this investigation were submitted to the NCBI's GeneBank database.

2.4 Statistical Analysis

The trials were carried out in triplicates, with the mean standard deviation (MSD) calculated.

3 Results

In this study, 10 bacterial endophytes were isolated from different parts of *Senecio glaucus* plant collected from 2 different places (4 isolates from the Mediterranean coastal plant and 6 isolates from the desert plant) on L.B agar medium under aseptic conditions and according to the difference in morphology as shown in Figures 2 and 3, and have codes (SGC-R, SGC-S, SGC-L, SGC-C) for the coastal samples and (SGD-R, SGD-S, SGD-L, SGD-C) for desert samples.

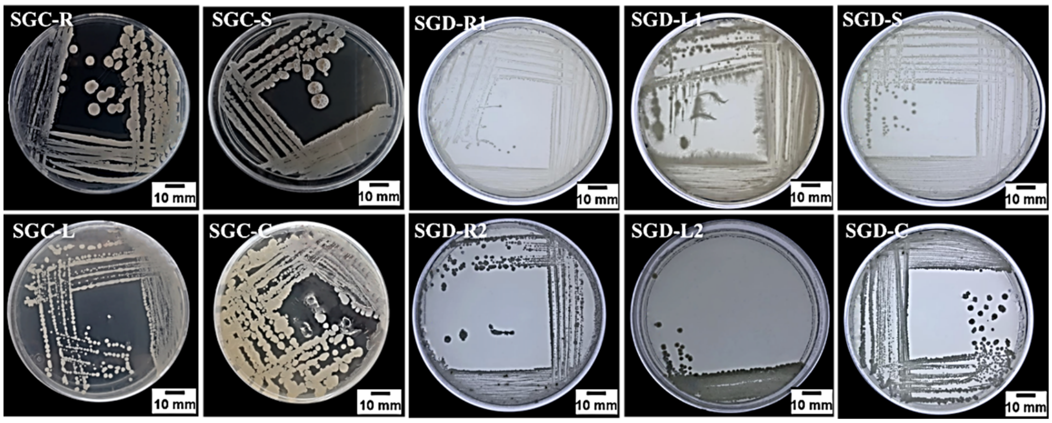

Figure 2. Bacterial endophytes isolated from *S. glaucus* SGC-R: *Senecio glaucus* Coastal-Root, SGC-S: -Stem, SGC-L: -Leaf, and SGC-C: -Capitula; SGD-R: *S. glaucus* Desert-Root, SGD-S: -Stem, SGD-L: -Leaf, and SGD-C: -Capitula.

The bacterial isolates were characterized morphologically according to colony shape, margin, elevation, texture, and pigmentation as shown in Table 1, and were scanned microscopically according to cell shape, whereas all isolates were rod shape and Gram stain, whereas the coastal sample showed 3 strains to be Gram-positive and 1 strain Gram-negative, by the other side the desert sample showed 3strains Gram-positive and 3 strains Gram-negative (Table 2).

The purified isolates were biochemically characterized according to enzymatic activity and function properties. The isolates showed an ability to produce a variety of enzymes (Tables 3 and 4).

Both coastal and desert bacterial isolates were able to produce indole with variable concentrations ranging from high to low compared with the control sample. The isolates SGC-L and SGC-C presented very weak results; while the isolates SGD-L2 and SGD-C did not indicate any positive results as shown in Table 4.

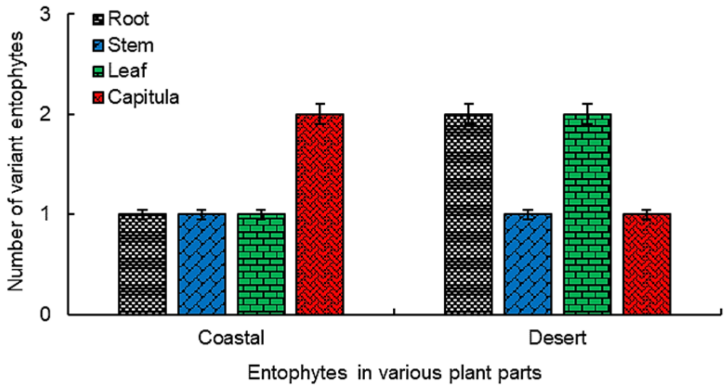

Figure 3. Number of the endophytic bacteria isolated from different tissues of the medicinal plant *S. glaucus* collected from coastal and desert habitats.

Table 1. Morphological characteristics of colonies of endophytic bacteria isolated from different tissues of the medicinal plant *S. glaucus* collected from coastal and desert habitats in Egypt.

Isolates	Tissue origin	Colony characterization					
		Size (mm)	Colony shape	Margin	Elevation	Texture	Pigmentation
Coastal sample							
SGC-R	Root	3.5	Irregular	Curled	Umbonate	Dry/Rough	Off-white
SGC-S	Stem	3.7	Irregular	Curled	Umbonate	Dry/Rough	Off-white
SGC-L	Leaf	1.5	Irregular	Lobate	Raised	Dry/Rough	Off-white
SGC-C	Capitula	2.5	Irregular	Curled	Umbonate	Dry/Rough	Off-white
Desert sample							
SGD-R1	Root	1.2	Circular	Entire	Raised	Creamy	Yellowish white
SGD-R2		1.4	Irregular	Lobate	Raised	Dry/Rough	Off-white
SGD-S	Stem	1.9	Circular	Entire	Raised	Creamy	Yellowish white
SGD-L1	Leaf	1.3	Irregular	Filamentous	Flat	Shiny Creamy	Pale Yellow
SGD-L2		2.1	Circular	Entire	Convex	Dry/Rough	Chalky White
SGD-C	Capitula	2.5	Irregular	Curled	Umbonate	Dry/Rough	Off-white

SGC-R: *Senecio glaucus* Coastal-Root, SGC-S: *S. glaucus* Coastal-Stem, SGC-L: *S. glaucus* Coastal-Leaf, and SGC-C: *S. glaucus* Coastal-Capitula; SGD-R: *S. glaucus* Desert-Root, SGD-S: *S. glaucus* Desert-Stem, SGD-L: *S. glaucus* Desert-Leaf, and SGD-C: *S. glaucus* Desert-Capitula.

Based on 16S rRNA gene sequence analysis, the isolated strains were identified as *Bacillus velezensis* strain CBMB205 (NR_116240.1), *Bacillus velezensis* strain CBMB205 (NR_116240.1), *Bacillus amyloliquefaciens* strain WS3-1 (MT579842.1), *Klebsiella aerogenes* strain ATCC13048 (NR_118556.1), *Enterobacter bugandensis* strain 247BMC (NR_148649.1), *Enterobacter hormaechei* subsp. *xiangfangensis* strain 10-17 (NR_126208.1), *Sphingobacterium faecium* strain DSM11690 (NR_025537.1), and *Kitasatospora aburaviensis* strain NBRC12830 (NR_112295.1); all the strains were correlated in the genetic distance as shown in Table 5 and Figure 4.

Table 2. Morphological characteristics of cells of endophytic bacteria isolated from different tissues of the medicinal plant *S. glaucus* collected from coastal and desert habitats in Egypt.

Isolates	Tissue origin	Cell Characteristics			
		Gram stain	Cell shape	Size (μm)	Motility
Coastal sample					
SGC-R	Root	Gram-positive	Rod	2.5	Motile
SGC-S	Stem	Gram-positive	Rod	2.5	Motile
SGC-L	Leaf	Gram-negative	Rod	1.2	Motile
SGC-C	Capitula	Gram positive	Rod	2.7	Motile
Desert sample					
SGD-R1	Root	Gram-negative	Rod	1.6	Motile
SGD-R2		Gram-negative	Rod	1.3	Motile
SGD-S	Stem	Gram-negative	Rod	1.7	Motile
SGD-L1	Leaf	Gram-positive	Rod	1.9	Motile
SGD-L2		Gram-positive	Rod	2.3	Motile
SGD-C	Capitula	Gram positive	Rod	1.8	Motile

SGC-R: *Senecio glaucus* Coastal-Root, SGC-S: *S. glaucus* Coastal-Stem, SGC-L: *S. glaucus* Coastal-Leaf, and SGC-C: *S. glaucus* Coastal-Capitula; SGD-R: *S. glaucus* Desert-Root, SGD-S: *S. glaucus* Desert-Stem, SGD-L: *S. glaucus* Desert-Leaf, and SGD-C: *S. glaucus* Desert-Capitula.

Table 3. Qualitative analysis of the biochemical characterization of the endophytic bacteria isolated from different tissues of the medicinal plant *S. glaucus* collected from different habitats in Egypt.

Isolates	Tissue organ	Biochemical Characterization					
		Catalase	Amylase	Cellulase	Protease	Lipase	H_2S
Coastal							
SGC-R	Root	+ve	+ve	+ve	+ve	+ve	-ve
SGC-S	Stem	+ve	+ve	+ve	+ve	+ve	-ve
SGC-L	Leaf	+ve	+ve	+ve	+ve	+ve	-ve
SGC-C	Capitula	+ve	+ve	+ve	+ve	+ve	-ve
Desert							
SGD-R1	Root	+ve	+ve	+ve	-ve	+ve	-ve
SGD-R2		+ve	+ve	+ve	+ve	+ve	-ve
SGD-S	Stem	+ve	+ve	+ve	-ve	+ve	-ve
SGD-L1	Leaf	+ve	+ve	+ve	-ve	+ve	-ve
SGD-L2		+ve	+ve	+ve	+ve	+ve	-ve
SGD-C	Capitula	+ve	+ve	+ve	+ve	+ve	-ve

+ve: positive reaction; -ve: negative reaction.

4 Discussion

Bacterial endophytes have long been known to be present in most healthy plant tissues (McInroy and Kloepper, 1995; Sturz, 1995; Frommel et al., 1993). Endophytic bacteria have been found in every plant species studied, according to Partida-Martínez and Heil (2011) as well as in this study. Several plant species have been found to have diverse endophytic bacterial communities that showed significant phenotypic and genotypic diversity (Santoyo et al., 2016; Miliute et al., 2015). The study of population diversity of bacterial endophytes isolated from the desert sample of *S. glaucus* showed more diverse species than the coastal sample (Figure 3). The plant host species, host specificity, and tissue types can strongly affect the type of endophytic community

(Ding and Melcher, 2016). Qualitative and quantitative variations between plant species in microbial colonization are mainly due to genotypic host-endophyte compatibility and ecological conditions (tropical versus temperate) (Rajan, 2012).

Table 4. Qualitative analysis of Plant growth promoting (PGP) parameters of the bacterial endophytes isolated from the medicinal plant *S. glaucus*.

Isolates	Tissue organ	Plant growth promoting			
		Phosphate solubilization	Nitrate reductase	IAA	GA3
Coastal					
SGC-R	Root	+ve	+ve	+ve	+ve
SGC-S	Stem	+ve	+ve	+ve	+ve
SGC-L	Leaf	+ve	+ve	+ve	+ve
SGC-C	Capitula	+ve	+ve	+ve	+ve
Desert					
SGD-R1	Root	+ve	+ve	+ve	+ve
SGD-R2		+ve	+ve	-ve	+ve
SGD-S	Stem	+ve	+ve	+ve	+ve
SGD-L1	Leaf	+ve	+ve	+ve	+ve
SGD-L2		+ve	+ve	+ve	+ve
SGD-C	Capitula	+ve	+ve	-ve	+ve

+ve: positive reaction; -ve: negative reaction.

The strains were isolated from roots, stems, leaves, and capitula tissues of *S. glaucus*. The highest population of endophytes was obtained from the internal tissues of the roots and leaves of the plant (Figure 3). The colonies' morphology indicated the endophytes variation. The tested isolates were chosen for their morphological variation as well as their dominance (Table 1). A large variety of both Gram-negative and Gram-positive bacteria are involved in the endophytic bacteria (Lodewyckx et al., 2002). Interestingly, Gram-positive was the most distributed population in the coastal sample than Gram-negative isolates of *S. glaucus*. On the other hand, the Gram-negative population was equal to the Gram-positive isolates of the desert sample (Table 2), as reported in several plants. An equal presence of Gram-negative and Gram-positive bacteria were identified (Zinniel et al., 2002). Literature has reported a predominance of Gram-negative bacteria in the tissues of various plants (Elbeltagy et al., 2000; Stoltzfus et al., 1997). These bacterial species could have coevolved with the plant to be adapted to a specific arid habitat that is nutrient-poor. In response to environmental conditions such as pH, temperature, and salinity, the Gram-positive bacteria from the isolated strains form spores, which may provide a survival advantage.

All morphology of the isolates cell shape showed to be bacilli/rod (Table 2). According to Jacobs et al. (1985), *Erwinia* sp., *Enterobacter* sp., *Bacillus* sp., *Pseudomonas* sp., *Micrococcus*, *Microbacterium*, *Stenotrophomonas*, *Pantoea*, *Burkholderia*, *Pseudomonas* and *Flavobacterium* sp. were the most common isolated bacterial genera of endophytic bacteria in several plants like tomato, cotton, soybean, rice, and maize (Chaturvedi et al., 2016; Hallmann et al., 1997).

Endophytic bacteria have been isolated from Senecio species tissues previously. Cheng et al. (2019); Singh et al. (2016) isolate the endophytes *Brevundimonas diminuta* and *Rhizobium leguminosarum* from *S. vulgaris; Sphingomonas aerolata, Sphingomonas faeni, Exiguobacterium sibiricum* and *Oxalobacteraceae* (OTU3) were characterized in leaves and roots of *S. vulgaris* (Gaspard and Rice, 1989; Koo et al., 2016; Vishnivetskaya et al., 2009).

In this study, the obtained isolates were biochemically characterized according to enzymatic activity and function properties (Table 3). The isolates showed a variety ability to produce a variety of enzymes such as catalase enzymes, amylolytic, cellulolytic, proteolytic, and lipolytic enzymes. However, no survey on these enzymes' secretion by endophytes has been conducted (Elbeltagy et al., 2000; Reinhold-Hurek and Hurek, 1998). Endophytic bacteria might act as virulence factors for plant pathogenic bacteria due to the cellulases and hydrolytic enzymes may play a role in the mechanisms which enter and persist in the host plant as reported for *Enterobacter asburiae* JM22 *Quadt-Hallmann1997* and *Azoarcus* sp. (Hurek et al., 1994).

Table 5. The 16S rRNA gene reference sequence of the strains in the GenBank database.

Serial	Plant parts	Similar Strain	Similarity %	NCBI sequence
Costal sample				
SGC-R	Root	*Bacillus velezensis* strain CBMB205 (NR_116240.1)	96.08 %	MZ520618.1
SGC-S	Stem	*Bacillus amyloliquefaciens* strain WS3-1 (MT579842.1)	99.80 %	OK148122.1
SGC-L	Leaf	*Klebsiella aerogenes* strain ATCC13048 (NR_118556.1)	94.31 %	MZ520791.1
SGC-C	Capitula	*Bacillus velezensis* strain CBMB205 (NR_116240.1)	99.80 %	MZ520618.1
Desert sample				
SGD-R1	Root	*Enterobacter bugandensis* strain 247BMC (NR_148649.1)	99.91 %	OK147922.1
SGD-R2		*Klebsiella aerogenes* strain ATCC13048 (NR_118556.1)	94.31 %	OK057209.1
SGD-S	Stem	*Enterobacter hormaechei* subsp. *xiangfangensis* strain 10-17 (NR_126208.1)	94.03 %	OK044126.1
SGD-L1	Leaf	*Sphingobacterium faecium* strain DSM11690 (NR_025537.1)	87.64 %	OK156473.1
SGD-L2		*Kitasatospora aburaviensis* strain NBRC12830 (NR_112295.1)	100 %	MZ477009.1
SGD-C	Capitula	*Bacillus velezensis* strain CBMB205 (NR_116240.1)	98.06 %	OK147924.1

Endophytic bacteria isolated from the coastal *S. glaucus* indicated the highest production of the studied enzymes than the desert isolates, despite the high diversity in the desert *S. glaucus* sample (Figures 4 and 5). All isolates secreted amylases, cellulases, protease, and lipase except *Enterobacter hormaechei* subsp. *Xiangfangensis* and *Sphingobacterium faecium* could not produce protease enzymes (Table

3). The cellulosic activity of these endophytes may give an advantage for intercellular entry and spreading of endophytes into the host plant, as the host plant's cell wall contains cellulose (Hallmann et al., 1997). Hydrolases, extracellular enzymes produced by endophytic bacteria, aid in the establishment of systemic resistance to pathogen invasion in plants (Singh et al., 2017b; Elbeltagy et al., 2000).

In addition, all isolates were biochemically characterized according to function properties (Table 4). The isolates showed ability to produce a variation of phytohormones that can help plants and can be used as PGPB indole acetic acid, and gibberellic acids as they also indicated their ability to solubilize phosphate and nitrate reductase. On the other hand, all isolates showed a negative result for hydrogen sulfide production. Likewise, the strains SGD-R2 and SGD-C isolated from the desert sample were negative for IAA.

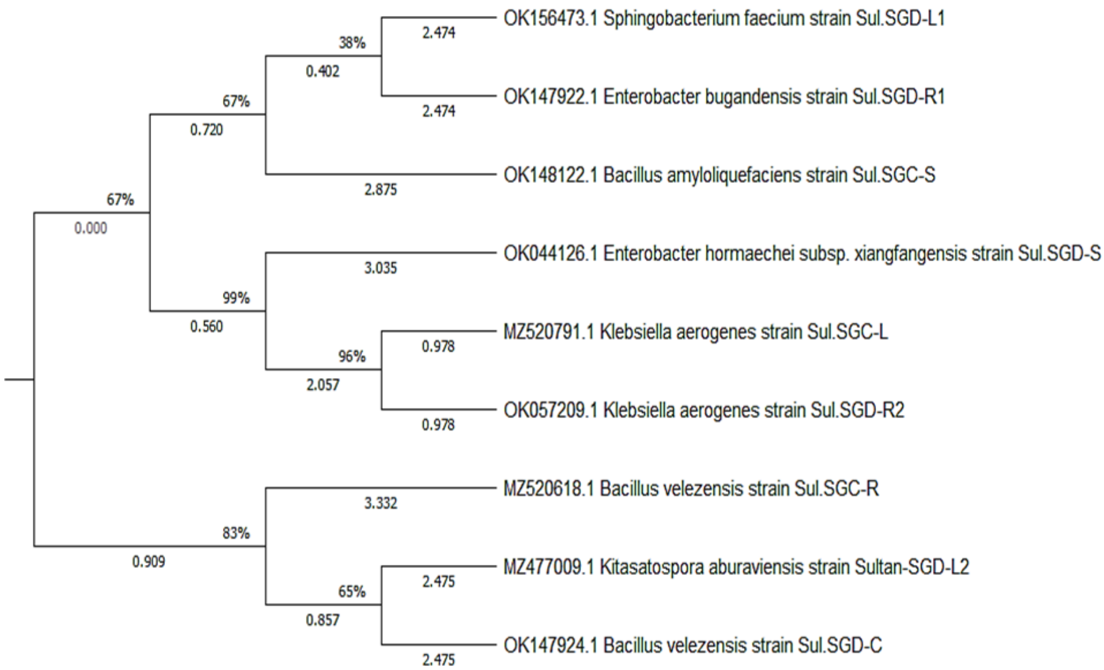

Figure 4. The phylogenetic tree derived from 16S rRNA gene sequences of the 10 bacterial endophytes strains.

Ten strains with various colony morphologies were isolated and their 16S rRNA gene sequences were analyzed for taxonomic relationships (Table 5 and Figure 6). Non *B. japonicum* bacteria were found in the isolates from surface-sterilized *S. glaucus* tissues studied, and most of them were morphologically unique. According to phylogenetic analysis, the isolates were shown to belong to four extremely different phyla already known to be plant-associated: Bacteroidetes, Proteobacteria, Actinobacteria, and Firmicutes (Reinhold-Hurek et al., 2015). Based on 16S rRNA gene sequence analysis, the isolated strains were identified as *Bacillus velezensis* strain CBMB205 and *Bacillus amyloliquefaciens* strain WS3-1 (Class: Bacilli), *Enterobacter bugandensis* strain 247BMC, and *Enterobacter hormaechei* subsp. *xiangfangensis* strain 10-17 (Class: Gamma Proteobacteria), *Sphingobacterium faecium* strain DSM 11690 (Class: Flavobacteria), *Klebsiella aerogenes* strain ATCC 13048 (Class: Gamma Proteobacteria), and *Kitasatospora aburaviensis* strain NBRC 12830 (*Streptomyces aburaviensis*) (Class: Actinomycetes). The sequence analysis revealed that the isolates may contain previously unknown bacterial species: strains from two phylotypes showed less than 98.7 % identity to previously reported 16S rRNA genes of known species. They are likely to represent at least unique species, given this value it has recently been proposed as a "gold standard" for distinguishing species (Stackebrandt, 2006). All the strains were correlated in genetic distance. The phylogenetic dendrogram illustrated the correlation among six isolates was conducted by MEGA-X program as shown in Figure 4.

5 Conclusion

This study confirmed the diversity and occurrence of bacterial endophytes in different parts of *Senecio glaucus* (Morrar) collected from different habitats in Egypt. These bacteria might be promising candidates for future applications. The isolation of Bacillus strains opens up biotechnological options for *S. glaucus* production and the prospective application of putatively unique species. Through the biochemical descriptions of these isolates, they show their ability to produce some decomposing enzymes such as cellulase, amylase, protease, catalase, and lipase. On the other hand, the descriptive analyzes showed a strong indication of their ability to produce some plant growth hormones that can increase growth and protect plants such as their ability to produce nitrate reductases, phosphate solubilization, indole, and gibberellins. The fact that these plants were successfully colonized by each microbe suggests that they could be used in many applications, such as bio-fertilizers, bioremediation, and biological control.

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