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Molecular Biodiversity of Bacteria Isolated from *Medicago sativa* Rhizosphere in Ha'il District, Saudi Arabia

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ABSTRACT

Worldwide biodiversity is impacted strikingly by global environmental change, and thus its impact is reflected in all life aspects. Identifying microorganisms in environmental samples, particularly soil could be a valuable interest to study their effect on soil quality and plant growth. Through this study, we conducted a molecular characterization of bacteria found in the rhizosphere of *Medicago sativa* plants grown in Hai'l soil and we highlighted their main properties. The analysis of sequences revealed that the main bacterial isolates were *Pseudarthrobacter*, *Metabacillus*, *Priestia*, and *Massilia* species. According to the sequence analyses and the phylogeny tree results, some of the identified bacteria were classified at the species level: one of the *pseudarthrobacter* isolates *was* identified clearly as *Pseudarthrobacter phenanthrenivorans*; *Metabacillus* isolates grouped with *Metabacillus sediminilitoris* and the two *Priestia* isolates closely related to *Priestia aryabhattai*. We concluded that Hai'l soil is a niche of diverse bacteria with a high interest in soil environment and ecosystems. Further studies are required for further classification of all identified bacteria and to define their specific role in the environment.

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Introduction

Biodiversity is a term given to all living things on the Earth, and biodiversity includes all organisms from the smallest to the largest and from the simplest to most sophisticated creatures. The term refers to genetic variation, ecosystem variation, and species variation within an area, biome, or planet (1). It is also defined as the variation of living organisms derived from all sources, including terrestrial and marine ecosystems. This variation includes biological diversity within species, as well as between different ecosystems.

In the last decades, the world faced changes in the environment due to climate change, which emerged as a priority crucial issue, which got global attention (2). Climate change affects all aspects of life including soil bacteria, which in turn affect agriculture leading to food insecurity (3).

To address this issue, the most efficient way is to work on the availability of basic information about the targeted bacteria and exploit them in addressing environmental emergent issues that affect life like pollution and abiotic stresses. The reason behind that is these bacteria work together in the consortium as a community in the environment to maintain it clean and maintain plant health and survival under environmental stresses (4,5). In addition, this approach is low-cost and eco-friendly technology.

In bioremediation and inoculation approaches either one uses the micro-organisms already present at the site of contamination which is called "intrinsic bioremediation" or inoculums composed of micro-organisms characterized by special catabolic properties which is called "bioaugmentation" (6). Therefore, this study was conducted to identify and characterize the beneficial bacteria found in the soil around *Medicago*

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sativa plants, thus contributing to the availability of basic information about the beneficial bacteria inhabiting Hai'l soil as a step toward application.

Materials and Methods

Study area

Ha'il is located in the northwestern region of the KSA (27° 31′ 0″ N, 41° 41′ 0″ E). This area is considered as an agricultural and pastoral province, characterized by rich water resources, fertile soil, and a temperate climate. This has resulted in agricultural development, based on agricultural products, e.g. grains, dates, vegetables, forage crops, and fruit production. In recent history, a large percentage of the Kingdom's wheat production came from Ha'il Province, as the area to the North East of Ha'il, 60 km to 100 km away, predominantly consists of irrigated gardens. Nowadays, different crops and fruit trees are cultivated in Ha'il, including barley, corn, vine trees, date palms, citruses, and other economical crops.

Isolation of Bacteria

Medicago sativa roots were collected from the Agricultural plantation garden (APG) in the study area. The APG is periodically replenished from the communal water supply system. The APG is located in a typical urban environment, in the North of the Ha'il city, and is exposed predominantly to traffic-related pollution. The plant roots were washed thoroughly in sterilized distilled water to remove larger contaminants. Bacterial cultures were initiated by transferring 100 µL of suspension from Yeast Extract Maintol Agar (YEMA) medium containing g/l: mannitol, 10; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.4; yeast extract, 1; agar, 15. The colonies were sub-cultivated several times to obtain purity. All rounds of cultivation were done at +27ºC. Colonies were stained according to Gram and checked under the microscope for stain reaction and purity of colonies.

DNA isolation and16srRNA gene amplification

We extracted the genomic DNA from the different isolates, then sent it to Macrogen, Inc., Seoul, Korea. The extracted DNA was used as a template in a 30-µL reaction mixture by using an EF-Taq DNA polymerase (SolGent, South Korea). To amplify 16srRNA, forward primer 27F5`(AGAGTTTGATCMTGGCTCAG)3`and reverse primer 1492R5`(TACGGYTACCTTGTTACGACTT)3` were used. PCR reactions were set as follows: 2 µl of 10x Taq PCR buffer, 1.6 µl dNTPs, 1 µl forward primer, 1 µl reverse primer, 1 µl template DNA, 0.2 µl KOMA *Taq* polymerase, and 13.2 µl double distilled water to make a total volume of 20 µl. PCR conditions were: one cycle of an initial denaturation at 95°C for 1 minute, 30 cycles of denaturation at 95°C for 30seconds, annealing at 55°C for 2 minutes, extension at 68°C for 1.5 minutes, and one cycle of final extension at 68°C for 10 minutes. PCR products were purified and sequenced.

16SrRNA Gene Sequencing

Sequencing was done by using Big Dye Terminator Cycle sequencing kit v.3.1 (Applied Biosy, USA). The primers used for sequencing were 785F 5' (GGA TTA GAT ACC CTG GTA) 3' and 907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'. The Sequences were resolved on Applied BioSystems model 3730 XL automated DNA Sequencing System at Macrogen, Inc., Seoul, Korea. The G+C content was calculated using APE (A plasmid Editor) software version 8.5.2.0. Sequences were deposited in GenBank under accession numbers: OM019337.1, OM021310, OM023870, OM023867, OM023887, OM033657, OM149777, and OM149810.

Data analysis

The algorithm Basic Local Alignment Search Tool (BLASTN) was used for sequence analyses of the different genes. Phylogeny tree analyses were done online at www.phylogeny.fr (7-11).

Results and discussion

The sequence analyses revealed that the isolates obtained from the rhizosphere of *Medicago sativa* grown in the Hail district are a genus of *Pseudarthrobacter*, *Metabacillus*, *Priestia*, and *Massilia*, two isolates for each (Table 1 and 2). The GC content of the different isolates obtained in this study including *Pseudarthrobacter* spp ranged from 53% to 57% at the time, and the GC% of the other *Pseudarthrobacter* strains studied before ranged from 64.4% to 70.8% (Table 1 and 2). These differences in GC% may be due to different environments from where the isolates were obtained and also may indicate that the isolates in this study are characterized by special

genetic traits. The importance of GC content represents in it is "effect on the accuracy of 16SrRNA sequencing" as a result of "PCR bias" (12).

Table 1. Different isolates obtained in this study and their GC contents

#	Isolate	GC %
1	*Pseudarthrobacter* Hail1	57
2	*Pseudarthrobacter* Hail2	55
3	*Metabacillus* Hail1	54
4	*Metabacillus* Hail2	53
5	*Priestia* Hail1	53
6	*Priestia* Hail2	53
7	*Massilia* Hail1	53
8	*Massilia* Hail2	53

Table 2. GC% of the different *Arthrobacter* studied before

Strain	GC%	References
Arthrobacter chlorophenolicus	65.1	(Westerberg et al., 2000)
Pseudarthrobacter phenanthrenivorans	65.7	(Kallimanis et al., 2009)
Arthrobacter defluvii	64.4	(Kim et al., 2017)
Arthrobacter siccitolerans	65.3	(Santacruz-Calvo et al. 2013)
Arthrobacter humicola	67	(Kageyama et al., 2008)
Arthrobacter oryzae	67	(Kageyama et al., 2008)
Arthrobacter niigatensis	70.8	(Ding et al., 2009)
Arthrobacter globiformis	65.9	(Sahoo et al., 2019)
Pseudarthrobacter enclensis	67.1	(Busse and Schumann, 2019)
Massilia rhizosphaerae	66.3	(Li et al., 2021).

One isolate of *Pseudarthrobacter* was found related to *Pseudarthrobacter enclensis*, *Arthrobacter nitrophenolicus* and *Arthrobacter oryzae* with identity 99.27%, 98.55%, and 98.40%, respectively. The second *Pseudarthrobacter* isolate was classified as *Pseudarthrobacter siccitolerans* with 99.40% identity, the same isolate also related to *Arthrobacter pass* with 98.81% identity. Both *Metabacillus* isolates were similar to *Metabacillus sediminilitoris* 99.71% and 99.77%. The two *Priestia* isolates are closely related to *Priestia aryabhattai* one with 99.71% identity and another with 99.89%. On the other hand, the sequence analyses ensured that *Massilia* isolates are *Massilia ginsengisoli* 98.10% and *Massilia rhizosphaerae* 97.57%.

The phylogeny tree ensures the sequence analyses results in BLASTN to some extent, it is shown that one *Pseudarthrobacter* isolated in this study from the rhizosphere of *Medicago sativa* plants grouped with *Pseudarthrobacter phenanthrenivorans*, *Pseudarthrobacter defluvii*, *Pseudarthrobacter siccitolerans*, *Arthrobacter oryzae*, *Arthrobacter humicola*, *Arthrobacter globiformis*, and *Pseudarthrobacter niigatensis*. However, the second isolate which was designated in this study as *Pseudarthrobacter* Hai'l2 was grouped with *Pseudarthrobacter phenanthrenivorans*. There is strong evidence of a genetic relationship between the isolates obtained in this study and the other *Pseudarthrobacter species* found in the same group (Figure 1). This result is partially the same as reported before by Santacruz-Calvo et al. 2013 (13) that *Arthrobacter siccitolerans* are genetically close to *Pseudarthrobacter phenanthrenivorans*. These findings indicate that not all bacteria isolated in this study can be identified at the species level because they were found to interfere and related to more than one species except *Pseudarthrobacter* Hai'l2 which was identified as *Pseudarthrobacter phenanthrenivorans*, the two *Metabacillus* isolates were grouped with *Metabacillus sediminilitoris* and *Priestia* Hai'l1 appeared as *Priestia aryabhattai* according to sequence analyses as mentioned above and the phylogeny tree (Figure 1). The reason for this may be the limitations of 16SrRNA to identify species, due to the possibility of genetic recombination and horizontal gene transfer occurrence which lead to sequence mosaicism (14,15,16). Another reason is that in "bacterial identification based on the analysis of 16SrRNA genes, closely related species cannot always be differentiated because of high levels of sequence conservation"(17). Despite this, the phylogeny tree analysis results seem to be more reliable than BLASTN, because the first show the exact place of the bacteria between the already identified species which help in the identification of the unknown species.

All the above-mentioned isolates are of environmental importance and play a crucial role in addressing environmental pollution through bioremediation processes, promoting plant growth, and addressing abiotic stresses. Previously it is reported that *Arthrobacter* and other plant-associated bacteria play role in environment cleaning and promoting plant growth. Despite this, there is a scarcity of information about these groups, as they are not documented well in Saudi Arabia according to our knowledge. This group of bacteria requires exploring their diversity and roles in addressing climate change challenges. The genus *Arthrobacter* is drought-tolerant and found in a wide range of the environment including human and animal specimens and diets (13, 18). These bacteria can grow

under harsh conditions because they are characterized by a versatile metabolism, which makes them relevant to the environment. Besides, they have the ability to establish a symbiotic association with different plant species (19) and mitigate stress in harsh environments (20). In addition, some *Arthrobacter* spp. are cold-tolerant which can be exploited at low environmental temperatures and applied in bioremediation (21).

There are many isolates of *Arthrobacter* and *Pseudarthrobacter* obtained from the soil in different countries and different environments were found to support this idea. For example, Kim et al. (22) isolated *Pseudarthrobacter siccitolerans* from the soil. Furthermore, *Arthrobacter defluvii* isolated from sewage in Korea was found to degrade 4-chlorophenol, it grows at an alkaline pH range of 6 –10 and NaCl up to 5 % (23) In addition to chlorophenol degradation, *Pseudarthrobacter defluvii also degrades* Phthalic acid esters, which are a class of biologically accumulated carcinogenic and teratogenic toxic chemicals that exist widely in the environment (24).

On the other hand, *Pseudarthrobacter phenanthrenivorans* which grows at 4–37℃)(25) was isolated from soil containing charred wood remnants and was found to contain several genes including *lgdA* gene which is responsible for levoglucosan dehydrogenase (LGDH) activity (26). Levoglucosan is the most prevalent soluble carbohydrate remaining after high-temperature pyrolysis of lignocellulosic biomass (26). Besides cleaning up the environment from pollutants, some isolates obtained in this study related to those characterized before and were reported to promote plant growth, exhibit antibiotic activities, and ameliorate and tolerate different environmental stresses. These isolates include *Arthrobacter siccitolerans*, which are isolated from the rhizosphere in Spain, and described as a "desiccation-tolerant and neuroprotectant-producing strain", it reduces nitrites to nitrogen, produces Indole and acetoin (7). In addition, *Arthrobacter oryzae* and *Arthrobacter humicola* were isolated from paddy soil in Japan, they tolerate up to 2 % and 3% NaCl and they have the ability to grow at pH values between 6 – 11 and 6 – 10, respectively (27). Like that, *Arthrobacter niigatensis* also grow at a temperature range of 5-40 ℃, tolerates 7 % NaCl and the pH range for its growth is 6 –11 (28).

As mentioned above in this study one of our isolates related to *Arthrobacter pascens* according to sequences analysis which was reported before to produce a growth-promoting substance (29). In addition, *Arthrobacter pascens* isolated from the rhizosphere of forest soil in China reported a salt-tolerant bacterial strain, that secretes IAA, dissolves inorganic phosphorus, and degrades persistent organic (POPs) pollutants like polycyclic aromatic hydrocarbons (PAHs) such as fluoranthene (30). Like that, *Arthrobacter globiformis* to which *Pseudarthrobacter* Hai'l2 is related was isolated from a cave deposit in India and contained biosynthetic genes for encoding putative secondary metabolites and other antibiotics and showed antimicrobial activity(31). *Arthrobacter globiformis* was also found to ameliorate salt stress, which suggests the possibility to use this strain as inoculum for plants growing in highly saline soils (32). This bacterium also contains important enzymes that can be harnessed in the industry like the Diamine Oxidase (DAO), which has great potential in the fishery industry and in detecting biogenic amines in the biological fluids and the environment that may give rise to health issues (33).

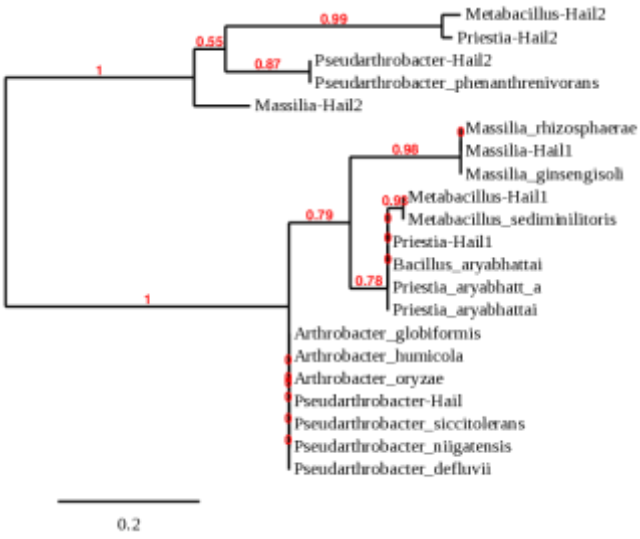

Figure 1. Phylogeny tree of the different isolates obtained from rhizosphere of *Medicago sativa*
*Both *Bacilus aryabhattai* and *Priestia aryabhattai* are used because they are deposited in the gene bank by different authors although the first is synonymous with the other)

The same as *Pseudarthrobacter*, *Priestia* spp. also plays a crucial role in environmental cleaning. For example, *Priestia aryabhattai* degrades benzoate as a sign of the ability to degrade aromatic compounds which can be useful in fertilizers and pesticide degradation. The presence of like these soil bacteria

which contain ligninolytic enzymes and tyrosinase may indicate an active role they play in soil fertility (34). It is able to degrade organophosphorus compounds (OPs) used as effective insecticides. As a result, using too many OPs causes the residues of pesticides to be washed away into the water or soil, not only polluting water and soil but also directly or indirectly affecting the environment and human health. They are very dangerous neurotoxins to humans, animals, and the environment. Using these strains shows the potential for biological decontamination of the pesticide and solving environmental pollution spots (35).

Recently, for the development of biological products, preference has been given to strains that combine such useful properties as the biocontrol of phytopathogens and stimulation of plant growth, as well as the ability of the biodegradation of xenobiotics (34). In addition to the role, the isolates in this study are expected to play in the environment, *Massilia* spp. were found to play role in the biological control of fungi like *Massilia rhizosphaerae* which was recently isolated from rhizosphere soil of rice and characterized as Gram-stain-negative and show antibacterial activity (30).

On the other hand, *Metabacillus sediminilitoris* obtained in this study was also characterized before as Gram-stain-positive, endospore-forming bacteria, grow at 16–47°C and alkaline pH 6.0–10.0 and tolerate NaCl up to 7% (w/v). The G+C content of its genomic DNA was 35.7mol% (36). Species of the genus *Metabacillus* are ubiquitous in natural environments and have been isolated from a wide variety of habitats, including soil, a hypersaline lake and a marine coastal region (37).

Based on the above discussion, the association of the different bacteria with *Medicago sativa* rhizosphere can be interpreted by the different roles these bacteria can play to contribute in plant growth, health, and availability of different plant exudates used by these microbes as energy sources. This assumption is supported by what was stated before that the interaction between the plants and their associated microbes is achieved by a consortium of different microbes, which integrate to help in more efficient nutrient mobilization (38).

The rhizosphere is a microecological area near the plant root, where rapid and numerous chemical interactions take place and its environment is more competitive than the soil mass. This environment is divided into three regions: internal, middle and external, based on proximity to the root and the extent of its impact. Compounds added to the soil by the roots are classified into four categories: exudates (passively removed from the roots), secretions (actively removed from the roots), dead cells, and gaseous compounds. The compounds in the substances left by the roots, by acidifying or changing the redox conditions in the rhizosphere or directly chelating the elements, help to provide nutrients such as nitrogen, phosphorus, iron, etc. As the soil dries, the hydraulic potential decreases, after which the root seepage begins to return water to the soil, increasing the degree of stability of the rhizosphere (39-48).

Finally, since the isolates obtained in this study are genetically related to the already characterized isolates, it is expected that they play the same role in the environment.

Conclusions

Diverse bacteria inhabit the rhizosphere of *Medicago sativa* grown in Hai'l soil; they may play a positive role in cleaning up the environment, promoting plant growth, and ameliorating different abiotic stresses that face plants. However, characterization of these bacteria using housekeeping genes can determine their species, and “Omics” studies are required to prove the ability of these bacteria to play the fore mentioned roles.

Interest conflict

The authors declare that they have no conflict of interest.

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References

1. Rawat US Agarwal NK. Biodiversity: Concept, threats and conservation. Environ Conserv J. 2015; 16(3) 19-28, 2015.
2. Ullah A, Nisar M, Ali H, Hazrat A, Hayat K, Keerio AA, Ihsan M, Laiq M, Ullah S, Fahad S, Khan A, Khan AA, Akbar A ,Yang X: Drought tolerance improvement

in plants: an endophytic bacterial approach. Appl Microbiol and Biotech, 2019; 103:7385-7397.
3. Choudhary DK, Sharma AK, Agarwal P, Varma A, Tuteja N: Volatiles and food security. Springer, Singapore. In: Vaishnav A, Shukla A.K, Sharma A, Kumar R, Choudhary DK, 2017.
4. Vaishnav A, Shukla AK, Sharma A, Kumar R, Choudhary DK. Endophytic Bacteria in Plant Salt Stress Tolerance: Current and Future Prospects. J. of Plan Grow Regul, 20.18; 20
5. Mart'ınez-Hidalgo P, Hirsch AM: The nodule microbiome: N2-fixing rhizobia do not live alone. Phytob 2017; 1-13.
6. Westerberg K, Elväng AM, Stackebrandt E, Jansson JK: *Arthrobacter chlorophenolicus* sp nov, a new species capable of degrading high concentrations of 4-chlorophenol. Int J Syst Evol Microbiol 2000; 50 (6):2083–2092. https://doi.org/10.1099/00207713-50-6-2083.
7. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S et al: Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Research, 2008;1; 36(Web Server issue): W465-9.
8. Edgar RC: Muscle, multiple sequence alignment with high accuracy and high throughput. Nucl Aci Res 2000; 32 (5): 1792-7.
9. Guindon S, Gascuel O: A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. System Biol 2003; 52(5): 696-704.
10. Anisimova M, Gascuel O: Approximate likelihood-ratio test for branches: A fast, accurate and powerful alternative. System Biol 2006; 55(4): 539-52.
11. Chevenet F, Brun C, Banuls AL, Jacq B, Chisten R: TreeDyn: towards dynamic graphics and annotations for analyses of trees. BMC Bioinform 2006; 7:439.
12. Laursen MF, Dalgaard MD and Bahl MI: Genomic GC-Content Affects the Accuracy of 16S rRNA Gene Sequencing Based Microbial Profiling due to PCR Bias. Front. Microbiol., 2017; 8:1934. doi: 10.3389/fmicb.2017.01934.
13. Santacruz-Calvo L, González-López J, Manzanera M. *Arthrobacter siccitolerans* sp. nov., a highly desiccation-tolerant, xeroprotectant producing strain isolated from dry soil. Int J Syst Evol Microbiol., 2013; 63(11):4174 -4180.
14. Neto IVR, Ribeiro RA, Hungria M: Genetic diversity of elite rhizobial strains of subtropical and tropical legumes based on the 16S rRNA and glnII genes. World J Microbiol Biotechnol., 2010; 26: 1291-1302.
15. van Berkum P, Terefework Z, Paulin L, Suomalainen S, Lindstrom K, Eardly BD: Discordant phylogenies within the rrn loci of rhizobia. J Bacteriol., 2003; 185: 2988–2998.
16. Vinuesa,P, Silva C, Werner D, Martinez-Romero E: Population genetics and phylogenetic inference in bacterial molecular systematics: the roles of migration and recombination in Bradyrhizobium species cohesion and delineation. Mol Phylogenet Evol 34: 29-54.
17. Martens M, Delaere M, Coopman R, De Vos P, Gillis M, Willems A: Multilocus sequence analysis of Ensifer and related species. Int J Syst Evol Microbiol., 2007; 57: 489-503.
18. Zhang J, Ma Y, Yu H: Arthrobacter cupressi sp. nov., an actinomycete isolated from the rhizosphere soil of *Cupressus sempervirens*. Int J Syst Evol Microbiol 2012; 62(11): 2731–2736.
19. Zhao S, Zhou N, Zhao ZY, Zhang K, Wu GH, Tian CY: Isolation of endophytic plant growth-promoting bacteria associated with the halophyte *Salicornia europaea* and evaluation of their promoting activity under salt stress. Curr Microbiol. 2016; 73: 574–581. In: Panaiotis M. S., Rossi, M., Borromeo, I., Capo, C., Beninati, S, Forni, C: Amelioration of salt stress tolerance in rapeseed (*Brassica napus*) cultivars by seed inoculation with *Arthrobacter globiformis*, Plan Biosyst -, 2020; DOI: 10.1080/11263504.2020.1857872.
20. Panaiotis MS, Rossi M, Borromeo I, Capo C, Beninati S, Forni C. Amelioration of salt stress tolerance in rapeseed (Brassicanapus) cultivars by seed inoculation with *Arthrobacter globiformis*, Plant Biosystems -, 2020; DOI: 10.1080/11263504.2020.1857872.
21. Abdulrasheed M, Zakaria NN, Roslee AFA, Shukor MY, Zulkharnain A, Napis S, Convey P, Alias SA, Gonzalez-Rocha G,Ahmad SA: Biodegradation of diesel oil by cold-adapted bacterial strains of *Arthrobacter* spp. from Antarctica. Antarctic Science: 2020; 13. doi:10.1017/S0954102020000206.
22. Kim J, Jang J, Maeng S, Kang M. Kim MK: A report of 14 unrecorded bacterial species in Korea isolated in 2017. J. of Spec Res, 7(2): 161-180.
23. Kim KK, Lee KC, Oh H, Kim MJ, Eom MK, Lee J,: *Arthrobacter defluvii* sp. nov., 4-chlorophenoldegrading bacteria isolated from sewage. Intern J of System and Evolution Microbiol 2008; 58, 1916–1921. DOI 10.1099/ijs.0.65550-0.
24. Chen F, Chen Y, Chen C, Feng L, Dong Y, Chen J, Lan J, Hou H: High-efficiency degradation of phthalic acid esters (PAEs) by *Pseudarthrobacter defluvii* E5: Performance, degradative pathway, and key genes. Scien Tot Environ 2021; 794: 148719.
25. Kallimanis A, Kavakiotis K, Perisynakis A, Spro¨er, C, Pukall R, Drainas C, Koukkou AI: *Arthrobacter phenanthrenivorans* sp. nov., to accommodate the phenanthrene-degrading bacterium *Arthrobacter* sp. strain Sphe3. Int J Syst Evol Microbiol., 2009; 59: 275–279.
26. Arya AS, Hang MTH, Eiteman MA: Isolation and characterization of levoglucosan metabolizing bacteria. Appl Environ Microbiol, 2021; 50
27. Kageyama A, Morisaki K, Omura S, Takahashi Y. *Arthrobacter oryzae* sp. nov. and *Arthrobacter humicola* sp. nov. Intern J System and Evolutio Microbiol 2008; 58: 53–56. DOI 10.1099/ijs.0.64875-0.
28. Ding L, Hirose T, Yokota A: Four novel *Arthrobacter* species isolated from filtration substrate. Int J Syst Evol Microbiol., 2009; 59: 856–862.
29. Chaplin C E: Life cycles in *Arthrobacter pascens* and *Arthrobacter terregens*. Can J of Microbiol 2011; 3(2): 103-106.

30. Li C, Cao P, Du, Zhang X, Bing H, Li L, Sun P, Xiang W, Zhao J, Wang Z: *Massilia rhizosphaerae* sp. nov., a rice-associated rhizobacterium with antibacterial activity against *Ralstonia solanacearum*. Inter J System and Evolution Microbiol 2021; 71 (9) (Abstract)
31. Sahoo D, Devi NJ, Ngashangva N, Momota P, Rojeena Y, Indira DS: Draft genome sequence of *Arthrobacter globiformis* mrc11, an antimicrobial agent isolated from a Khangkhui cave deposit. Microbiol Resour Announc., 2019; 8:e01620-18
32. Nouioui, I., Carro, L., GarcIa-Lopez, M., Meier-Kolthoff, J.P., Woyke, T., Kyrpides, N.C., Pukall, R., Klenk, H.-P., Goodfellow, M., Goker, M: Genome-based taxonomic classification of the phylum Actinobacteria, Front Microbiol 2018; 9:2007.
33. Razali NN, Hashim NH, Leow ATC, Salleh A: Conformational design and characterization of a truncated diamine oxidase from *Arthrobacter globiformis*. HigThrough 2018; (7) 21: 16 page. doi:10.3390/ht7030021
34. Esikova TZ, Anokhina TO, Abashina TN, Suzina, N. E, Solyanikova IP: Characterization of soil bacteria with the potential to degrade benzoate and antagonistic to fungal and bacterial phytopathogens. Microorg, 2021; 9, 755. https://doi.org/10.3390/ microorganisms9040755.
35. Le TH, Hoang QC, Vu DD, Vo T HT: Biodegradation of organophosphorus insecticide methyl parathion by soil microorganisms. E3S Web of Conferences 265, 03002 APEEM 2021. https://doi.org/10.1051/e3sconf/202126503002.
36. Mao H, Wei Y, Gao Y, Pei, J, Zhang Y, Fang J: *Metabacillus sediminilitoris* sp. nov., a marine bacterium isolated from tidal sediment. Int J Syst Evol Microbiol., 2020; 70: 5211–5216 DOI 10.1099/ijsem.0.004392.
37. Patel S, Gupta RS: A phylogenomic and comparative genomic framework for resolving the polyphyly of the genus *Bacillus*: Proposal for six new genera of *Bacillus* species, *Peribacillus* gen. nov., *Cytobacillus* gen. nov., *Mesobacillus* gen. nov., *Neobacillus* gen. nov., *Metabacillus* gen. nov., and *Alkalihalobacillus* gen. nov. Int J Syst Evol Microbiol, 70: 406–438. In: Mao H, Wei Y, Gao Y, Pei J, Zhang Y, and Fang J. *Metabacillus sediminilitoris* sp. nov., a marine bacterium isolated from tidal sediment. Int J Syst Evol Microbiol 2020; 70: 5211–5216 DOI 10.1099/ijsem.0.004392.
38. Kumar V, Pathak DV, Dudeja SS, Saini R, Narula S, Anand RC:. Legume nodule endophytes more diverse than endophytes from roots of legumes or non-legumes in soils of Haryana, India. J. Microbiol. Biotechnol. Res., 2013; 3(3): 83 92.
39. Moosavi S, Siadat S, Koochekzadeh A, Parmoon G, Kiani S. Effect of Seed Color and Size on Cardinal Temperatures of Castor Bean (Ricinus Communis L.) Seed Germination. Agrotech Ind Crops 2022; 2(1): 1-10. doi: 10.22126/atic.2022.7417.1041
40. Luo Z. Analysis of the effect of reasonable close planting on respiration characteristics of alfalfa (Medicago sativa L.) artificial grassland. Turk J Agric For 2021;45(5):533-40.
41. Jalilian, S., Mondani, F., Fatemi, A., Bagheri, A. Evaluating the Effect of Farmyard Manure and Green Manure on Soil Physicochemical Traits and Growth Yield of Organic Sesame (*Sesamum indicium* L.). Agrotech Ind Crops 2022; 2(1): 19-31. doi: 10.22126/atic.2022.7332.1039.
42. Borzoo S, Mohsenzadeh S, Moradshahi A, Kahrizi D, Zamani H, Zarei M. Characterization of physiological responses and fatty acid compositions of *Camelina sativa* genotypes under water deficit stress and symbiosis with Micrococcus yunnanensis. Symbiosis. 2021;83(1):79-90. https://doi.org/10.1007/s13199-020-00733-5
43. Zarei B, Kahrizi D, Mousavi SA, Nasrollah Nezhad Qomi AA. Agrobacterium rhizogense-mediated transformation of Atropa belladonna. Agric Biotech J 2013 Aug 23;5(2):59-68.
44. Hosseini Beryekhani, S., Parsa, Z. A Review of the Effects of Using Municipal Treated Wastewater on Some Characteristics of Cotton Plants and Soil of Irrigated Fields. Agrotech Ind Crops 2021; 1(4): 182-187. doi: 10.22126/atic.2022.7297.1035.
45. Borzoo S, Mohsenzadeh S, Moradshahi A, Kahrizi D. Water-deficit stress and genotype variation induced alteration in seed characteristics of *Camelina sativa*. Rhizosphere. 2021; Volume 20, https://doi.org/10.1016/j.rhisph.2021.100427.
46. Shao T, Long X, Liu Y, Gao X, Liu M, Rengel Z. Effect of industrial crop Jerusalem artichoke on the micro-ecological rhizosphere environment in saline soil. Appl Soil Ecol 2021;166:104080.
47. Li C, Cao P, Du C, Zhang X, Bing H, Li L, Sun P, Xiang W, Zhao J, Wang X. Massilia rhizosphaerae sp. nov., a rice-associated rhizobacterium with antibacterial activity against Ralstonia solanacearum. Int J System Evolution Microbiol 2021;71(9):005009.
48. Busse HJ, Schumann P. Reclassification of Arthrobacter enclensis as Pseudarthrobacter enclensis comb. nov., and emended descriptions of the genus Pseudarthrobacter, and the species Pseudarthrobacter phenanthrenivorans and Pseudarthrobacter scleromae. Int J System Evolution Microbiol 2019;69(11):3508-11.