Supporting information for

A synthetic bacterial community derived from a desert rhizosphere confers salt stress resilience to tomato in the presence of a soil microbiome

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Figures

Fig. S1: Former agricultural areas in the Jizan desert, which suffer from high salinity, are scarcely populated with indigofera.



Fig. S2: Venn diagram showing number of depleted OTUs in the Rhizo and EC of *I. argentea* in comparison to the Soil. See Dataset S2.





Fig. S3: Growth promotion of arabidopsis by the Jizan strains Ensifer sp. SA403 and Bacillus sp. SA436. Ten arabidopsis seeds were grown on a plate which was the experimental unit. There were four experimental units per inoculation which also included a sterile control. Seedling fresh weight was defined as the average weight per experimental unit and determined eleven days post bacterial inoculation. The y-axis shows the average seedling fresh weights with the standard deviation as error bars. An asterisk is a significant difference (p < 0.05) from the control per Dunnett's test. Strains SA436 and SA403 were grown in 5 mL 1/10th TSB or 5 mL YEM liquid medium, respectively, for 48 hours at 28°C. Bacterial cells were washed and resuspended in 10 mM MgSO₄ and the final suspension had an OD₆₀₀ of 1.0 (~109 cfu/mL). Seeds of Arabidopsis thaliana Columbia-0 were surface steriled by washing with ethanol and soaking in 1/4 strength commercial bleach for 10 minutes. Seeds were transferred to wet filter paper in Petri dishes. After incubating at 4°C for 3 days, 10 seeds were sown on plates containing 50 mL 1/2th Murashige Skoog medium. Two µL of the bacterial suspension was applied to the root tips of one-week-old seedlings. Control plants were inoculated with 2 μ L of 10 mM MgSO₄.

Fig. S4: Maximum likelihood trees based on the AMPHORA gene alignments of nine different

genera. (a) *Massilia*, (b) *Streptomyces*, (c) *Ensifer*, (d) *Bacillus*, (e) *Enterobacter*, (f) *Acinetobacter*, (g) *Ochrobactrum*, (h) *Pseudomonas*, (i) *Ralstonia*. Each tree contains at least one of the strains found in the Jizan SynCom, representative genomes and plant-associated strains for that genus. All trees are rooted on a suitable outgroup and the Jizan strains are colored in red. The distance scale indicates the number of differences between sequences.

а







С



Rhizobium tropici CIAT 899 Ensifer adhaerens X097 Ensifer sp. Root423 Ensifer sp. Root558 Ensifer adhaerens Casida A Ensifer sp. Root1298 Ensifer sp. Root1312 Ensifer sp. Root954 Ensifer sp. Root74 Sinorhizobium sp. GL28 Ensifer sp. Root278 Ensifer sp. Root1252 Ensifer sp. Root231 Ensifer sp. Root258 Ensifer sp. Root127 Ensifer sp. Root31 Ensifer sp. NM-2 Ensifer sp. Root142 Ensifer sp. 1H6 Sinorhizobium terangae USDA4894 Ensifer sp. MPMI2T Sinorhizobium meliloti BL225C Sinorhizobium meliloti AK83 Sinorhizobium sp. M4_45 Sinorhizobium arboris LMG 14919 Sinorhizobium medicae WSM1115 Sinorhizobium saheli USDA4893 Ensifer sp. WSM1721 Ensifer aridi LMR001 Ensifer aridi LEM451 Ensifer aridi LMR013 Ensifer aridi TW10 Ensifer alkalisoli YIC4027 Ensifer sp. LCM 4579 Ensifer sojae CCBAU 05684 Ensifer sp. SA403 Sinorhizobium fredii CCBAU 45436 Sinorhizobium fredii CCBAU 25509 Sinorhizobium sp. BJ1 Ensifer sp. BR816 Sinorhizobium americanum CCGM7 Sinorhizobium sp. NG07B Sinorhizobium fredii NXT3 Sinorhizobium sp. FG01



Lysinibacillus sphaericus LMG 22257 Bacillus coagulans R11 Bacillus acidiproducens DSM 23148 Bacillus ginsengihumi M2.11 Bacillus lentus NCTC4824 Bacillus freudenreichii NCTC4823 Bacillus circulans PK3_109 Bacillus taxi M5HDSG1-1 Bacillus kyonggiensis NB22 Bacillus mesophilum IITR-54 Bacillus canaveralius ATCC 29669 Bacillus licheniformis BL-010 Bacillus haynesii S16 Bacillus paralicheniformis MDJK30 Bacillus subtilis RO-NN-1 Bacillus mojavensis RO-H-1 Bacillus halotolerans ZB201702 Bacillus pumilus MTCC B6033 Bacillus stratosphericus LK33 Bacillus altitudinis GR-8 Bacillus aerophilus C772 Bacillus safensis BRM1 Bacillus aciditolerans YN-1 Bacillus alkalitelluris DSM 16976 Bacillus megaterium NCT-2 Bacillus aryabhattai T61 Bacillus sp. SA436 Bacillus endophyticus FH5 Bacillus filamentosus Hbe603 Bacillus luciferensis CH01 Bacillus acidiceler 1.17 Bacillus solisilvae NEAU-cbsb5 Bacillus funiculus W18-1 Bacillus panaciterrae DSM 19096 Bacillus pseudomycoides FSL H8-0534 Bacillus mycoides Gnyt1 Bacillus wiedmannii GOE6 Bacillus thuringiensis YBT-1520 Bacillus albus PG26 Bacillus paranthracis PR1 Bacillus pacificus RC-1 Bacillus tropicus CD3-2 Bacillus anthracis CZC5 Bacillus cereus S2-8





Alkanindiges illinoisensis DSM 15370 Acinetobacter puyangensis ANC 4466 Acinetobacter apis ANC 5114 Acinetobacter radioresistens SA188 Acinetobacter radioresistens NBRC 102413 Acinetobacter seifertii S21 Acinetobacter baumannii Ab03 Acinetobacter lactucae QL-1 Acinetobacter pittii PHEA-2 Acinetobacter calcoaceticus CA16 Acinetobacter oleivorans DR1 Acinetobacter sp. 2JN-4 Acinetobacter haemolyticus HW-2A Acinetobacter sp. C16S1 Acinetobacter parvus CIP 108168 Acinetobacter junii 65 Acinetobacter junii Izh-X15 Acinetobacter gyllenbergii NIPH 230 Acinetobacter dispersus NCCP 16014 Acinetobacter sp. MN12 Acinetobacter beijerinckii CIP 110307 Acinetobacter baylyi ADP1 Acinetobacter ursingii CIP 107286 Acinetobacter larvae BRTC-1 Acinetobacter pullicarnis S23 Acinetobacter rudis CIP 110305 Acinetobacter gerneri CIP 107464 Acinetobacter guillouiae NBRC 110550 Acinetobacter defluvii WCHA30 Acinetobacter sichuanensis WCHAc060041 Acinetobacter shaoyimingii 323-1 Acinetobacter Ianii 158 Acinetobacter chinensis WCHAc010005 Acinetobacter tandoii CIP 107469 Acinetobacter sp. ANC 5084 Acinetobacter harbinensis HITLi 7 Acinetobacter kookii ANC 4667 Acinetobacter equi 114 Acinetobacter indicus UBT1 Acinetobacter bouvetii CIP 107468 Acinetobacter cumulans WCHAc060092 Acinetobacter wanghuae dk386 Acinetobacter mesopotamicus GC2 Acinetobacter idrijaensis MII

Mesorhizol Ochrobacti Brucella ab Brucella ce Brucella m Ochrobacti Ochrobacti

Mesorhizobium australicum WSM2073 Ochrobactrum sp. CNPMS2088 Ochrobactrum pituitosum AA2 Ochrobactrum pseudogrignonense W13P3 Ochrobactrum pseudogrignonense K8 Ochrobactrum grignonense OgA9a Ochrobactrum pituitosum BU72 Ochrobactrum quorumnocens A44 Ochrobactrum rhizosphaerae PR17 Brucella ovis ATCC 25840 Brucella neotomae NCTC10084 Brucella melitensis 16M Brucella abortus 2308 Brucella abortus A19 Brucella canis ATCC 23365 Brucella suis 1330 Brucella ceti TE10759-12 Brucella microti CCM 4915 Ochrobactrum anthropi ALM4 Ochrobactrum anthropi DE2010 Ochrobactrum lupini LUP21 Ochrobactrum lupini LUP23 Ochrobactrum anthropi ATCC 49188 Ochrobactrum anthropi T16R-87 Ochrobactrum anthropi LMG 2136 Ochrobactrum anthropi DSM 14396 Ochrobactrum sp. DDT2 Ochrobactrum anthropi CTS-325 Ochrobactrum anthropi PBO Ochrobactrum anthropi W13P3 Ochrobactrum anthropi ML7 Ochrobactrum cytisi IPA7.2 Ochrobactrum tritici TA93 Ochrobactrum anthropi FRAF13 Ochrobactrum oryzae OA447 Ochrobactrum pecoris 08RB2639 Ochrobactrum sp. 3-3 Ochrobactrum pseudintermedium CCUG 34735 Brucella intermedia NCTC12171 Ochrobactrum intermedium SA148 Ochrobactrum sp. EGD-AQ16 Brucella intermedia OiC8-6 Brucella intermedia RH1CCR112 Brucella intermedia Tara1



Pseudomonas luteola FDAARGOS 637 Pseudomonas sp. SA613 Pseudomonas savastanoi ICMP 19499 Pseudomonas syringae B728a Pseudomonas syringae DC3000 Pseudomonas caspiana FBF102 Pseudomonas lutea DSM 17257 Pseudomonas versuta L10.10 Pseudomonas simiae PCL1751 Pseudomonas canadensis 2-92 Pseudomonas rhodesiae NL2019 Pseudomonas lactis SS101 Pseudomonas marginalis H21 Pseudomonas trivialis IHBB745 Pseudomonas fildesensis KG01 Pseudomonas veronii R02 Pseudomonas yamanorum LBUM636 Pseudomonas umsongensis CY-1 Pseudomonas brassicacearum 3Re2-7 Pseudomonas frederiksbergensis SI8 Pseudomonas corrugata RM1-1-4 Pseudomonas glycinae MS586 Pseudomonas vranovensis DSM 16006 Pseudomonas alkylphenolica Neo Pseudomonas donghuensis P482 Pseudomonas putida KT2440 Pseudomonas monteilii B5 Pseudomonas taiwanensis DSM 21245 Pseudomonas capeferrum WCS358 Pseudomonas hydrolytica DWY01 Pseudomonas argentinensis SA190 Pseudomonas argentinensis CCUG 50743 Pseudomonas seleniipraecipitans D1-6 Pseudomonas stutzeri 28a24 Pseudomonas sp. SA244 Pseudomonas furukawaii KF707 Pseudomonas lalkuanensis PE08 Pseudomonas resinovorans DSM 21078 Pseudomonas aeruginosa HIAE PA01 Pseudomonas multiresinivorans populi Pseudomonas nitritireducens WZBFD3-5A2 Pseudomonas humi CCA1 Pseudomonas citronellolis P3B5



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Fig. S5: Tomato salt stress assay. The salt stress assay was adapted from a protocol by Awlia et al., 2016. Pots with plants are soaked in a saline solution for about 30 minutes. As the WHC is always maintained at 60%, there is a certain volume of water already present in the substrate. When the saline solution enters through the bottom of the cups during the soaking, the salt concentration lowers as the solution is diluted by the water present in the substrate. The volume of saline solution that enters the pot is equal to the remaining 40% WHC plus extra air. This extra air volume is a result of the supersaturation by the soaking method which exceeds the amount predicted by 100% WHC. As the WHC returns to 60% due to evaporation and transpiration, the diluted saline solution will concentrate to a target concentration in the substrate.



Fig. S6: Salt tolerance test of tomato plants grown with or without SynCom inoculation. Shoot dry weight of 21-day-old tomato plantlets exposed to 0, 100, 200, 300 mM NaCl (for 14 days) and inoculated with or without SynCom C. Plants were grown in the non-sterile substrate. An asterisk represents a statistically significant (p < 0.05) difference between the two inoculations per Student's t-test.



0 mM NaCl

200 mM NaCl



e



SA187 SA436 SA444









0 mM NaCl

200 mM NaCl



Fig. S8: Tomato plants were grown in the non-sterile substrate and inoculated with SynCom C or individually with the five SynCom C strains. A control condition without inoculum was also included. Plants were exposed to 0 or 200 mM NaCl one week after sowing. Shoot tissue was harvested at ten days post salt imposition for fresh weight and ion content analysis. The standard deviation is shown by the error bars. (a & b) Average shoot fresh weights (n=15) for the 0 & 200 mM NaCl treated plants, respectively. An asterisk indicates a significant difference (p < 0.05) from the control per Dunnett's test. (c & d) Average shoot ion content as determined by ion chromatography for the 0 & 200 mM NaCl salt levels, respectively. Five random plants were selected from the control and SynCom C inoculated plants. The left axis shows the concentration in mg / g of dry weight for each ion. The right axis shows the dimensionless ratio between the concentrations of the Sodium and Potassium ions. An asterisk is a significant difference (p < 0.05) per Student's t-test.

Fig. S9: ASVs of Jizan strains which did not pass the detection criteria for the root colonization of tomato.



Acinetobacter sp. SA188 (ASV10)

EC

EC

Fig. S10: Positive correlation between bacterial ASVs and salt concentration. Log2-transformed normalized read counts of ASVs found in the Rhizo (a) and EC (b) of tomato growing in the non-sterile substrate and inoculated with the Jizan SynCom. The ASVs that were enriched in any of the salt levels and with a positive correlation to the salt gradient are shown here. Crosses indicate mean log2-transformed normalized read counts of each ASV under each salt level. Blue crosses indicate ASVs matching a SynCom strain detected in this analysis.

