

T.R. Elsayed, N.A.M. El-Said, F.A. Safhi, N.El-Houda A. Reyad (2024). *Bacillus velezensis* B63 and chitosan control root rot, improve growth and alter the rhizosphere microbiome of geranium. *Phytopathologia Mediterranea* 63(1): 137–154. doi: 10.36253/phy1to-15093

Table S1. Primers used in this study.

| Target gene | Primers used | Sequence | Temp | Size (bp) |
|---------------------------------|--------------|---|------|-----------|
| 16S rRNA | Eub338 | ACTCCTACGGGAGGCAGCAG | 55°C | 123 |
| | Eub518 | ATTACCGCGGCTGCTGG | | |
| <i>nifH</i> | DVV | ATIGCRAAICCICRCAIACIACRTC | 53°C | 454 |
| | IGK3 | GCIWTHHTAYGGIAARGGIGGIATHGGIAA | | |
| <i>bacC</i> | Bacc1f | GAAGGACACGGAGAGAGTC | 55°C | 875 |
| | Bacc1r | CGCTGATGACTGTTTCATGCT | | |
| <i>ituD</i> | | GATGCGATCTCCTTGGATGT | 55°C | 647 |
| | | ATCGTCATGTGCTGCTTGAG | | |
| 16S Amplicon PCR Forward Primer | | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGC CTACGGGNGGCWGCAG | | |
| 16S Amplicon PCR revers Primer | | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACTACHVGGGTATCTAATCC | | |

Table S2. Relative abundance of dominant genera in the rhizosphere of geranium under the effect of chitosan, B63 compared to untreated control plants.

| Genera | Control | Chitosan | B63 | Genera | Control | Chitosan | B63 |
|---|---------|----------|------|---|---------|----------|------|
| Kaistobacter | 3.5 | 6.1 | 6.7 | <i>Micromonosporaceae_unclassified</i> | 0.71 | 0.63 | 1.42 |
| Agrobacterium | 6.51 | 0.64 | 3.64 | Planctomyces | 1.36 | 1.06 | 0.97 |
| <i>Acidobacteria</i> iii1-15_unclassified | 2.67 | 6.22 | 3.86 | Rhodoplanes | 0.76 | 1.36 | 0.57 |
| <i>Comamonadaceae_unclassified</i> | 1.73 | 1.05 | 4.94 | <i>Rhodospirillales_unclassified</i> | 1.34 | 1.21 | 0.97 |
| <i>Rhizobiales_unclassified</i> | 4.15 | 2.72 | 3.64 | <i>Pirellulaceae_unclassified</i> | 1.26 | 1.26 | 1.19 |
| Gemmata | 1.58 | 3.47 | 1.76 | <i>Alphaproteobacteria_unclassified</i> | 1.07 | 0.6 | 1.25 |
| <i>Bacillales_unclassified</i> | 2.25 | 3.44 | 0.91 | <i>Kineosporiaceae_unclassified</i> | 1.25 | 0.22 | 0.11 |
| <i>Nocardioideaceae_unclassified</i> | 3 | 1.49 | 2.44 | Sinorhizobium | 0.73 | 0.89 | 1.19 |
| <i>Gaiellaceae_unclassified</i> | 1.6 | 2.74 | 2.33 | Devosia | 1.19 | 0.31 | 1.02 |
| <i>Bacteria_unclassified</i> | 2.29 | 1.69 | 2.73 | <i>Solirubrobacterales_unclassified</i> | 1.15 | 1.09 | 0.85 |
| <i>Enterobacteriaceae_unclassified</i> | 0.81 | 2.22 | 0.63 | Sphingomonas | 0.89 | 0.49 | 1.14 |
| <i>Actinomycetales_unclassified</i> | 2.01 | 0.88 | 1.25 | mb2424_unclassified | 0.3 | 1.14 | 0.51 |
| <i>Micrococcaceae_unclassified</i> | 2.01 | 0.68 | 0.4 | <i>Promicromonosporaceae_unclassified</i> | 1.03 | 0.41 | 0.28 |
| <i>Sphingomonadaceae_unclassified</i> | 1.31 | 1.31 | 1.93 | <i>Acidimicrobiales_unclassified</i> | 0.96 | 1.01 | 0.97 |
| <i>Micrococcales_unclassified</i> | 1.12 | 1.78 | 1.48 | <i>Chthoniobacteraceae_unclassified</i> | 0.53 | 0.97 | 0.74 |
| <i>Xanthomonadaceae_unclassified</i> | 0.85 | 0.63 | 1.7 | <i>Myxococcales_unclassified</i> | 0.49 | 0.75 | 0.97 |
| <i>Rhodospirillaceae_unclassified</i> | 1.09 | 1.63 | 1.53 | Steroidobacter | 0.86 | 0.92 | 0.68 |
| WD2101_unclassified | 0.89 | 1.47 | 1.59 | Bacillus | 0.92 | 0.88 | 0.23 |
| <i>Rhizobiaceae_unclassified</i> | 1.56 | 0.5 | 1.42 | <i>Chitinophagaceae_unclassified</i> | 0.4 | 0.59 | 0.91 |
| Paracoccus | 1.25 | 1.53 | 0.51 | Acinetobacter | 0.23 | 0.91 | 0.63 |
| <i>Gemmataceae_unclassified</i> | 0.93 | 1.46 | 0.68 | <i>Sinobacteraceae_unclassified</i> | 0.64 | 0.88 | 0.8 |
| Balneimonas | 1.23 | 1.44 | 1.02 | <i>Erythrobacteraceae_unclassified</i> | 0.84 | 0.28 | 0.85 |
| Streptomyces | 1.07 | 0.47 | 1.42 | Pirellula | 0.43 | 0.82 | 0.63 |

(Continued)

Table S2. (Continued).

| Genera | Control | Chitosan | B63 | Genera | Control | Chitosan | B63 |
|--|---------|----------|------|---|---------|----------|------|
| RB41_unclassified | 0.19 | 0.82 | 0.68 | Actinoplanes | 0.26 | 0.08 | 0.57 |
| Rubellimicrobium | 0.8 | 0.55 | 0.63 | <i>Bradyrhizobiaceae_unclassified</i> | 0.52 | 0.56 | 0.45 |
| Mycobacterium | 0.77 | 0.15 | 0.45 | <i>Microbacteriaceae_unclassified</i> | 0.55 | 0.11 | 0.23 |
| <i>Solirubrobacteraceae_unclassified</i> | 0.75 | 0.44 | 0.23 | <i>Cytophagaceae_unclassified</i> | 0.48 | 0.53 | 0.34 |
| <i>Gemmatimonadetes_unclassified</i> | 0.25 | 0.65 | 0.68 | <i>Solibacterales_unclassified</i> | 0.21 | 0.53 | 0.45 |
| Erwinia | 0.18 | 0.68 | 0.17 | <i>Betaproteobacteria_unclassified</i> | 0.37 | 0.51 | 0.51 |
| Agromyces | 0.67 | 0.59 | 0.4 | <i>Verrucomicrobiaceae_unclassified</i> | 0.42 | 0.39 | 0.51 |
| JG30-KF-CM45_unclassified | 0.65 | 0.43 | 0.4 | Rhizobium | 0.26 | 0.1 | 0.51 |
| Aeromicrobium | 0.49 | 0.25 | 0.57 | | | | |

Table S3. Relative abundance of dominant phyla and classes in the rhizosphere of geranium under the effect of chitosan, B63 compared to untreated control plants.

| Phylum | Class | Control | Chitosan | B63 |
|-----------------------|------------------------------------|---------|----------|-------|
| Proteobacteria | | 43.98 | 39.25 | 47.78 |
| | <i>Alphaproteobacteria</i> | 33.62 | 25.91 | 33.18 |
| | <i>Betaproteobacteria</i> | 2.9 | 2.75 | 6.48 |
| | <i>Deltaproteobacteria</i> | 1.66 | 2.44 | 1.88 |
| | <i>Gammaproteobacteria</i> | 5.54 | 7.97 | 5.85 |
| | <i>Proteobacteria_unclassified</i> | 0.24 | 0.15 | 0.4 |
| Actinobacteria | | 27.83 | 19.93 | 21.59 |
| | <i>Acidimicrobiia</i> | 2.42 | 2.92 | 2.1 |
| | <i>Actinobacteria</i> | 20.31 | 10.28 | 14.6 |
| | <i>Actinobacteria_unclassified</i> | 0.94 | 1.8 | 0.85 |
| | <i>Nitriliruptoria</i> | 0.07 | 0.09 | 0.23 |
| | <i>Rubrobacteria</i> | 0.31 | 0.32 | 0.23 |
| | <i>Thermoleophilia</i> | 3.78 | 4.53 | 3.58 |
| Planctomycetes | | 6.92 | 10.06 | 7.56 |
| | <i>Phycisphaerae</i> | 0.91 | 1.54 | 1.65 |
| | <i>Planctomycetia</i> | 5.94 | 8.41 | 5.85 |
| | <i>Planctomycetes_unclassified</i> | 0.02 | 0.06 | 0 |
| Acidobacteria | | 5.06 | 12.57 | 8.41 |
| | <i>Chloracidobacteria</i> | 1.33 | 3.36 | 3.07 |
| | <i>Acidobacteria_unclassified</i> | 0.02 | 0.03 | 0.06 |
| | <i>Acidobacteria</i> | 3.32 | 8.32 | 4.72 |
| | <i>Solibacteres</i> | 0.22 | 0.56 | 0.4 |
| Firmicutes | | 4.7 | 5.18 | 1.53 |
| | <i>Bacilli</i> | 4.6 | 5.03 | 1.48 |
| | <i>Clostridia</i> | 0.09 | 0.14 | 0.06 |
| Chloroflexi | | 3.61 | 3.92 | 2.78 |
| | <i>Anaerolineae</i> | 0.4 | 0.33 | 0.34 |
| | <i>Chloroflexi</i> | 0.84 | 0.66 | 0.8 |
| | <i>Chloroflexi_unclassified</i> | 0.11 | 0.11 | 0 |
| | <i>Thermomicrobia</i> | 0.77 | 0.48 | 0.45 |
| Bacteria_unclassified | <i>Bacteria_unclassified</i> | 2.29 | 1.69 | 2.73 |
| Bacteroidetes | | 1.9 | 1.81 | 2.44 |
| | <i>Rhodothermi</i> | 0.06 | 0.06 | 0 |

(Continued)

Table S2. (Continued).

| Phylum | Class | Control | Chitosan | B63 |
|------------------|--------------------------------------|---------|----------|------|
| Verrucomicrobia | <i>Saprospirae</i> | 0.67 | 0.9 | 1.14 |
| | <i>Bacteroidetes_unclassified</i> | 0.03 | 0.04 | 0.23 |
| | <i>Cytophagia</i> | 0.64 | 0.63 | 0.74 |
| | <i>Flavobacteriia</i> | 0.2 | 0.09 | 0.11 |
| | <i>Sphingobacteriia</i> | 0.3 | 0.1 | 0.23 |
| | | 1.61 | 2.34 | 2.05 |
| | <i>Pedosphaerae</i> | 0.22 | 0.34 | 0.17 |
| | <i>Spartobacteria</i> | 0.72 | 1.43 | 1.25 |
| | <i>Opitutae</i> | 0.14 | 0.15 | 0.17 |
| | <i>Verrucomicrobiae</i> | 0.51 | 0.41 | 0.45 |
| Gemmatimonadetes | | 0.82 | 1.63 | 1.59 |
| | <i>Gemmatimonadetes</i> | 0.35 | 0.76 | 0.85 |
| | <i>Gemmatimonadetes_unclassified</i> | 0.45 | 0.795 | 0.67 |
| TM7 | | 0.7 | 0.51 | 0.91 |
| Nitrospirae | | 0.19 | 0.6 | 0.17 |
| Cyanobacteria | | 0.11 | 0.12 | 0.06 |
| Armatimonadetes | | 0.06 | 0.06 | 0 |
| | <i>Fimbriimonadia</i> | 0.05 | 0.05 | 0 |
| Chlamydiae | | 0.05 | 0.01 | 0 |
| Elusimicrobia | | 0.01 | 0.05 | 0 |

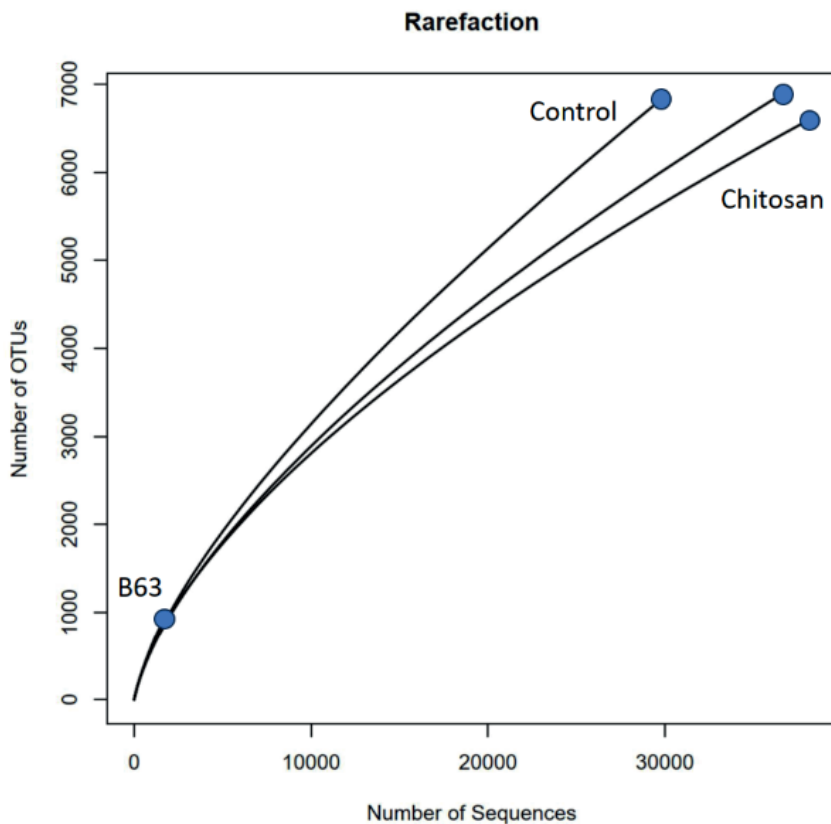


Figure S1. Rarefaction curve.