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SHORT COMMUNICATION

In Vitro Antimicrobial Activity of Endophytes, Isolated from *Moringa peregrina* Growing in Eastern Region of Saudi Arabia

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Abstract Seven endophytes bacteria were isolated from *Moringa peregrina* and identified using 16S ribosomal RNA sequences, which revealed two bacterial genuses, namely *Proteus mirabilis* and *Bacillus*; three species belong to *Bacillus*: *B. licheniformis, B. subtilis subsp. Inaquosorum* and *B. pumilus.* The antimicrobial activity of the isolate was screened against 11 human pathogens, and the potency of the selected isolate was qualitatively and quantitatively analyzed by the cross-streak method and minimum inhibitory concentrations (MICs), respectively. Minimum inhibitory concentration (MIC) of the organic extract of selected isolate *B. licheniformis* MpKL1 was found to exhibit appreciable growth inhibition between 125 µg/ml and 500 µg/ml against most tested human pathogens and less influence against *Acinetobacter baumanii*, *Enterobacter aerogenes, Streptococcus pyogenes* and *Candida albicans* with MIC > 1000.

Keywords Antimicrobial · Endophytes · *Moringa peregrina*

Medicinal plants are the most useful source of lifesaving drugs for humans, animals and plants. Bioactive compounds that are usually extracted from plants are used as medicines, food, insecticides and other industrially important chemicals. One such medicinal plant is *Moringa peregrina*, belonging to the family Moringaceae, which is mainly found in the tropical and subtropical areas. *M. peregrina* species is commonly known as the miracle tree, ranging in size from a tiny plant to massive tree [1]. Almost all the parts of *M. peregrina* are edible and have been consumed as a vegetable and used to treat many ailments such as tumors, hysteria, scurvy, paralysis, sores and skin infections [2, 3]. *M. peregrina* is an economically important plant of the Arabian and North African region [4, 5]. The occurrence of the associated microbial flora of this important plant is yet to be studied; therefore, looking at the importance and the potential of the plant to withstand extreme environmental conditions, we aimed to assess the endophytes flora of the plant in the present study.

Bacteria and fungi live asymptomatically within the plant tissue without manifesting their presence in the host tissue [6].

A wide variety of endophytes can be observed as colonizing the plant parts, these colonies remain symptomless, and the plant tissue remains unaffected and functional. Several endophytes have been isolated from the tissues of aquatic and terrestrial plants [7, 8]. The wide presence of an endophyte in plant tissue creates an effective barrier preventing an attack of the pathogens to the host plant. It has been observed that metabolites produced by endophytes inhibit the growth of pathogens. Furthermore, the endophytic association of plant enables it to withstand the environmental stress and also from various predations. The plant, in turn, provides shelter and nutrients to the endophytes [9]. The present study aims to isolate endophytes from the selected medicinal plant, *M. peregrina*, and to explore their antimicrobial activities against a number of

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Table 1 Endophytic isolates from *M. peregrina* based on the 16S rRNA sequencing

Code number	16rRNA sequence	Name of the organism	Similarity
MpKL1	GKGTMGAGTC TGACGGAGCA CGCCGCGTGA GTGATGAAGG TTTTCGGATC GTAAAACTCT GTTGTTAGGG AAGAACAAGT ACCGTTCGAA TAGGGCGGTA CCTTGACGGT ACCTAACCAG AAAGCCACGG CTAACTACGT GCCAGCAGCC GCGGTAATAC GTAGGTGGCA AGCGTTGTCC GGAATTATTG GGCGTAAAGC GCGCGCAGGC GGTTCYTTAA GTCTGATGTG AAAGCCCCCG GCTCAACCGG GGAGGGTCAT TGGAAACTGG GGAACTTGAG TGCAGAAGAG GAGAGTGGAA TTCCACGTGT AGCGGTGAAA TGCGTAGAGA TGTGGAGGAA CACCAGTGGC RAAGCGACTC TCTGGTCTGT AACTGACGCT GAGGCGCGAA AGCGTGGGGA GSGAACAGGA TTAGATACYK KGKWRKTCAC	*Bacillus licheniformis*	98.80
MpDT1	GGTCGCAGCC TGATGCAGCC ATGCCGCGTG TATGAAGAAG GCCTTAGGGT TGTAAAGTAC TTTCAGCSGG GAGGAAGGTG ATAAGGTTAA TACCCTTRTC AATTGACGTT ACCCGCAGAA GAAGCACCGG CTAACTCCGT GCCAGCAGCC GCGGTAATAC GGAGGGTGCA AGCGTTAATC GGAATTACTG GGCGTAAAGC GCACGCAGGC GGTCAATTAA GTCAGATGTG AAAGCCCCGA GCTTAACTTG GGAATTGCAT CTGAAACTGG TTGGCTAGAG TCTTGTAGAG GGGGGTAGAA TTCCATGTGT AGCGGTGAAA TGCGTAGAGA TGTGGAGGAA TACCGGTGGC GAAGGCGGCC CCCTGGACAA AGACTGACGC TCAGGTGCGA AAGCGTGGGG AGCAAACAGG ATTAGATACC TKGGGGTAGT CCA	*Proteus mirabilis*	99.53
MpKT2	GMGTAAGTCT GACGGGAGCA CGCCGCGTGA GTGATGAAGG TTTTCGGATC GTAAAGCTCT GTTGTTAGGG AAGAACAAGT ACCGTTCGAA TAGGGCGGTA CCTTGACGGT ACCTAACCAG AAAGCCACGG CTAACTACGT GCCAGCAGCC GCGGTAATAC GTAGGTGGCA AGCGTTGTCC GGAATTATTG GGCGTAAAGG GCTCGCAGGC GGTTTCTTAA GTCTGATGTG AAAGCCCCCG GCTCAACCGG GGAGGGTCAT TGGAAACTGG GGAACTTGAG TGCAGAAGAG GAGAGTGGAA TTCCACGTGT AGCGGTGAAA TGCGTAGAGA TGTGGAGGAA CACCAGTGGC GAAGGCGACT CTCTGGTCTG TAACTGACGC TGAGGAGCGA AAGCGTGGGG AGCGAACAGG ATTAGTTKKG GGGGWWAKTC MAA	*Bacillus subtilis subsp. inaquosorum*	96.71
MpKT3	AGAGAAGTCT GACGGAGCAC GCCGCGTGAG TGATGAAGGT TTTCGGATCG TAAAACTCTG TTGTTAGGGA AGAAYAAGTA CCGTTCGAAT AGGGCGGTAC CTTGACGGTA CCTAACCAGA AAGCCACGGC TAACTACGTG CCAGCAGCCG CGGTAATACG TAGGTGGCAA GCGTTGTCCG GAATTATTGG GCGTAAAGCG CGCGCAGGCG GTTTCTTAAG TCTGATGTGA AAGCCCCCGG CTCAACCGGG GAGGGTCATT GGAAACTGGG GAACTTGAGT GCAGAAGAGG AGAGTGGAAT TCCACGTGTA GCGGTGAAAT GCGTAGAGAT GTGGAGGAAC ACCAGTGGCG AAGGCGACTC TCTGGTCTGT AACTGACGCT GAGGCGCGAA	*Bacillus licheniformis*	99.23
MpKT4	GMGTAAGTCT GACGGAGCAC GCCGCGTGAG TGATGAAGGT TTTCGGATCG TAAAGCTCTG TTGTTAGGGA AGAACAAGTG CGAGAGTAAC TGCTCGCACC TTGACGGTAC CTAACCAGAA AGCCACGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT AGGTGGCAAG CGTTGTCCGG AATTATTGGG CGTAAAGGGC TCGCAGGCGG TTTCTTAAGT CTGATGTGAA AGCCCCCGGC TCAACCGGGG AGGGTCATTG GAAACTGGGA AACTTGAGTG CAGAAGAGGA GAGTGGAATT CCACGTGTAG CGGTGAAATG CGTAGAGATG TGGAGGAACA CCAGTGGCGA AGGCGACTCT CTGGTCTGTA ACTGACGCTG AGGAGCGAAA GCGTGGGGAG CGAACAGGAT TAGATATKSK GGKWWRKYCA AA	*Bacillus pumilus*	98.82

Table 1 continued

Code number	16rRNA sequence	Name of the organism	Similarity
MpDT5	CRKGGMGAGT CTGACGGAGC ACGCCGCGTG AGTGATGAAG GTTTTCGGAT CGTAAAGCTC TGTTGTTAGG GAAGAACAAG TACCGTTCGA ATAGGGCGGT ACCTTGACGG TACCTAACCA GAAAGCCACG GCTAACTACG TGCCAGCAGC CGCGGTAATA CGTAGGTGGC AAGCGTTGTC CGGAATTATT GGGCGTAAAG GGCTCGCAGG CGGTTTCTTA AGTCTGATGT GAAAGCCCCC GGCTCAACCG GGGAGGGTCA TTGGAAACTG GGGAACTTGA GTGCAGAAGA GGAGAGTGGA ATTCCACGTG TAGCGGTGAA ATGCGTAGAG ATGTGGAGGA ACACCAGTGG CGAAGGCGAC TCTCTGGTCT GTAACTGACG CTGAGGAGCG AAAGCGTGGG GAGCGAACAG GATTAGAACK GKGGGKRWAK YMCA	*Bacillus subtilis subsp. inaquosorum*	98.34
MpKT6	AKCAGAAGTC TGACGGAGCA CGCCGCGTGA GTGATGAAGG TTTTCGGATC GTAAAGCTCT GTTGTTAGGG AAGAACAAGT GCGAGAGTAA CTGCTCGCAC CTTGACGGTA CCTAACCAGA AAGCCACGGC TAACTACGTG CCAGCAGCCG CGGTAATACG TAGGTGGCAA GCGTTGTCCG GAATTATTGG GCGTAAAGGG CTCGCAGGCG GTTTCTTAAG TCTGATGTGA AAGCCCCCGG CTCAACCGGG GAGGGTCATT GGAAACTGGG AAACTTGAGT GCAGAAGAGG AGAGTGGAAT TCCACGTGTA GCGGTGAAAT GCGTAGAGAT GTGGAGGAAC ACCAGTGGCG AAGGCGACTC TCTGGTCTGT AACTGACGCT GAGGAGCGAA AGCGTGGGGA GCGAACAGGA TTAGATACTG GGGKRWRKTC AMA	*Bacillus pumilus*	98.12

human pathogens. This study is driven by the fact that infectious microorganisms are developing resistance to the available antibiotics; hence, there is a need for discovering new antibiotics and therapeutic agents that are highly effective against a wide range of pathogens, less toxic and cost-effective. Endophytes were isolated using the method described by [10]. From the various parts of *M. peregrina* such as the root, twig and leaves, growing in the eastern region (Dammam and Alkobar) of Saudi Arabia, samples were collected from each region in a sterilized poly-bag during transportation and stored at 4 °C until use. Then, they were thoroughly washed in running tap water and surface sterilized with 75% (v/v) ethanol. The outer tissues of the root and twig were removed and cut into smaller pieces, and the leaf tissues were excised and macerated with sterile distilled water by sterile mortar and pestle. Then, they were plated separately into minimal media (agar 1.5% w/v) supplemented with antibiotic cycloheximide 30 µg/ml and incubated at 37 ± 2 °C. The isolates were characterized and identified both morphologically and physiologically. The identification of isolates was based on the 16S ribosomal RNA sequences by the Genetic Unit in the Institute for Research and Medical Consultations. Test organisms included: *E. coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Klebsiella ocytota* ATCC700324, *K. pneumonia* ATCC100324, *Acinetobacter baumanii* ATCCmra747, *Enterobacter aerogenes* ATCC13048, *Streptococcus dgalacticae* ATCC12336, *Streptococcus pyogenes* ATCC 19615, *Staphylococcus epidermis* ATCC12228, *Staphylococcus aureus* ATCC24213 and *Candida albicans*. The strains were maintained on agar slants at 4 °C and activated at 37 °C for 24 h on nutrient agar prior to any study. All the endophytes isolates were inoculated on nutrient agar by a single streak of inoculum at the center of the Petri dish, while the unstreaked nutrient agar plate was assigned as a control plate. After 2 days of incubation at 37 °C, all the plates including the control plates were seeded with test bacteria by a single streak at a 90° angle (perpendicular) to the endophytes. Both experimental and the control plates were incubated at 37 °C. The assay was carried out in replicates. Based on the results from the cross-streak method, endophytes isolates from *M. peregrina* leaves (MpKL1) were selected and further inoculated in five numbers of 250-ml Erlenmeyer flasks, each containing 50 ml nutrient broth at 37 °C for 48 h. The seed culture was transferred to five numbers of the 2-L Erlenmeyer flask, each containing 1000 ml nutrient broth at 37 °C for 7 days to obtain a good quantity of pellet. The culture cell pellet was obtained by centrifugation (15000 rpm), and the supernatant was extracted with two types of organic solvents: chloroform/methanol 5:1 (MpKL1 cm) and ethyl acetate (MpKL1ea) in a separating funnel with vigorous shaking. The organic extract was recovered and then the organic solvent was evaporated by rotary evaporation at 30 °C. The dry weight of organic extract was taken and dissolved in dimethyl sulfoxide (DMSO) for further studies [11]. The *Moringa* plant species are a valuable source of useful metabolites that has

Table 2 Screening of endophytic isolates for their antimicrobial activity using Cross-streak method

Identification results	Isolate code	Test organisms (pathogens)*										
		Gram-negative Bacteria						Gram-positive bacteria				Yeast
		1	2	3	4	5	6	7	8	9	10	11
Bacillus licheniformis	MpKL1=5	+++	+++	+++	+++	++	+	+++	++	+++	+++	+++
Proteus mirabilis	MpDT1	+++	+++	+	–	–	+	++	+	+++	+++	++
Bacillus subtilis subsp. inaquosorum	MpKT2	+	+++	+	+	–	+	–	–	++	–	+
Bacillus licheniformis	MpKT3	+	+++	–	–	–	–	+	+	++	–	+
Bacillus pumilus	MpKT4	++	–	–	+	+++	+	–	–	++	–	–
Bacillus subtilis subsp. inaquosorum	MpDT5	+	+	–	–	–	–	–	–	–	+++	++
Bacillus pumilus	MpKT6	–	+	–	–	–	–	–	–	–	–	+

+ = less active, ++ = moderately active, +++ = highly active, - =No activity

*(1) *E. coli* ATCC25922, (2) *Pseudomonas aeruginosa* ATCC27853, (3) *Klebsiella ocytota* ATCC700324, (4) *Klebsiella pneumonia* ATCC100324, (5) *Acinetobacter baumanii* ATCCmra747, (6) *Enterobacter aerogenes* ATCC13048, (7) *Streptococcus dgalacticae* ATCC12336, (8) *Streptococcus pyogenes* ATCC 19615, (9) *Staphylococcus epidermis* ATCC12228, (10) *Staphylococcus aureus* ATCC24213, (11) *Candida albicans*

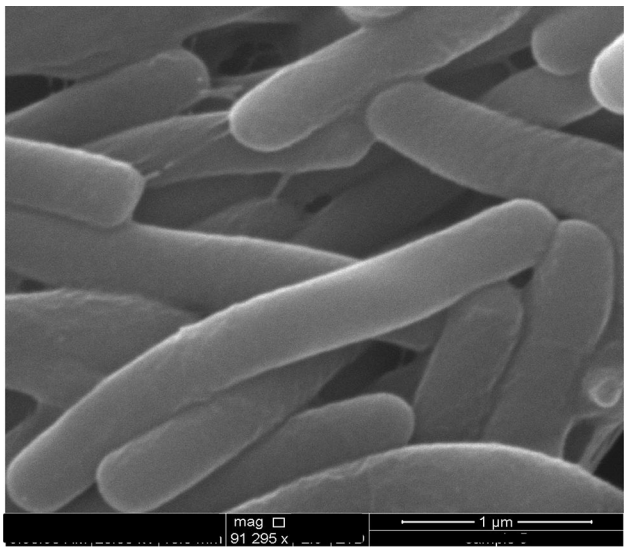

Fig. 1 Scanning electron microscopy of *B. licheniformis* MpKL1

been known for decades. In the present study, seven endophytic isolates were obtained from the samples of *M. peregrina*, and this result emphasizes that all types of plant species harbor endophytic bacteria. This finding is inconsistent with other researchers where they had stated that the aerial plant parts have a higher endophytic content than underground parts [12]. The result based on the 16S rRNA sequencing revealed two bacterial genus *Proteus mirabilis* and *Bacillus*; three species belong to *Bacillus*: *B. licheniformis, B. subtilis* subsp. *Inaquosorum* and *B. pumilus.* This finding revealed that the *Bacillus* species is prevalent in this plant (Table 1). Few studies reported endophytic isolate from *M. peregrina*. Khan et al. [13] isolated two fungi genus *Aspergillus caespitosus* LK12 and *Phoma* sp. LK13. Interestingly, there is one study that reported endophytic bacterial isolate from this plant by Khan *et al.* [14] who isolated five endophytic bacteria: *Methylobacterium radiotolerans*, two species of *Sphingomonas* sp. and two strains of *Bacillus subtilis*. However, isolation of endophytic bacteria from different plants was reported by Amaresan et al. [15] who isolated 37 endophytic bacteria from chili plant. Endophytes are a group of microorganisms that could serve as dependable source of new and highly useful compounds with potential for exploitation in pharmaceutical and agricultural areas [16]. As pathogenic organisms are developing resistance to the majority of antibiotics available, it is important to find new antibiotics to tackle this problem. The result reveals that all endophytic isolates exhibited antagonistic activity against two or more of the pathogenic human microbes (Table 2). Moreover, *M. peregrina* Alkobar leaf 1 (MpKL1) isolate showed promising antimicrobial activity against all the test organisms with moderate to less activity against only a few organisms such as *A. baumanii*, *S. pyogenes* and *E. aerogenes*. The results of this study in agreement with several researches showed endophytic bacteria exhibit antimicrobial activity against human pathogenic microbes [17]. Also, using low concentrations of the active substance negatively affects their efficacy. Thus, it is important to determine the MIC of the active ingredient and, moreover, to reduce the possible cell toxicity risk of the active substance.

MpKL1 was further studied for its MIC values. The MIC of MpKLea (ethyl acetate extract) showed a high inhibitory effect against gram-negative bacteria *E. coli*, *P. aeruginosa* at 125 µg/ml and *K. ocytota*, *K. pneumonia* at 250 µg/ml. Also gram-positive bacteria *S. epidermis* and *S. aureus* highly inhibited at 250 µg/ml (Fig. 1).

Table 3 MIC (μg/ml) determination of the organic extract of MpKL1 against human pathogens (1–11)

Organic extract	Test organisms (pathogens)*										
	Gram-negative bacteria						Gram-positive bacteria				Yeast
	1	2	3	4	5	6	7	8	9	10	11
MpKLea	125	125	250	250	>1000	>1000	500	>1000	500	250	500
MpKLcm	250	250	250	250	>1000	>1000	500	>1000	125	500	>1000
Positive control Ciprofloxacin	0.5	2	2	0.5	8	4	8	4	0.25	0.25	4

*(1) *E. coli* ATCC25922, (2) *Pseudomonas aeruginosa* ATCC27853, (3) *Klebsiella ocytota* ATCC700324, (4) *Klebsiella pneumonia* ATCC100324, (5) *Acinetobacter baumanii* ATCCmra747, (6) *Enterobacter aerogenes* ATCC13048, (7) *Streptococcus dgalacticae* ATCC12336, (8) *Streptococcus pyogenes* ATCC 19615, (9) *Staphylococcus epidermis* ATCC12228, (10) *Staphylococcus aureus* ATCC24213, (11) *Candida albicans*

Moreover, MpKLcm (chloroform/methanol extract) also showed high inhibition effect at 250 μg/ml against gram-negative bacteria *E.coli*, *P. aeruginosa*, *K. ocytota* and *K. pneumonia*, whereas MIC of MpKLcm raised up to 500 μg/ml to inhibit the growth of gram-positive bacteria *S. dgalacticae* and *S. aureus.* Also, MIC of MpKLea raised up to 500 μg/ml to inhibit the growth of *S. dgalacticae*, *S. epidermis* and *Candida albicans.* In general, both the extracts were not found very active against *A. baumanii*, *E. aerogenes, S. pyogenes* and *Candida albicans* with MIC > 1000. Ciprofloxacin was used as a standard antibiotic (Table 3).

This assay confirms that there is an appreciable degree of antibacterial activity against human pathogenic organisms. The future prospect of the study is the economic feasibility of the antibacterial drug development from these isolated endophytes. This finding is in agreement with several studies which revealed that endophytic bacteria have been proven as reliable sources of novel bioactive compounds and the richest source of secondary metabolites.

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References

1. Alaklabi A (2015) Genetic diversity of *Moringa peregrina* species in Saudi Arabia with ITS sequences. Saudi J Biol Sci. https://doi.org/10.1016/j.sjbs.2014.09.01
2. El-Alfy TS, Ezat SM, Hegazy AK, Amer AMM, Kamel GM (2011) Isolation of biologically active constituents from *Moringa peregrina* (Forsk.) Fiori (Family: Moringaceae) growing in Egypt. Pharmacogn Mag 26:109–115
3. Rahman IM, Barua S, Begum ZN, Rahman AM, Hasegawa H (2009) Physicochemical properties of *Moringa oleifera* Lam. Seed oil of the indigenous cultivar. J Food Lipids 16:540–553
4. Ghodsi R, Sadeghi HM, Asghari G, Torabi S (2014) Identification and cloning of putative water clarification genes of *Moringa peregrina* (Forssk.) Fiori in *E. coli* Xl1 blue cells. Adv Biomed Res 27:3–57
5. Hegazy AK, Hammouda O, Lovett-Doust J, Gomaa NH (2008) Population dynamics of *Moringa peregrina* along altitudinal gradient in the northwestern sector of the Red Sea. J Arid Environ 72:1537–1551
6. Firáková S, Šturdíková M, Múčková M (2007) Bioactive secondary metabolites produced by microorganisms associated with plants. Biologia. https://doi.org/10.2478/s11756-007-0044-1
7. Dutta D, Puzari KC, Gogoi R, Dutta P (2014) Endophytes: exploitation as a tool in plant protection. Braz Arch Biol Technol. https://doi.org/10.1590/S1516-8913201402043
8. Hardoim PR, van Overbeek LS, Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol. https://doi.org/10.1016/j.tim.2008.07.008
9. Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic, London
10. Arnold AE, Henk DA, Eells RL, Lutzoni F, Vilgalys R (2007) Diversity and phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and environmental PCR. Mycologia. https://doi.org/10.3852/mycologia.99.2.185
11. Amanda SM, Florencia CO, Adriano B (2004) Screening for antimicrobial activity among bacteria isolated from the Amazon basin. Braz J Microbiol 35:307–310
12. El-Deeb B, Khalaf F, Youssuf G (2013) Isolation and characterization of endophytic bacteria from *Plectranthus tenuiflorus* medicinal plant in Saudi Arabia desert and their antimicrobial activities. J Plant Interact 8:56–64
13. Al Khan, Waqas M, Hussain J, Al-Harrasi A, Al-Rawahi A, Al-Hosni K (2014) Endophytes *Aspergillus caespitosus* LK12 and *Phoma* sp. LK13 of *Moringa peregrina* produce gibberellins and improve rice plant growth. J Plant Interact. https://doi.org/10.1080/17429145.2014.917384
14. Al Khan, Boshra A, Elyassi A, Ali S, Al-Hosni K, Hussain J, Al-Harrasi A, In Jung L (2016) Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum*. Electron J Biotechnol 21:58–64
15. Amaresan N, Jayakumar V, Thajuddin N (2014) Isolation and characterization of endophytic bacteria associated with chilli.

(*Capsicum annuum*) grown in coastal agricultural ecosystem. Indian J Biotechnol 13:247–255
16. Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev. https://doi.org/10.1128/mmbr.67.4.491-502.2003
17. Seo W, Lim W, Kim E, Yun H, Lee Y, Cho K (2010) Endophytic bacterial diversity in the young radish and their antimicrobial activity against pathogens. J Korean Soc Appl Biol Chem 53:493–503