Supplementary Material

Comparative analysis of the cultured and total bacterial community in the wheat rhizosphere microbiome using culture-dependent and culture-independent approaches

Sameh H. Youseif, a Fayrouz H. Abd El-Megeed, a Ethan A. Humm, b Maskit Maymon, b
Akram H. Mohamed, a Saleh A. Saleh, c Ann M. Hirsch b,d

a Department of Microbial Genetic Resources, National Gene Bank (NGB), Agricultural Research Center (ARC), Giza 12619, Egypt
b Department of Molecular, Cell & Developmental Biology, University of California-Los Angeles (UCLA), 621 Charles Young Drive South, Los Angeles, CA 90095-1606, U.S.A.
c Agricultural Microbiology Research Department, Soils, Water and Environment Research Institute, Agricultural Research Center (ARC), Giza, Egypt
d Molecular Biology Institute, UCLA, 621 Charles Young Drive South, Los Angeles, CA 90095-1606, U.S.A.
TABLE S1 Physical and chemical analysis of the three collected soil samples

| Property                          | El Matanah, Luxor | Mallawi, Minya | Nubaria region, El Beheira |
|-----------------------------------|-------------------|----------------|---------------------------|
| Latitude                          | 25°25'27.97"N     | 27°43'17.03"N  | 30°54'16.25"N             |
| Longitude                         | 32°32'18.17"E     | 30°43'20.91"E  | 29°52'43.59"E             |
| Particle size distribution (%)    |                   |                |                           |
| Sand                              | 21.90             | 88.70          | 54.30                     |
| Silt                              | 32.60             | 5.90           | 25.10                     |
| Clay                              | 45.50             | 5.40           | 20.60                     |
| Saturation percent (%)            |                   |                |                           |
| CaCO$_3$ (%)                      | 1.94              | 2.80           | 18.80                     |
| pH (soil paste)                   | 8.00              | 7.93           | 8.51                      |
| E.C (dS m$^{-1}$, at 25°C)        | 0.62              | 0.33           | 0.84                      |
| Soluble cations (meq L$^{-1}$)    |                   |                |                           |
| Ca$^{++}$                         | 3.10              | 0.90           | 2.59                      |
| Mg$^{++}$                         | 0.80              | 0.64           | 1.55                      |
| Na$^+$                            | 1.21              | 1.31           | 4.21                      |
| K$^+$                             | 1.04              | 0.59           | 0.14                      |
| Soluble anions (meq L$^{-1}$)     |                   |                |                           |
| CO$_3^-$                          | 0.00              | 0.00           | 0.00                      |
| HCO$_3^-$                         | 1.96              | 1.43           | 2.41                      |
| Cl$^-$                            | 1.77              | 0.58           | 3.98                      |
| SO$_4^{2-}$                       | 2.42              | 1.43           | 2.10                      |
| Total-N (%)                       | 0.067             | 0.020          | 0.015                     |
| Total soluble-N (mg kg$^{-1}$)    | 52.20             | 14.30          | 16.40                     |
| Available-P (mg kg$^{-1}$)        | 9.90              | 4.40           | 3.40                      |
| Available-K (mg kg$^{-1}$)        | 331.80            | 180.00         | 127.00                    |
| Organic matter (%)                | 0.72              | 0.16           | 0.21                      |
| DTPA-extractable (mg kg$^{-1}$)   |                   |                |                           |
| Fe                                | 4.82              | 3.16           | 0.61                      |
| Mn                                | 2.41              | 1.16           | 0.46                      |
| Zn                                | 1.82              | 0.21           | 0.38                      |
| Cu                                | 0.25              | 0.02           | 0.09                      |

DTPA: Di-ethylene tri-amine penta acetic acid.
### TABLE S2 List of isolated bacteria and their respective soil type and media preparations

| No. | Isolate | Soil type | Media preparation** | No. | Isolate | Soil type | Media preparation |
|-----|---------|-----------|---------------------|-----|---------|-----------|-------------------|
| 1   | NGB-R1  | Clay      | LB-Phytigel™        | 90  | NGB-R90 | Calcareous | LB-Phytigel™      |
| 2   | NGB-R2  | Clay      | LB-Phytigel™        | 91  | NGB-R91 | Calcareous | LB-Phytigel™      |
| 3   | NGB-R3  | Clay      | LB-Phytigel™        | 92  | NGB-R92 | Calcareous | LB-Phytigel™      |
| 4   | NGB-R4  | Clay      | LB-Phytigel™        | 93  | NGB-R93 | Calcareous | LB-Phytigel™      |
| 5   | NGB-R5  | Clay      | LB-Phytigel™        | 94  | NGB-R94 | Calcareous | LB-Phytigel™      |
| 6   | NGB-R6  | Clay      | LB-Phytigel™        | 95  | NGB-R95 | Calcareous | LB-Phytigel™      |
| 7   | NGB-R7  | Clay      | LB-Phytigel™        | 96  | NGB-R96 | Calcareous | LB-Phytigel™      |
| 8   | NGB-R8  | Clay      | LB-Phytigel™        | 97  | NGB-R97 | Calcareous | LB-Gelrite®       |
| 9   | NGB-R9  | Clay      | LB-Phytigel™        | 98  | NGB-R98 | Calcareous | LB-Gelrite®       |
| 10  | NGB-R10 | Clay      | LB-Phytigel™        | 99  | NGB-R99 | Calcareous | LB-Gelrite®       |
| 11  | NGB-R11 | Clay      | LB-Phytigel™        | 100 | NGB-R100| Calcareous | LB-Gelrite®       |
| 12  | NGB-R12 | Clay      | LB-Agar             | 101 | NGB-R101| Calcareous | LB-Gelrite®       |
| 13  | NGB-R13 | Clay      | LB-Agar             | 102 | NGB-R102| Calcareous | LB-Gelrite®       |
| 14  | NGB-R14 | Clay      | LB-Agar             | 103 | NGB-R103| Calcareous | LB-Gelrite®       |
| 15  | NGB-R15 | Clay      | LB-Agar             | 104 | NGB-R104| Calcareous | LB-Gelrite®       |
| 16  | NGB-R16 | Clay      | LB-Agar             | 105 | NGB-R105| Calcareous | LB-Gelrite®       |
| 17  | NGB-R17 | Clay      | LB-Agar             | 106 | NGB-R106| Calcareous | LB-Gelrite®       |
| 18  | NGB-R18 | Clay      | LB-Gelrite®         | 107 | NGB-R107| Calcareous | LB-Gelrite®       |
| 19  | NGB-R19 | Clay      | LB-Gelrite®         | 108 | NGB-R108| Calcareous | LB-Gelrite®       |
| 20  | NGB-R20 | Clay      | LB-Gelrite®         | 109 | NGB-R109| Calcareous | LB-Gelrite®       |
| 21  | NGB-R21 | Clay      | JM-Agar             | 110 | NGB-R110| Calcareous | LB-Gelrite®       |
| 22  | NGB-R22 | Clay      | JM-Agar             | 111 | NGB-R111| Calcareous | LB-Gelrite®       |
| 23  | NGB-R23 | Clay      | JM-Agar             | 112 | NGB-R112| Calcareous | LB-Gelrite®       |
| 24  | NGB-R24 | Clay      | JM-Agar             | 113 | NGB-R113| Calcareous | LB-Gelrite®       |
| 25  | NGB-R25 | Clay      | JM-Agar             | 114 | NGB-R114| Sandy      | LB-Agar           |
| 26  | NGB-R26 | Clay      | JM-Agar             | 115 | NGB-R115| Sandy      | LB-Agar           |
| 27  | NGB-R27 | Clay      | JM-Agar             | 116 | NGB-R116| Calcareous | JM-Agar           |
| 28  | NGB-R28 | Clay      | JM-Agar             | 117 | NGB-R117| Calcareous | JM-Agar           |
| 29  | NGB-R29 | Clay      | SJM-Phytigel™       | 118 | NGB-R118| Calcareous | JM-Agar           |
| 30  | NGB-R30 | Clay      | SJM-Phytigel™       | 119 | NGB-R119| Calcareous | JM-Agar           |
| 31  | NGB-R31 | Clay      | SJM-Phytigel™       | 120 | NGB-R120| Calcareous | JM-Agar           |
| 32  | NGB-R32 | Clay      | SJM-Phytigel™       | 121 | NGB-R121| Calcareous | JM-Agar           |
| 33  | NGB-R33 | Clay      | SJM-Phytigel™       | 122 | NGB-R122| Calcareous | JM-Agar           |
| 34  | NGB-R34 | Clay      | SJM-Phytigel™       | 123 | NGB-R123| Calcareous | JM-Agar           |
| 35  | NGB-R35 | Clay      | SJM-Phytigel™       | 124 | NGB-R124| Calcareous | SJM-Agar          |
| 36  | NGB-R36 | Clay      | SJM-Gelrite®        | 125 | NGB-R125| Calcareous | SJM-Agar          |
| 37  | NGB-R37 | Clay      | SJM-Gelrite®        | 126 | NGB-R126| Calcareous | SJM-Agar          |
| 38  | NGB-R38 | Clay      | SJM-Gelrite®        | 127 | NGB-R127| Calcareous | SJM-Agar          |
| 39  | NGB-R39 | Clay      | SJM-Gelrite®        | 128 | NGB-R128| Calcareous | SJM-Agar          |
| 40  | NGB-R40 | Clay      | SJM-Gelrite®        | 129 | NGB-R129| Calcareous | SJM-Agar          |
| 41  | NGB-R41 | Clay      | SJM-Gelrite®        | 130 | NGB-R130| Calcareous | SJM-Agar          |
| 42  | NGB-R42 | Sandy     | LB-Agar             | 131 | NGB-R131| Calcareous | SJM-Agar          |
| 43  | NGB-R43 | Sandy     | LB-Agar             | 132 | NGB-R132| Calcareous | SJM-Phytigel™     |
| 44  | NGB-R44 | Sandy     | LB-Agar             | 133 | NGB-R133| Calcareous | SJM-Phytigel™     |
| 45  | NGB-R45 | Sandy     | LB-Agar             | 134 | NGB-R134| Calcareous | SJM-Phytigel™     |
| 46  | NGB-R46 | Sandy     | LB-Agar             | 135 | NGB-R135| Calcareous | SJM-Phytigel™     |
| 47  | NGB-R47 | Sandy     | LB-Agar             | 136 | NGB-R136| Calcareous | SJM-Phytigel™     |
| 48  | NGB-R48 | Sandy     | LB-Agar             | 137 | NGB-R137| Calcareous | SJM-Phytigel™     |
| 49  | NGB-R49 | Sandy     | LB-Agar             | 138 | NGB-R138| Calcareous | SJM-Phytigel™     |
| 50  | NGB-R50 | Sandy     | LB-Agar             | 139 | NGB-R139| Calcareous | SJM-Phytigel™     |
| 51  | NGB-R51 | Sandy     | LB-Agar             | 140 | NGB-R140| Calcareous | SJM-Phytigel™     |
from Luxo

JM: Jensen medium prepared by autoclaving phosphates and solidifying agent together. SJM: Jensen medium

* Bacterial colonies failed to grow.

** Agar, Phytagel™, and Gelrite® were used in the following concentration 1.5%, 1%, and 0.75% respectively.

JM: Jensen medium prepared by autoclaving phosphates and solidifying agent together. Clay, sandy, and calcareous soils were collected from Luxor, Minya, and Nubaria sites, respectively.
| No. | Phylum                        | Luxor (Clay soil) | Minya (sandy soil) | Nubaria (calcareous soil) |
|-----|-------------------------------|-------------------|---------------------|---------------------------|
| 1   | Acidobacteria                 | 4.323             | 3.291               | 3.116                     |
| 2   | Actinobacteria                | 17.189            | 17.527              | 15.963                    |
| 3   | Armatimonadetes               | 0.045             | 0.051               | 0.019                     |
| 4   | Bacteroidetes                 | 3.862             | 4.099               | 11.684                    |
| 5   | Candidatus Bipolaricaulota    | 0.000             | 0.007               | 0.000                     |
| 6   | Candidatus Melainabacteria    | 0.002             | 0.002               | 0.000                     |
| 7   | Candidatus Saccharibacteria   | 0.004             | 0.002               | 0.030                     |
| 8   | Candidatus Tectomicrobia      | 0.054             | 0.073               | 0.560                     |
| 9   | Chlamydiae                    | 0.002             | 0.000               | 0.048                     |
| 10  | Chlorobi                      | 0.007             | 0.000               | 0.005                     |
| 11  | Chloroflexi                   | 10.214            | 13.471              | 4.983                     |
| 12  | Chlorophyta                   | 0.000             | 0.000               | 0.087                     |
| 13  | Cyanobacteria                 | 0.509             | 0.411               | 0.841                     |
| 14  | Deinococcus-Thermus           | 0.073             | 0.246               | 0.152                     |
| 15  | Elusimicrobia                 | 0.004             | 0.000               | 0.012                     |
| 16  | Fibrobacteres                 | 0.326             | 0.243               | 0.129                     |
| 17  | Firmicutes                    | 4.627             | 7.950               | 10.257                    |
| 18  | Fusobacteria                  | 0.009             | 0.002               | 0.004                     |
| 19  | Gemmatimonadetes              | 2.641             | 2.772               | 2.471                     |
| 20  | Ignavibacteriaae              | 0.028             | 0.042               | 0.062                     |
| 21  | Nitrospinae                   | 0.000             | 0.000               | 0.004                     |
| 22  | Nitrospirae                   | 0.584             | 0.476               | 0.576                     |
| 23  | Planctomycetes                | 3.452             | 4.700               | 4.706                     |
| 24  | Proteobacteria                | 40.757            | 36.572              | 32.533                    |
| 25  | Rhodothermaceota              | 0.000             | 0.009               | 0.014                     |
| 26  | Spirochaetes                  | 0.000             | 0.000               | 0.012                     |
| 27  | Synergistetes                 | 0.000             | 0.000               | 0.005                     |
| 28  | Tenericutes                   | 0.019             | 0.018               | 0.048                     |
| 29  | Thermotogae                   | 0.000             | 0.000               | 0.009                     |
| 30  | Verrucomicrobia               | 2.464             | 1.964               | 3.079                     |
| No. | Class             | Luxor (clay) | Minya (sandy) | Nubaria (calcareous) | No. | Class             | Luxor (clay) | Minya (sandy) | Nubaria (calcareous) |
|-----|------------------|--------------|---------------|----------------------|-----|------------------|--------------|---------------|----------------------|
| 1   | Acetothermiia    | 0.000        | 0.007         | 0.000                | 36  | Gammaproteobacteria | 4.543        | 7.171         | 8.674                |
| 2   | Acidimicrobiia   | 3.762        | 1.128         | 0.984                | 37  | Gemmatimonadetes  | 2.641        | 2.772         | 2.471                |
| 3   | Acidithiobacillia| 0.000        | 0.020         | 0.021                | 38  | Gloeobacteria     | 0.000        | 0.009         | 0.000                |
| 4   | Acidobacteria    | 0.000        | 2.581         | 2.464                | 39  | Holophagae        | 1.130        | 0.502         | 0.505                |
| 5   | Actinobacteria   | 10.705       | 11.852        | 10.405               | 40  | Ignavibacteria    | 0.028        | 0.042         | 0.062                |
| 6   | Alphaproteobacteria | 14.403   | 15.723        | 11.062               | 41  | Ktedonobacteria   | 0.022        | 0.004         | 0.000                |
| 7   | Anaerolineae     | 3.105        | 3.902         | 1.791                | 42  | Melainabacteria   | 0.002        | 0.002         | 0.000                |
| 8   | Ardenticatenia   | 0.007        | 0.000         | 0.000                | 43  | Mollicutes        | 0.019        | 0.018         | 0.048                |
| 9   | Armatimonadia    | 0.002        | 0.015         | 0.000                | 44  | Negativicutes     | 0.000        | 0.000         | 0.002                |
| 10  | Bacilli           | 1.865        | 6.193         | 7.010                | 45  | Nitriliruptoria   | 0.250        | 0.197         | 0.189                |
| 11  | Bacteriodetes    | 0.050        | 0.000         | 0.000                | 46  | Nitrospina        | 0.000        | 0.000         | 0.004                |
| 12  | Bacteroidia      | 1.029        | 0.429         | 3.842                | 47  | Nitrospira        | 0.584        | 0.476         | 0.576                |
| 13  | Betaproteobacteria | 8.609   | 5.439         | 4.040                | 48  | Oligoflexia       | 0.041        | 0.230         | 0.254                |
| 14  | Blastocatellia   | 0.140        | 0.208         | 0.147                | 49  | Opitutae         | 1.343        | 0.509         | 1.367                |
| 15  | Caldilineae      | 0.261        | 0.659         | 0.375                | 50  | Oscillatoriophyceae | 0.000     | 0.104         | 0.000                |
| 16  | Candidatus Brocadiae | 0.032     | 0.049         | 0.152                | 51  | Phyccisphaerae    | 0.688        | 0.484         | 0.429                |
| 17  | Chitinophagia    | 0.908        | 1.163         | 1.477                | 52  | Planctomycetia   | 2.732        | 4.167         | 4.125                |
| 18  | Chlamydia        | 0.002        | 0.000         | 0.048                | 53  | Rhodothermaeae   | 0.000        | 0.000         | 0.048                |
| 19  | Chlorobia        | 0.007        | 0.000         | 0.005                | 54  | Rhodothermia     | 0.000        | 0.000         | 0.014                |
| 20  | Chloroflexia     | 3.577        | 4.353         | 1.168                | 55  | Rhodothermaeota  | 0.002        | 0.000         | 0.000                |
| 21  | Chthonomonadetes | 0.032        | 0.024         | 0.009                | 56  | Rubrobacteria     | 2.527        | 1.203         | 0.954                |
| 22  | Closstridia      | 2.497        | 1.659         | 2.782                | 57  | Saccharibacteria | 0.004        | 0.002         | 0.030                |
| 23  | Conoidasida      | 0.000        | 0.009         | 0.000                | 58  | Saprospira       | 0.060        | 0.069         | 0.413                |
| 24  | Cyanophyceae     | 0.509        | 0.299         | 0.841                | 59  | Spartobacteria   | 0.028        | 0.252         | 0.071                |
| 25  | Cytophagia       | 1.020        | 1.807         | 4.098                | 60  | Sphingobacteria  | 0.449        | 0.338         | 1.480                |
| 26  | Dehalococcoidia  | 1.128        | 1.405         | 0.956                | 61  | Spirochaeta      | 0.000        | 0.000         | 0.012                |
| 27  | Deinococcii      | 0.073        | 0.246         | 0.152                | 62  | Synergistia      | 0.000        | 0.000         | 0.005                |
| 28  | Deltaproteobacteria | 13.014  | 7.967         | 8.437                | 63  | Tectomicrobia    | 0.000        | 0.073         | 0.560                |
| 29  | Elusimicrobia    | 0.004        | 0.000         | 0.012                | 64  | Thermoanaerobacterales | 0.043     | 0.000         | 0.000                |
| 30  | Epsilonproteobacteria | 0.147     | 0.022         | 0.044                | 65  | Thermoleophilica | 2.999        | 3.148         | 3.431                |
| 31  | Erysipelotrichia | 0.216        | 0.077         | 0.436                | 66  | thermomicobia    | 2.113        | 3.148         | 0.694                |
| 32  | Fibrobacteria    | 0.326        | 0.243         | 0.129                | 67  | Thermotogae      | 0.000        | 0.000         | 0.009                |
| 33  | Fimbriomonadina  | 0.011        | 0.011         | 0.011                | 68  | Tissierellia     | 0.006        | 0.020         | 0.026                |
| 34  | Flavobacteria    | 0.343        | 0.292         | 0.325                | 69  | Ulvophyceae      | 0.000        | 0.000         | 0.087                |
| 35  | Fusobacteria     | 0.009        | 0.002         | 0.004                | 70  | Verrucomicrobia  | 1.093        | 1.203         | 1.641                |
**TABLE S5** Variability of the top genera detected in the three soils, their percentages, and taxonomy based on the metagenomic analyses

| No. | Genus              | Luxor (clay soil) | Minya (sandy soil) | Nubaria (calcareous soil) | Taxonomy (class; phylum)                        |
|-----|--------------------|-------------------|--------------------|---------------------------|------------------------------------------------|
| 1   | *Pelobacter*       | 6.399             | 3.568              | 2.742                     | Deltaproteobacteria; Proteobacteria             |
| 2   | *Bacillus*         | 1.317             | 4.497              | 5.328                     | Bacilli; Firmicutes                             |
| 3   | *Candidatus nitrososphaera* | 5.768      | -                  | -                          | Nitrososphaeria; Thaumarchaeota                 |
| 4   | *Rhodopseudomonas* | 3.228             | 0.829              | 0.442                     | Alphaproteobacteria; Proteobacteria             |
| 5   | *Gemmatimonas*     | 2.641             | 2.772              | 2.471                     | Gemmatimonadetes; Gemmatimonadetes              |
| 6   | *Acidobacterium*   | 2.391             | 2.031              | 1.934                     | Acidobacteriaia; Acidobacteria                  |
| 7   | *Nitrosospira*     | 2.279             | 0.869              | 0.392                     | Betaproteobacteria; Proteobacteria              |
| 8   | *Rubrobacter*      | 2.197             | 1.068              | 0.526                     | Rubrobacteria; Actinobacteria                   |
| 9   | *Chloroflexus*     | 1.928             | 2.955              | 0.938                     | Chloroflexia; Chloroflexi                      |
| 10  | *Arthrobacter*     | 0.821             | 4.371              | 1.185                     | Actinomycetia; Actinobacteria                   |
| 11  | *Methylocaldum*    | 0.004             | 2.688              | 0.005                     | Gammaproteobacteria; Proteobacteria             |
| 12  | *Sphaerobacter*    | 1.330             | 2.564              | 0.622                     | Thermomicrobia; Chloroflexi                    |
| 13  | *Levilinea*        | 0.699             | 2.143              | 0.723                     | Anaerolineae; Chloroflexi                      |
| 14  | *Cytophaga*        | 0.218             | 0.358              | 3.328                     | Cytophagia; Bacteroidetes                      |
| 15  | *Steroidobacter*   | 1.046             | 1.141              | 3.187                     | Gammaproteobacteria; Proteobacteria             |
| 16  | *Ohtaekwangia*     | 0.490             | 0.270              | 3.076                     | Cytophagia; Bacteroidetes                      |
| 17  | *Streptomyces*     | 0.352             | 0.352              | 2.014                     | Actinomycetia; Actinobacteria                   |
**FIG S1** Comparison of metagenomic (culture-independent) and cultured bacteria datasets at the class level distributed in the three rhizospheric soil samples (a) Luxor, (b) Minya and (c) Nubaria of *T. aestivum* L. plants. JM (Jensen medium prepared by autoclaving phosphate and solidifying agent together), SJM (Jensen medium prepared by autoclaving phosphates and solidifying agent separately).
FIG S2 The percentages of cultured genera from the three analyzed soils obtained on LB and Jensen media using the standard culture protocols (LB agar and JM-agar) and the modified media preparations used in this study (LB-phytagel, LB-gelrite, SJM-agar, SJM-phytagel, and SJM-gelrite) compared to the metagenomic datasets.
FIG S3 GIS map of the three soil sites generated by Google® Earth Pro 7.1.7.2600