**Supplementary Table S1.** Number of randomly picked CFUs/isolates secured from positive MPN enrichment tubes\*, representing both plant compartments (endo-rhizosphere and endo-phyllosphere), developed on all tested culture media (leaf strips/root segments) under various oxygen conditions (ambient/limited), and further sub-cultured for 16S rRNA gene sequencing.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Incubation conditions** | **Plant compartments/ Culture Media** | **Total picked CFUs** | **Successfully sub-cultured isolates** | **Isolates of good quality sequence** |
| Ambient oxygen | Endo-rhizosphere/Leaf strip (RL) | 177 | 82 | 22 |
| Endo- rhizosphere/ Root segment (RR) | 150 | 70 | 23 |
| Endo-phyllosphere/Leaf strip (PL) | 86 | 68 | 29 |
| Endo-phyllosphere/Root segment (PR) | 90 | 80 | 42 |
| Limited oxygen | Endo-rhizosphere/Leaf strip (RL) | 176 | 64 | 16 |
| Endo- rhizosphere/ Root segment (RR) | 139 | 79 | 8 |
| Endo-phyllosphere/Leaf strip (PL) | 145 | 56 | 11 |
| Endo-phyllosphere/Root segment (PR) | 106 | 67 | 12 |
| **Total** | **1068** | **566** | **163** |

\*Two positive MPN tubes were selected to represent the first two dilutions (10-1 and 10-2 for endo-phyllosphere and 10-2 and 10-3 for endo-rhizosphere) of tested culture media (leaf strips/root segments-based culture media) and growth conditions (ambient/limited oxygen). From each tube, 20 μl of homogenized surface/sub-surface pellicle were aseptically transferred to an agar plate of corresponding culture media; then carefully surface-spread with a sterile glass spatula , with which a second agar plate (palate 2) was surface- inoculated and further used as well to inoculate a more diluted third plate .After incubation (under ambient/limited oxygen) at 25oC for 7-15 days, all separated colonies developed on the more diluted plate 3 ( >30-100 colonies) were picked for further single colony isolation.

### Supplementary Table S3. Genera of culturable endophytic bacteria of sunflower isolated by *in situ similis* culturomic strategies compared to other conventional culturing methods reported in literature and to 16S rRNA Metagenomic Analysis (NGS).

|  |  |  |  |
| --- | --- | --- | --- |
| **Bacterial genera** | ***In situ similis* MPN culture method a** | ***In situ similis* leaf surface method b** | **Conventional culturing methods reported in literature c,1-6 and NGS analysis c,7** |
| *Achromobacter*(or *Alcaligenes*) | - | - | Yes 3,6 |
| *Acidipila* | - | - | Yes 7 |
| *Acidovorax* | - | - | Yes 7 |
| *Acinetobacter* | Yes | - | Yes 6 |
| *Actinoallomurus* | - | - | Yes 7 |
| *Actinomadura* | - | - | Yes 7 |
| *Actinospica* | - | - | Yes 7 |
| *Aequorivita* | - | - | Yes 7 |
| *Aeromicrobium* | - | - | Yes 7 |
| *Agrobacterium* | Yes | - | Yes 6 |
| *Agrococcus* | - | - | Yes 7 |
| *Algoriphagus* | - | - | Yes 7 |
| *Altererythrobacter* | - | - | Yes 7 |
| *Amaricoccus* | - | - | Yes 7 |
| *Arenimonas* | - | - | Yes 7 |
| *Arsenophonus* | - | - | Yes 7 |
| *Arthrobacter* | - | - | Yes 7 |
| *Asticcacaulis* | - | - | Yes 6,7 |
| *Aurantimonas* | - | - | Yes 7 |
| *Aureimonas* | - | Yes | - |
| *Azospirillum*  | - | - | Yes 6 |
| *Azotobacter* | - | - | Yes 5 |
| *Bacillus* | Yes | Yes | Yes 1,2,3,4,5,7 |
| *Bacteriovorax* | - | - | Yes 7 |
| *Bartonella* | - | - | Yes 7 |
| *Bdellovibrio* | - | - | Yes 7 |
| *Bosea* | Yes | - | Yes 7 |
| *Brevibacillus* | - | - | Yes 7 |
| *Brevundimonas* | Yes | - | Yes 7 |
| *Burkholderia* | - | - | Yes 6,7 |
| *Caenimonas* | - | - | Yes 7 |
| *Candidatus Portiera* | - | - | Yes 7 |
| *Candidimonas* | - | - | Yes 7 |
| ***Caulobacter*** | Yes | - | - |
| *Chitinophaga* | Yes | - | Yes 7 |
| *Conexibacter* | - | - | Yes 7 |
| *Chryseobacterium* | - | - | Yes 6,7 |
| *Curtobacterium* | - | Yes | Yes 7 |
| *Cytophaga* | - | - | Yes 7 |
| *Delftia* | - | - | Yes 7 |
| *Devosia* | - | - | Yes 7 |
| *Dokdonella* | - | - | Yes 7 |
| *Duganella* | - | - | Yes 7 |
| *Dyadobacter* | - | - | Yes 7 |
| *Dyella* | - | - | Yes 7 |
| *Edaphobacter* | - | - | Yes 7 |
| *Ensifer (Sinorhizobium)* | - | - | Yes 7 |
| *Enterobacter* | Yes | - | Yes 4,6,7 |
| *Erwinia* | - | Yes | Yes 7 |
| *Erythrobacter* | - | - | Yes 7 |
| *Escherichia* | - | - | Yes 7 |
| *Flavisolibacter* | - | - | Yes 7 |
| *Flavobacterium* | - | - | Yes 7 |
| *Fluviicola* | - | - | Yes 7 |
| *Frateuria* | - | - | Yes 7 |
| *Gemmobacter* | - | - | Yes 7 |
| *Geobacillus* | - | - | Yes 7 |
| *Gillisia* | - | - | Yes 7 |
| *Gordonia* | - | - | Yes 7 |
| *Granulicella* | - | - | Yes 7 |
| *Grimontella*  | - | - | Yes 6 |
| *Gryllotalpicola* | - | - | Yes 7 |
| *Gynumella* | - | - | Yes 7 |
| *Halobacillus* | - | - | Yes 7 |
| *Herbaspirillum*  | - | - | Yes 6 |
| *Hydrocarboniphaga* | - | - | Yes 7 |
| *Hydrogenophaga* | - | - | Yes 7 |
| *Hyphomicrobium* | - | - | Yes 7 |
| *Janthinobacterium* | - | - | Yes 7 |
| *Jatrophihabitans* | - | - | Yes 7 |
| *Jeotgalibacillus* | - | - | Yes 7 |
| *Kaistia* | - | - | Yes 7 |
| *Klebsiella* | - | - | Yes 4,6 |
| *Kluyvera* | - | - | Yes 7 |
| *Kocuria* | - | Yes | Yes 7 |
| *Kosakonia* | - | Yes | - |
| *Kribbella* | - | - | Yes 7 |
| *Laceyella* | - | - | Yes 7 |
| *Lactobacillus* | - | - | Yes 7 |
| *Leclercia* | - | - | Yes 7 |
| *Legionella* | - | - | Yes 7 |
| *Leifsonia* | - | - | Yes 7 |
| *Leuconostoc* | - | - | Yes 7 |
| *Limnobacter* | - | - | Yes 7 |
| *Loktanella* | - | - | Yes 7 |
| *Luteimicrobium* | - | - | Yes 7 |
| *Lysobacter* | - | - | Yes 7 |
| *Malikia* | - | - | Yes 7 |
| *Mariniflexile* | - | - | Yes 7 |
| *Marmoricola* | - | - | Yes 7 |
| *Massilia* | - | - | Yes 7 |
| *Mesorhizobium* | - | - | Yes 7 |
| *Methylobacterium* | - | - | Yes 2,7 |
| *Methylophaga* | - | - | Yes 7 |
| *Methylophilus* | - | - | Yes 7 |
| *Methylotenera* | - | - | Yes 7 |
| *Methylovorus* | - | - | Yes 7 |
| *Microbacterium* | - | Yes | Yes 6,7 |
| *Mitsuaria*  | - | - | Yes 6 |
| *Moraxella*  | - | - | Yes 6 |
| *Mucilaginibacter* | - | - | Yes 7 |
| *Mycobacterium* | - | - | Yes 7 |
| *Nakamurella* | - | - | Yes 7 |
| *Niabella* | - | - | Yes 7 |
| *Nocardia* | - | - | Yes 7 |
| *Nocardioides* | - | - | Yes 7 |
| *Novosphingobium* | Yes | - | Yes 6,7 |
| *Oceanobacillus* | - | - | Yes 7 |
| *Ochrobactrum* | - | - | Yes 7 |
| *Ornithinibacter* | - | - | Yes 7 |
| *Oxalicibacterium* | - | - | Yes 7 |
| *Paenibacillus* | Yes | Yes | Yes 1,7 |
| *Paenisporosarcina* | - | - | Yes 7 |
| *Panacagrimonas* | - | - | Yes 7 |
| *Pantoea* | Yes | Yes | Yes 6,7 |
| *Paracoccus* | Yes | Yes | Yes 7 |
| *Parvibaculum* | - | - | Yes 7 |
| *Patulibacter* | - | - | Yes 7 |
| *Pedobacter* | - | - | Yes 7 |
| *Peredibacter* | - | - | Yes 7 |
| *Phenylobacterium* | - | - | Yes 7 |
| *Phyllobacterium* | - | - | Yes 7 |
| *Planococcus* | - | - | Yes 7 |
| *Planomicrobium* | - | - | Yes 7 |
| *Pontibacter* | - | - | Yes 7 |
| *Propionibacterium* | - | - | Yes 7 |
| *Pseudaminobacter* | - | - | Yes 7 |
| *Pseudolabrys* | - | - | Yes 7 |
| *Pseudomonas* | Yes | - | Yes 5,6,7 |
| *Pseudonocardia* | - | - | Yes 7 |
| *Pseudorhodobacter* | - | - | Yes 7 |
| *Pseudoxanthomonas* | Yes | - | Yes 7 |
| *Pusillimonas* | - | - | Yes 7 |
| *Rathayibacter* | - | - | Yes 7 |
| *Rhizobium* | Yes | Yes | Yes 6,7 |
| *Rhizomicrobium* | - | - | Yes 7 |
| *Rhodanobacter* | - | - | Yes 7 |
| *Rhodococcus* | - | - | Yes 7 |
| *Rhodoferax* | - | - | Yes 7 |
| *Rhodoplanes* | - | - | Yes 7 |
| *Rhodopseudomonas* | - | - | Yes 7 |
| *Rugamonas* | - | - | Yes 7 |
| *Saccharopolyspora* | - | - | Yes 7 |
| *Salinibacterium* | - | - | Yes 7 |
| *Salinimicrobium* | - | - | Yes 7 |
| *Salinirepens* | - | - | Yes 7 |
| ***Scandinavium*** | Yes | - | - |
| *Segetibacter* | - | - | Yes 7 |
| *Serratia*  | - | - | Yes 6 |
| *Shinella* | - | - | Yes 6,7 |
| *Sinorhizobium* | - | - | Yes 7 |
| *Skermanella* | - | - | Yes 7 |
| *Solimonas* | - | - | Yes 7 |
| *Sphingobacterium* | Yes | - | Yes 7 |
| *Sphingobium* | Yes | - | Yes 6 |
| *Sphingomicrobium* | - | - | Yes 7 |
| *Sphingomonas* | Yes | Yes | Yes 7 |
| *Sphingopyxis* | - | - | Yes 7 |
| *Sphingosinicella* | - | - | Yes 7 |
| *Sporichthya* | - | - | Yes 7 |
| *Sporosarcina* | - | - | Yes 7 |
| ***Starkeya*** | Yes | - | - |
| *Staphylococcus* | - | - | Yes 7 |
| *Stenotrophomonas* | - | Yes | Yes 6,7 |
| *Streptococcus* | - | - | Yes 7 |
| *Streptomyces* | - | - | Yes 7 |
| *Sulfuricella* | - | - | Yes 7 |
| *Thermoactinomyces* | - | - | Yes 7 |
| *Thermomonas* | - | - | Yes 7 |
| *Trabulsiella* | - | - | Yes 7 |
| *Variovorax* | - | - | Yes 6,7 |
| *Virgibacillus* | - | - | Yes 7 |
| *Vitellibacter* | - | - | Yes 7 |
| *Williamsia* | - | - | Yes 7 |
| *Winogradskyella* | - | - | Yes 7 |
| *Xanthomonas*  | - | - | Yes 6 |

Yes, isolated; -, not isolated; bold genera, only found in *in situ similis* MPN culture method

**a** Current manuscript

**b** Nemr, R. A., Khalil, M., Sarhan, M. S., Abbas, M., Elsawey, H., et al. (2020). “*In situ similis*” culturing of plant microbiota: a novel simulated environmental method based on plant leaf blades as nutritional pads. *Front. Microbiol*. 11, 1–15. doi: 10.3389/fmicb.2020.00454

**c** Conventional culturing methods reported in literature:

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2. Campos, K. G., Luisa, M. C., Cattelan, A. J., Nogueira, M. A., Guilherme, C. P., and Luiz, A. M. (2012). Biochemical and molecular characterization of high population density bacteria isolated from Sunflower. *J Microbiol Biotechnol.* 22, 437–447. doi: 10.4014/jmb.1109.09007
3. Forchetti, G., Masciarelli, O., Alemano, S., Alvarez, D., and Abdala, G. (2007). Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. *Appl. Microbiol. Biotechnol.* 76, 1145–1152. doi: 10.1007/s00253-007-1077-7
4. Liu, X., Li, X., Li, Y., Li, R., and Xie, Z. (2017). Plant growth promotion properties of bacterial strains isolated from the rhizosphere of the Jerusalem artichoke (*Helianthus tuberosus* L.) adapted to salt-alkaline soils and their effect on wheat growth. *Can. J Microbiol*. 63, 228–237. doi: 10.1139/cjm- 2016-0511
5. Raval, A. A., and Desai, P. B. (2012). Rhizobacteria from rhizosphere of sunflower (*Helianthus* *annuus* L.) and their effect on plant growth. *Res. J. Recent Sci*. 1, 58–61.
6. Ambrosini, A., Beneduzi, A., Stefanski, T., Pinheiro, F. G., Vargas, L. K., and Passaglia, L. M. P. (2012). Screening of plant growth promoting rhizobacteria isolated from sunflower (*Helianthus annuus* L.). *Plant and Soil* 356, 245–264. doi: 10.1007/s11104-011-1079-1
7. Tamošiūnė, I., Gelvonauskiene, D., Haimi, P., Mildažienė, V., Koga, K., Shiratani, M., et al. (2020). Cold plasma treatment of sunfl ower seeds modulates plant-associated microbiome and stimulates root and lateral organ growth. *Front Plant Sci.* 28, 1–13. doi.org/10.3389/fpls.2020.568924