

**MICROBIAL CONSORTIUM FOR PLANT GROWTH AND METHOD FOR
PREPARING THE SAME**

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority to the Singapore application no. 10202251275V filed October 5, 2022, and the contents of which are hereby incorporated by reference in their entirety for all purposes.

TECHNICAL FIELD

[0002] The present disclosure generally relates to a microbial consortium for plant growth and a method for preparing a microbial consortium for a target plant.

BACKGROUND

[0003] Increasing food demand, limited land resources and disruption in the food supply chain globally have created a need to develop sustainable solutions for urban farming. As usage of engineered plants or crops and fertilizer are known to cause long-term side effects, utilizing plant growth promoting microbes in agricultural industry are considered as nature-derived sustainable solution for enhancing plant health and resilience. However, conventional screening and selection procedures for obtaining microbes with the plant growth promoting abilities are time consuming and laborious. Adoption of the microbes at a wider scale for different plants or soil often fails due to the stringent growth requirements.

[0004] Thus, there is a need for a microbial consortium for improving plant growth, in which the microbes form syntrophic relationships, and a need for a method of selecting microbial consortium for a target plant which incorporates computational and experimental approaches, so that a customized microbial consortium with plant growth promoting benefits can be made for specific plant in a shorter time.

SUMMARY

[0005] In one aspect, the present application discloses a microbial consortium for plant growth, comprising: at least two microbial strains, wherein each of the at least two microbial strains has at least a plant growth-promoting genetic element; and wherein one of the at least two microbial strains forms a syntrophic relationship with the remaining of the at least two microbial strains.

[0006] In some embodiments, the at least two microbial strains are selected from the group consisting of *Streptomyces sp.*, *Agromyces sp.*, *Bacillus subtilis*, *Priestia aryabhatai*, *Pseudomonas sp.*, and *Atlantibacter sp.*.

[0007] In another aspect, the present application discloses a method for preparing a microbial consortium for a target plant, comprising: collecting a sample from a community of the target plant; identifying microbial strains in the sample; selecting at least two microbial strains identified in the sample; isolating the selected at least two microbial strains from the sample; predicting plant growth-promoting potential of each of the isolated microbial strains or a combination of the at least two microbial strains; and validating the predicted plant growth-promoting potential with the target plant, wherein the step of selecting the at least two microbial strains comprises selecting microbial strains with each having at least a plant growth-promoting genetic element; and selecting at least one microbial strain that is capable of forming a syntrophic relationship with the remaining of the at least two microbial strains; and wherein the selected microbial strains are syntrophic.

[0008] In some embodiments, the step of predicting includes applying *in-silico* genomic and metabolic characterization and conducting a chemical assay on each of the isolated microbial strains or on the combination of the at least two microbial strains.

[0009] In another aspect, the present application discloses use of a microbial consortium of the present disclosure for treating a target plant to promote plant growth, comprising contacting

seeds, seedlings, any part of roots or any part of the target plant or a combination thereof, or rhizosphere-associated soil with the microbial consortium.

[0010] In some embodiments, the target plant is a member of the *Brassicaceae* family.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Various embodiments of the present disclosure are described below with reference to the following drawings:

FIG. 1 is a flowchart of a method for preparing a microbial consortium according to an embodiment of the present disclosure. FIG. 1 depicts 1. An urban farm (101); 2. A microbiome assessment involving 3 GLV, 4 niches, 2 growth stage and 910 genomes (102); 3. A microbial ecology (103); 4. Microbial isolation involving approximately 150 isolates (104); 5. Genomics, modelling & predictions involving 55 WGA and 400 plant growth-promotion rhizobacteria (PGPR) genes (105); and 6. Product testing (106).

FIGS. 2A and 2B depict the choy sum plant phenotype after 20 days of growth in the growth room (FIG. 2A: consortium SAB (*Streptomyces sp.*, *Agromyces sp.*, and *Bacillus subtilis*) treated, FIG. 2B: untreated).

FIGS. 3A and 3B are box plots showing the fresh biomass difference in shoot and root sampled from the consortia SAB treated, individual isolates treated, and untreated control choy sum plant (FIG. 3A: shoot fresh biomass, FIG 3B: root fresh biomass). The seeds were treated just one time before sowing according to the method as disclosed herein. The data was collected after 20 days of plant growth in the growth room.

FIGS. 4A and 4B are box plots showing the fresh biomass difference in shoot and root after 22 days of growth, sampled from the consortia SAB treated, individual isolates treated, and untreated control choy sum plant (FIG. 4A: shoot fresh biomass, FIG 4B: root fresh biomass), which involved the treatment with the individual bacteria and the consortia at both seed and

seedling stage. The plants were transferred to new bigger pots on the 13th day and microbes were applied by spraying in root regions.

FIGS. 5A and 5B are box plots showing the fresh biomass difference in shoot and root of the choy sum plant when applied with different dosages of consortia SAB (FIG. 5A: shoot fresh biomass, FIG 5B: root fresh biomass).

FIGS. 6A and 6B depict the choy sum plants phenotype after 30 days of growth in the greenhouse using non-sterile soil (FIG. 6A: consortia SAB treated, FIG. 6B: untreated). Two-time treatment was used.

FIG. 6C is a box plot showing the fresh biomass difference in shoot sampled from consortia SAB treated and untreated control of choy sum plant.

FIGS. 6D and 6E depict the choy sum plants phenotype showing enhanced shoot and root growth under SAB treatment in greenhouse conditions. Both peat-moss soil (left) and nutrient-poor garden soil (right) were used separately in the study.

FIG. 7A is a bar plot showing the increase in percentage germination rate of choy sum seeds treated with SAB compared to untreated seeds in nutrient-rich peat moss and nutrient-poor garden soil under greenhouse conditions.

FIGS. 7B and 7C are box plots showing a significant increase in shoot and root fresh biomass of choy sum plants treated with SAB compared to untreated plants in nutrient-rich peat moss soil.

FIGS. 7D to 7G are box plots showing a significant increase in shoot fresh biomass, root fresh biomass, root length, and root area of choy sum plants treated with SAB compared to untreated plants in nutrient-poor garden soil. Each point in the plot represents an individual plant grown in a separate pot.

FIG. 8A depicts the growth dynamics of leaf area between SAB treated and untreated choy sum plants. The leaf area was digitally measured at regular intervals of time. The leaf area was

digitally measured at regular intervals of time. Leaf area was measured from three different plants.

FIGS. 8B and 8C are box plots showing the significant increase in shoot and root fresh biomass of choy sum plants treated with consortium SAB compared to untreated plants at three different time points during plant growth. The boxes represent the median, interquartile range, and 95% confidence interval, while the whiskers indicate the range of data points. The plots clearly demonstrate the significant increase in both shoot and root fresh biomass in plants treated with SAB compared to untreated plants at all three time points.

FIG. 8D is a box plot represents the increase in maximal photochemical efficiency of photosystem II (PSII) with time in plants treated with consortium SAB compared to untreated plants. The Fv/Fm ratio, which indicates PSII efficiency, was determined using CIRAS-3, and the box plot displays the differences in Fv/Fm ratio between treated and untreated plants over time. The plot clearly shows a significant increase in Fv/Fm ratio in plants treated with SAB compared to untreated plants.

FIG. 8E is a box plot illustrating the differences in stomatal conductance over time in plants treated with consortium SAB compared to untreated plants. The stomatal conductance was measured using CIRAS-3, and the average values from all the leaves for each plant are represented by each point in the graph. A significant increase in stomatal conductance was observed in plants treated with SAB at later stages compared to untreated plants. These results highlight the positive impact of SAB treatment on plant growth and development.

FIG. 9A depicts the phenotype comparison of *Arabidopsis* treated with and without consortia SAB.

FIGS. 9B and 9C are box plots showing the fresh biomass difference in shoot and root of the *Arabidopsis* treated with and without consortia SAB.

FIG. 9D and 9E are box plots showing the root length and root area of the *Arabidopsis* treated with and without consortia SAB after 30 days of growth.

FIG. 10A depicts the phenotype comparison of kailan treated with and without consortia SAB.

FIGS. 10B and 10C are box plots showing the fresh biomass difference in shoot and root of the kailan treated with and without consortia SAB after 30 days of growth.

FIGS. 10D, 10E, 10F and 10G are box plots showing the fresh and dry biomass difference in shoot and root of the kailan treated with and without consortia SAB after 30 days of growth.

FIGS. 11A and 11B depict the co-occurrence networks of rhizospheric bacterial community in young (a) and adult (b) stages of green leafy vegetables, based on SparCC correlation between ASVs in the rhizosphere region of an urban farm. The edges with strong (corr more than 0.6) and significant (P-value less than 0.05) correlation are displayed, while the nodes represent ASVs, with three highlighted genera depicted as larger nodes respectively. The community exhibits a single module in the young stage, while three distinct modules are evident at the adult stage, as represented by three dense clusters. Notably, *Agromyces sp.* is a keystone species observed to positively interact with the target bacteria in both stages.

FIG. 12A depicts a predicted growth curve of each microbial strain in consortium SAB.

FIG. 12B depicts a predicted exchange of the metabolites between each microbial strain in consortium SAB.

FIGS. 13A illustrates comparative growth curve of the isolates and consortium SAB in M9 media with glucose.

FIGS. 13B – 13F depict experimental time-dependent change in the concentration of the selected metabolites from consortium culture filtrate. The concentration was determined by using targeted liquid chromatography with triple-quadrupole mass spectrometry (LC-QQQ) based-analysis and then normalized using colony forming unit (CFU) count to give a rough estimate of concentration for each colony. Error bars represent the standard deviation (n=3).

FIG. 14 is a high-resolution scanning electron microscopy (SEM) image showcases the interaction between *Bacillus* sp. SS3 and *Agromyces* sp. SS2, with the bacterial cells visibly attached to the branch-like hyphae via flagella/nanotubes. The image is magnified at 20,000X, providing intricate details of the cellular structures and their interactions.

FIG. 15A is a boxplot showing the variations in the Shannon diversity indices of the microbiome in three niches (rhizosphere, endosphere, and phyllosphere) of the consortium SAB-treated and untreated choy sum plants. The facets in each niche depict the differences between young and adult stages.

FIG. 15B is a canonical correspondence analysis (CCA) plot based on Bray-Curtis dissimilarity showing the differences between consortium SAB treated and untreated choy sum plants at two growth stages in three different niches (rhizosphere, endosphere, and phyllosphere).

FIG. 16 illustrates the permutational multivariate analysis of variance (PERMANOVA) results from the amplicon data of the SAB treated and untreated choy sum plants from three different niche and at two different time points.

FIG. 17 illustrates the phenotypic changes in choy sum plant under a different period of drought conditions which were exposed to them after 15 days of growth in normal conditions with adequate watering in growth room condition.

FIG. 18 is a boxplot showing the fresh biomass difference in shoot sampled from treated (individual isolate *Priestia*, two bacteria consortia, PA, consisting of *Pseudomonas* sp. and *Atlantibacter*, and three member consortia, PPA, consisting of *Priestia* and PA) and untreated choy sum plant after 20 days of growth.

FIG. 19 depicts a predicted exchange of the metabolites between each microbial strain in consortium PPA.

DETAILED DESCRIPTION

[0012] The following detailed description is made with reference to the accompanying drawings, showing details and embodiments of the present disclosure for the purposes of illustration. Features that are described in the context of an embodiment may correspondingly be applicable to the same or similar features in the other embodiments, even if not explicitly described in these other embodiments. Additions and/or combinations and/or alternatives as described for a feature in the context of an embodiment may correspondingly be applicable to the same or similar feature in the other embodiments.

[0013] In the context of various embodiments, the articles “a”, “an” and “the” as used with regard to a feature or element include a reference to one or more of the features or elements.

[0014] In the context of various embodiments, the term “about” or “approximately” as applied to a numeric value encompasses the exact value and a reasonable variance as generally understood in the relevant technical field, e.g., within 10% of the specified value.

[0015] As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items.

[0016] As used herein, “comprising” means including, but not limited to, whatever follows the word “comprising”. Thus, use of the term “comprising” indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present.

[0017] As used herein, “consisting of” means including, and limited to, whatever follows the phrase “consisting of”. Thus, use of the phrase “consisting of” indicates that the listed elements are required or mandatory, and that no other elements may be present.

[0018] As used herein, the term “microorganism” or “microbe” should be taken broadly. It includes, but is not limited to, the two prokaryotic domains, Bacteria and Archaea, as well as eukaryotic fungi and protists.

[0019] “Microbial strain” means a group of species with one or more characteristics that distinguish it from other subgroups of the same species of the strain.

[0020] As used herein, the term “microbial consortium” or “microbial consortia” means a mixture of two or more selected microbial species or strains of a species. It includes compatible beneficial microbes, which can be described as involving in or associating in producing desirable plant phenotypic traits. For example, it can be described as able to perform plant growth promoting functions. The term “consortium” or “consortia” are used interchangeably in the present disclosure.

[0021] As used herein, the term “syntrophic” or “syntrophic relationship” means a state or a relationship involving cross-feeding or exchange of metabolites between at least two different microbial species.

[0022] As used herein, the term “inherent” means naturally occurring or existing without laboratory-based genetic modification.

[0023] In one aspect, the present disclosure relates to a microbial consortium for plant growth comprising at least two microbial strains, wherein each of the at least two microbial strains has at least a plant growth-promoting genetic element; and wherein one of the at least two microbial strains forms a syntrophic relationship with the remaining of the at least two microbial strains.

[0024] Each of the plant growth-promoting genetic element is inherent in any of the at least two microbial strains.

[0025] In various embodiments, the microbial consortium may comprise a combination of two microbial strains, a combination of three microbial strains, a combination of four microbial strains, a combination of five microbial strains or a combination of six microbial strains.

[0026] In some embodiments, the microbial consortium comprises two or more microbial strains selected from the group consisting of *Streptomyces sp.*, *Agromyces sp.*, *Bacillus subtilis*, *Priestia aryabhattai*, *Pseudomonas sp.*, and *Atlantibacter sp.*

[0027] In one embodiment, the microbial consortium comprises microbial strains including *Streptomyces sp.*, *Agromyces sp.*, and *Bacillus subtilis* deposited with NAIMCC under Accession No. NAIMCC-IDA-5. In another embodiment, the microbial consortium comprises microbial strains including *Priestia aryabhattai*, *Pseudomonas sp.*, and *Atlantibacter sp.*, deposited with NAIMCC under Accession No. NAIMCC-IDA-6.

[0028] The microbial strains of the present disclosure may be isolated in substantially pure or mixed cultures.

[0029] In another aspect, a method for preparing a microbial consortium for a target plant is provided. Referring to FIG. 1, an exemplary method of the present disclosure is described. The method depicted in FIG. 1 is merely embodiment and is not intended to limit the scope of the present disclosure as described herein. For example, the steps recited in the method descriptions may be executed in any order and are not limited to the order presented. With reference to FIG. 1, the method comprises (1) collecting a sample from a community of the target plant; (2) identifying microbial strains in the sample; (3) selecting at least two microbial strains identified in the sample; (4) isolating the at least two microbial strains from the sample; (5) predicting plant growth-promoting potential of each of the isolated microbial strains or a combination of the at least two microbial strains; and (6) validating the predicted plant growth-promoting potential with the target plant, wherein the step of selecting the at least two microbial strains comprises selecting microbial strains with each having at least a plant growth-promoting genetic element; and selecting at least one microbial strain that is capable of forming a syntrophic relationship with the remaining of the at least two microbial strains; and wherein the selected microbial strains are syntrophic.

[0030] In some embodiments, the sample collected is the soil surrounding the target plant. In other embodiments, the sample collected is the soil surrounding the roots of the target plant. In further embodiments, the sample collected is the soil from the rhizosphere of the target plant. In yet other embodiments, the sample collected is the soil in contact with the rhizoplane of the target plant. In yet further embodiments, the sample collected is the soil from both seedling and adult stages of plants.

[0031] In some embodiments, the identification method of the microbial strains comprises 16s rRNA sequencing.

[0032] In some embodiments, the step of predicting the plant growth-promoting potential of each of the isolated microbial strains or a combination of the at least two microbial strains comprising applying *in-silico* genomic and metabolic characterization and conducting a chemical assay on each of the isolated microbial strains or on the combination of the at least two microbial strains. The *in-silico* genomic and metabolic characterization that can be used in the method of the present disclosure include, but are not limited to, genome sequencing, microbial ecological network construction and genome scale metabolic model simulation. Non-limiting examples of chemical assay that can be used with the present disclosure include, but are not limited to, biofilm forming capacity, siderophore, Indole Acetic Acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC) deaminase production and phosphate solubilization capacity.

[0033] In some embodiments, the selecting, the predicting, and the validating in the present disclosure occur iteratively for syntropic microbial strains in the microbial consortium. In other embodiments, the selecting, the predicting, and the validating in the present disclosure can be performed in any order or in parallel.

[0034] The method of the present disclosure further includes combining the at least two isolated microbial strains to form a microbial consortium, wherein the at least two microbial

strains are selected from the group consisting of *Streptomyces sp.*, *Agromyces sp.*, *Bacillus subtilis*, *Priestia aryabhattai*, *Pseudomonas sp.*, and *Atlantibacter sp.* In one embodiment, the microbial consortium comprises microbial strains including *Streptomyces sp.*, *Agromyces sp.*, and *Bacillus subtilis*, deposited with NAIMCC under Accession No. NAIMCC-IDA-5. In another embodiment, the microbial consortium comprises microbial strains including *Priestia aryabhattai*, *Pseudomonas sp.*, and *Atlantibacter sp.*, deposited with NAIMCC under Accession No. NAIMCC-IDA-6.

[0035] After a suitable microbial consortium is identified, isolated and prepared, the microbial consortium may be provided to at least one part of the target plant or soil in an effective amount to treat the target plant. This can be carried out for the purpose of validating the predicted plant growth-promoting potential or for the purpose of promoting plant growth of the target plant. In some embodiments, the microbial consortium is provided to the at least one part of the target plant by contacting seeds, seedlings, any part of roots or any part of the target plant or a combination thereof with the microbial consortium. In other embodiments, the microbial consortium is provided to the soil by contacting rhizosphere-associated soil with the microbial consortium. In certain embodiments, the microbial consortium is provided to the at least one part of the target plant by contacting seeds, seedlings or a combination thereof of the target plant with the microbial consortium.

[0036] In some embodiments, the step of validating in the present disclosure includes conducting pot experiments to monitor the biomass of the target plant treated with the microbial consortium. An increase in the biomass of the target plant over a predetermined duration indicates that the microbial consortium is a suitable candidate for promoting plant growth of the target plant.

[0037] In another aspect, the present disclosure relates to a microbial consortium obtained by the method disclosed herein.

[0038] In yet another aspect, the present disclosure relates to use of the microbial consortium of the present disclosure for treating a target plant to promote plant growth, comprising contacting seeds, seedlings, any part of roots or any part of the target plant or a combination thereof, or rhizosphere-associated soil with the microbial consortium.

[0039] The microbial consortium of the present disclosure may be applied to any target plant or any plant of interest. In some embodiments, the target plant may include urban plant or crop, green leafy vegetables such as choy sum (*Brassica chinensis* var. *parachinensis*), kailan (*Brassica oleracea* var. *alboglabra*), bayam (*Amaranthus*) etc.

[0040] In one example, the microbial consortium in the present disclosure may be applied to the target plant as seed dressing or seed treatment. The seed dressing can occur at least one time before sowing to the sterilized surface of a seed. In another example, the microbial consortium in the present disclosure may be applied to the target plant two times. In yet another example, the microbial consortium in the present disclosure may be applied to the target plant by spraying on the roots of the target plant after the treated seed enters the plant seedling stage.

[0041] In a further example, the microbial consortium in the present disclosure may be applied as soil dressing or soil treatment. The soil dressing can occur by applying the microbial consortium by spraying on the soil before seed or seedling transplantation.

[0042] In another example, the microbial consortium in the present disclosure may be applied to the target plant in a concentration of at least 1.2×10^8 to 2.0×10^8 cells.

[0043] In some embodiments, the present disclosure relates to use of the microbial consortium of the present disclosure on a target plant, wherein the target plant is a member of the *Brassicaceae* family.

[0044] The present disclosure utilizes microbes to promote the growth a plant. Examples of the plant growth-promoting traits are increased growth (increased biomass), increased yield, increased drought tolerance and increased phosphorus, etc. In the present disclosure, the

microbial strains used for designing suitable microbial consortium for a target plant are derived from an agricultural farm, in particular, from soil collected from the agricultural farm. Soil is a natural product and thus, there is no ecological disturbances or harmful effects resulting from the use of microbial strains isolated from soil samples from the community of the target plant. Moreover, the microbial consortium prepared by the method of the present disclosure is self-stable as the microbial consortium is a result of natural microbial associations. This makes it suitable for long term storage and applications. It also reduces variation in plant performance when compared with application of individual isolates.

[0045] Non-limiting examples of the disclosure will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the disclosure.

EXAMPLES

Example 1 - Methods

[0046] Isolated Microbes Source Material

[0047] The microbes were obtained from the soil of a local urban farm cultivating green leafy vegetables. The microbes were isolated from the same samples from which DNA was extracted for the amplicon study.

[0048] Microbial Culture Techniques

[0049] The microbes were cultured using isolation media plates using different sample dilutions. For example, the isolation media are nutrient agar, soil extract media and Luria-Bertani media. The grown colonies were morphologically characterized and for initial 16S rRNA identification of isolated colonies were carried out. The bacterial colonies can be maintained aerobically in liquid media, media plates and in the form of glycerol stock stored at -80°C. Whole genome sequencing by both Illumina (NovaSeq 6000) and Nanopore (Oxford Nanopore PromethION) were done for the isolates.

[0050] **Colony Microscopic Imaging**

[0051] In one example, one of the isolates whose sequencing resulted in the identification of two different genomes were further subjected to several rounds of serial dilutions, but two different colonies were not observed. The single colony of the isolate was then cultured, processed, and imaged by using Scanning Electron Microscope (SEM) to observe the existence of two different types of bacterial cells at different levels of resolution.

[0052] **Plant Growth-Promotion Rhizobacteria (PGPR) assay**

[0053] The various plant growth promoting properties of the isolates were tested by chemical assays. In one example, the microbes were tested for their biofilm forming capacity, siderophore, indole acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production and phosphate solubilization capacity. All the assays were performed in replicates of five.

[0054] **Isolates Genome Sequencing and Improved Hybrid Assembly**

[0055] Genomes of the isolated bacteria were sequenced by both Illumina (Illumina) and Nanopore (Oxford Nanopore Technologies) sequencing technology. Genomic DNA extraction was performed using Zymo DNA extraction kit. Prior to sequencing, the quality of extracted genomic DNA was assessed by both agarose gel electrophoresis and Qubit Fluorometer (Thermo Fisher Scientific). For the sequencing by Illumina, NovaSeq 6000 was used. The DNA library was prepared before sequencing and the adapter was removed after the sequences were generated and the raw reads were used for further downstream processing. For the sequencing by Nanopore, Oxford Nanopore PromethION was used with Flowcell version R9.4.1. Raw sequences were basecalled using Guppy v3.2.10.

[0056] **Hybrid Genome Assembly**

[0057] Quality assessment of the Illumina short reads were done by using FastQC and for the Nanopore long reads, Nanoplot was used. Processing of both the long and short reads and

generation of the final genome assembly was done by the method as described herein by Tikariha et. Al., (2021). The pipeline for the hybrid assembly method uses Flye (long read assembler) followed by polishing of short reads. It detects multiple genomes, if there are co-occurring bacteria present together. It also provides opportunity to detect plasmids in the bacteria. The pipeline has been integrated in the form of integrated pipeline on the Nextflow platform.

[0058] **Genome Taxonomic Classification and Functional annotation**

[0059] For taxonomic classification, 16S rRNA gene sequence was submitted in the Silva database server and whole genomes were used for Average Nucleotide Identity (ANI) based classification by GTDB-Tk (Chaumeil *et al.*, 2020). General function was predicted using Prokka v1.13 (Seemann, 2014). For pathway level prediction, the amino acid fasta file was processed using KEGG kofam KOALA (Aramaki *et al.*, 2020) with predefined threshold and evaluate cutoff of 0.01 and then output submitted in KEGG Mapper. The potential PGPR genes were identified using a customized in-house gene list.

[0060] **Rhizosphere sampling and amplicon sequencing**

[0061] Local urban greenhouse farms were selected for sampling where three green leafy vegetables, such as kai lan, choy sum, and bayam are grown throughout the year. The sampling and processing of samples were like the methods as described by Bandla *et al.* (Bandla *et al.*, 2020) but in this case, instead of shotgun metagenomes, amplicon sequencing was carried out for rhizosphere and rhizoplane. Samples from both seedling and adult stages of plants were collected to account for changes in microbiome during different growth stages. For amplicon, V6-V8 hypervariable regions of the 16S rRNA gene were amplified using universal primers 926F (5'-AACTYAAAKGAATTGACGG-3') and 1392R (5'-ACGGGCGGTGTGTRC-3').

[0062] **Amplicon data analysis and ecological network construction**

[0063] The 16S rRNA amplicon data were processed by using the DADA2 error model pipeline (Callahan *et al.*, 2016). Adapters and primers were removed from raw reads using cutadapt v3.0. After trimming, high-quality reads were paired and followed with denoising, chimera removal, and final ASV table generation. Taxonomic assignment was performed using the SILVA v138.1 database.

[0064] The Phyloseq (McMurdie and Holmes, 2013) object was created in R, grouping the ASV and taxonomic sequence data for all the 47 samples for further processing. Twenty-three samples belonging to the seedling stage and 24 samples for the adult stage of all the three green leafy vegetables combined were processed separately for network generation. Those ASVs with read counts less than 2 and prevalent in 50% of the samples were removed to account for only those ASVs which are proper representative of the green leafy vegetables. The tool used for network construction here consider the data type of microbiome where simple Pearson and Spearman correlation may not be a good option. Moreover, at every step stringent cutoff were selected to avoid any type of false correlation and spurious outcomes. For ecological correlation network creation, the SparCC algorithm implemented in the FastSpar (Watts *et al.*, 2019) was considered due to its fast-processing capability. Data was processed with correlation cutoff threshold set at 0.6 and 1000 bootstraps were done for calculating p-value with its cutoff set at 0.05. Positive and significant correlation were visualized as network by Cytoscape (Su *et al.*, 2014) and the genomes of interest were highlighted. In one example, the network was generated and visualized using Gephi v0.9.7 (Bastian *et al.*, 2009). The ASV corresponding to the sequenced genome was identified based on the local blast analysis carried out between all the generated ASVs and 16S rRNA gene sequences from the genome. The matches with 99 percent identity or more than that were only considered.

[0065] **Genome scale metabolic model construction, curation, and simulation**

[0066] Genome-scale metabolic model (GMM) based study was incorporated to get the metabolic basis of cooperation between the bacteria, in the consortium. The high-quality assembled genome was taken for model construction. Draft metabolic network was reconstructed by using gapseq (Zimmermann *et al.*, 2021). The gap filling was done by using TSB (Tryptic soy broth) media. The model quality was evaluated by Memote (Lieven *et al.*, 2020), and sanity check was performed by COBRA Toolbox (Heirendt *et al.*, 2019). Metabolic interactions potential between the members of the consortium was analyzed by using BacArena (Bauer *et al.*, 2017). Existence of cross-feeding between the bacteria at two different time points during their growth were identified and further cross-checked with KEGG pathway mapping. Cross-feeding between the bacteria at two different time points during their growth was identified and visualized using Cytoscape (Su *et al.*, 2014).

[0067] **Soil, seed, and culture preparation for pot experiment**

[0068] BVB peat moss soil and sand were mixed in 3:1 ratio as it helps in maintaining proper aeration in the soil. The premixed soil was then autoclaved twice at 121°C for 30 minutes and around 125g of soil were filled in each pot. In some examples, to stimulate actual condition, the soil was not autoclaved. Single colony for each isolate was selected from Luria-Bertani (LB) agar plates and inoculated into LB broth in a flask and allowed to grow overnight in a shaking incubator maintained at 37°C.

[0069] The next day, the culture was centrifuged at 6000rpm for 10 min to pellet down the bacterial cells. The pellet was resuspended in 5 to 15 ml sterile MgSO₄ and washed twice. The OD was measured at 600 nm. The bacterial cultures were diluted to get 1.6×10^8 cells with 10mM MgSO₄.

[0070] In one example, for seed preparation, choy sum seeds were imbibed in sterile water for an hour. After that surface sterilization of the seeds were carried by transferring them in a solution containing 50% bleach and 1% Tween-20 for 5 min. The seeds were washed multiple

times with MilliQ water to remove traces of bleach and Tween in it. For seed dressing, the microbial adhesion onto sterilized seeds is allowed by immersing the seeds in 5ml of bacterial treatment suspensions (1.6×10^8 cells) for 30 to 60 minutes with shaking at 50 to 100 rpm. Seeds soaked in MgSO_4 only were used as untreated control.

[0071] In other example, the treated and untreated seeds were removed from suspension and one seed was transferred to one pot. The pots were then put in the growth rack in the growth room maintained at temperature 24°C and humidity of 72%. The light intensity was maintained around $300 \mu\text{mol}/\text{m}^2\text{s}$. Pots were watered on alternate days with MilliQ water and after 20 days of growth the plants were taken from the pots for data analysis. For two-time treatment experiment, the seeds were initially sown in smaller pots and then at 13th day when it reaches the two-leaf stage, they are transferred to bigger pots, during which bacteria strains/consortium are sprayed on the roots.

[0072] **Pot Experiment**

[0073] Experiment 1: The control, individual isolate-treated, and consortia-treated seeds were sowed in the soil on day 0 (one seed per pot). Ten replicates were taken for each treatment. The pots were kept in the growth room fitted with a rack system. The light intensity was $300 \mu\text{mol}/\text{m}^2\text{s}$, temp 23°C , and 75-80% humidity. The pots were watered every alternate day. After day 20 the plants were uprooted, and biomass was measured for shoot and root separately. For measurement of root length and area, it was scanned and analyzed using WinRhizo (Pang *et al.*, 2011). For dry biomass measurement, the shoots and roots were kept in aluminium foil in an oven for drying, after which their weight was measured. All the statistical analysis was carried out in R and a student t-test was used to check the significant differences between the treatment and control.

[0074] Experiment 2: For growth rate measurement the control and consortia treated seeds were potted and experiment settings were followed as mentioned earlier. For the leaf growth

rate monitoring, the images of leaves were taken on days 7, 10, 13, 17, and 20 by the imaging system, and the area was measured by ImageJ. The shoot biomass was measured on days 11, 14, and 16 while the leaf photosynthetic parameters were monitored on days 12, 15, and 17.

[0075] Experiment 3: A pot experiment was also carried out for Arabidopsis and Kailan. In the case of these plants, the shoot and root biomass were measured after 30 days of growth, and the results were analyzed like the first round.

[0076] Experiment 4: To ascertain that the consortia can be applied in normal urban farms and meet some of the standard practices, a greenhouse trial of the consortia with choy sum was carried out. In this experiment, consortia-treated seeds, and control (seeds without any treatment) were sowed in both peat moss soil and another set-in normal soil. The small pots were kept in the greenhouse for 11 days after which the small plantlets were transferred to bigger pots. The germinated rate of seeds in both types of soil was recorded. During the transfer, the consortia were applied again near the root region of the consortia treated set. After 30 days of growth, the biomass was measured for all the plants. Plant leaf area and height were determined by Phenospex.

[0077] **Drought Tolerance Experiment**

[0078] The PPA consortium was prepared in a manner similar to the preparation for the SAB consortium and mode of application was the same. To determine the days of survival, 10 batches of pots (5 treated consortium and 5 untreated consortium as control) were prepared with 5 pots in each batch. Watering was withheld in 8 batches after day 15 and gradually allowed in each of the 8 batches separately, after 3, 5, 7 and 9 days of drought, for checking revival capacity of the plants. After 2 days of revival (which was 2 days after watering began after the drought), plants were harvested to record their various characteristics for comparisons as carried out in case of SAB consortium.

[0079] **Metabolomics Validation**

[0080] Consortium SAB growth study was carried out using minimal media in 50ml flask was set up in three replicates. Based on its previous growth curve study at three different time points 10 ml samples were aliquoted from each flask and colony forming units were also calculated for each time point. The samples were centrifuged at 10000 rpm for 10 min to settle down the bacterial cells. The culture filtrate was collected and stored at -80°C and the bacterial cell pellet was discarded. The filtrate which may contain extracellular metabolites was then lyophilized to dryness and reconstituted in 2 mL of 50% acetonitrile pre-cooled to -20°C, and vortex for 1 min. It was then filtered with 0.2-µm PTFE or PES syringe filter and transferred to a 2-mL conical centrifuge tube. Then incubated at -20°C overnight for salt precipitation. After this, it was centrifuged at 8000 rpm for 10min at 4°C. From this 700 µL of the upper phase was withdrawn and transferred to a 2-mL amber LC vial. Similarly, 1000 µL of the lower phase was withdrawn, filtered with 0.2-µm PTFE or PES syringe filter, and transferred to a 2-mL amber LC vial. Both the upper and lower phases were kept in a -80 °C freezer before LC-MS analysis. For each of the five metabolites (putrescine, L-cysteine, α -ketoglutaric acid, sucrose, and D-fructose) targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was carried out in Agilent 1290 Infinity coupled to an Agilent 6400 series Triple Quadrupole. UHPLC system was integrated with Agilent 6495C Triple Quadrupole, and the liquid chromatography system was controlled by Agilent Mass-Hunter Workstation Data Acquisition B.06.00 (Build 6.0.6025.4 SP4).

[0081] **Curation and assembly**

[0082] The erroneous k-mers were removed from the paired reads using run_rcorrector perl script (https://github.com/mourisl/Rcorrector/blob/master/run_rcorrector.pl). Trim Galore was used for removing low-quality and short length reads. Kraken2 was used to remove bacterial and human contaminations (Raghavan *et al.*, 2022). All the clean reads from 24 samples were passed for *de novo* assembly by Trinity v2.14.0 (Hass *et al.*, 2013). Bowtie2 (Langmead and

Salzberg, 2012) was used to quantify read support for the assembly. BUSCO v5 (Seppey *et al.*, 2019) was used for assessing assembly quality and completeness using brassicales_odb10.2019-11-20 lineage as the dataset. Coding regions in the generated assembly was predicted by TransDecoder (<https://github.com/TransDecoder/TransDecoder/wiki>) For functional annotation, Trinotate (Bryant *et al.*, 2017) was used with various databases along with blast against *Arabidopsis thaliana* TAIR10 database with e-value cutoff of 1e-3. Cross-checking was done between results of various databases for their consistency and finally result from TAIR match gene ID were used for the downstream analysis.

[0083] **DNA extraction and amplicon data analysis (with and without consortium treatment)**

[0084] DNA was extracted from the phyllosphere, endosphere and rhizosphere samples of 8th and 20th day old choy sum plants with and without treatment. A total of 64 samples were collected with 5-6 replicates per sample. Amplicon metagenomic sequencing was performed for all the samples and downstream analysis was carried out by dada2 (Callahan *et al.*, 2016) and phyloseq packages (McMurdie and Holmes, 2013).

[0085] **Gas exchange and Fv/Fm measurements**

[0086] Stomatal conductance (gs) and maximal photochemical efficiency of PS II (Fv/Fm) were measured on all the leaves from both consortia treated and untreated choy sum plants. All the measurements were performed using CIRAS-3 (PP Systems, Amesbury, MA). The CO₂ concentration (390 $\mu\text{mol mol}^{-1}$), photosynthetic photon flux density was 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, leaf temperature (25°C), and relative humidity (62.61 $\pm$ 2.51%) were maintained using an automatic control device on the CIRAS-3 (Pandey *et al.*, 2018). Fv/Fm measurements were carried out using CIRAS-3 after the leaves were dark-adapted 30min.

Example 2 - Microbial strains for consortium design

[0087] Table 1 shows the genomic properties of the isolated microbes used to form

consortium SAB (*Streptomyces* sp. SS1, *Agromyces* sp. SS2, *Bacillus subtilis* sp. SS3) and consortium PPA (*Priestia aryabhatai* SS4, *Pseudomonas* sp. SS5, *Atlantibacter* sp. SS6).

[0088] Table 1. Genomic properties of the bacteria used to form consortium SAB and consortium PPA.

	Genome Size	Contigs	N50	GC	Completeness	CDS	rRNA	tRNA	tmRNA	misc_RNA
<i>Streptomyces</i> sp. SS1	7,419,346	3	7,190,718	73.27	99.58	6501	21	85	1	41
<i>Agromyces</i> sp. SS2	3,792,276	1	3,792,276	71.36	100.00	3552	6	48	1	10
<i>Bacillus subtilis</i> SS3	4,175,804	1	4,175,804	43.90	99.81	4080	30	88	1	91
<i>Priestia aryabhatai</i> SS4	5,141,273	2	5,064,416	38.32	99.43	5150	42	131	1	102
<i>Pseudomonas</i> sp. SS5	5,419,665	2	4,752,644	63.96	100.00	4967	12	105	2	58
<i>Atlantibacter</i> sp. SS6	4,563,392	1	4,563,392	55.35	99.11	4178	22	84	1	116

[0089] Both 16S rRNA sequences and genome comparison show that the isolated microbes (microbial strains) used in the consortium design are unique. The nearest reference genome for *Streptomyces* sp. SS1, *Agromyces* sp. SS2, and *Pseudomonas* sp. SS5 have less than 95% similarity and those of *Bacillus subtilis* sp. SS3, *Priestia aryabhatai* SS4, and *Atlantibacter* sp. SS6 are only around 95-97% similar. Moreover, as the quality of assembled genome is high, there are chances of more uniqueness in these strains in comparison to other reported similar microbial strain (bacteria).

Example 3 - Ecologically relevant microbes

[0090] The present disclosure describes the ecological relevance of the microbes used to form the consortium. Co-occurrence network analysis based on rhizosphere communities of three leafy vegetables revealed that *Streptomyces* sp., *Agromyces* sp., and *Bacillus subtilis* (SAB) co-occurred in the largest subnetwork in both the seedling (FIG. 10A) and adult (FIG. 10B) stages. They had more positive interactions than negative ones at both stages, however the number of interacting members belonging to the three genera increased five-fold in the adult

stage compared to the seedling stage indicating an increased complexity in the microbial community at the adult stage. *Agromyces sp.* met the criteria as a keystone member in the seedling stage. To develop consortium that would be effective across different green leafy vegetables, we hypothesized that keystone members and their interacting partners that are known to have PGP functions and are central in early plant growth stage could provide boost at least at an early stage of crop development.

[0091] The ecologically meaningful microbes are those that are present in the natural settings and are interacting positively with each other. The usage of such microbes was proposed as a consortium will help in alleviating the harmful effect of introduction of alien species and moreover will boost the functioning of native microbial communities. In the context of plant microbe interactions, it will be easy for such microbes to strengthen the benefits that they are providing to source plants from where they have been collected.

[0092] Three microbes were isolated from the natural plant rhizosphere environment and through chemical assay found to have various plant growths promoting capacity. Moreover, the presence of several PGPR genes in these microbial genomes strengthen their functional capacity to provide beneficial services to the plants. The ecological relevance of these microbes was also overlaid by the correlation network where two of the microbes (*Streptomyces sp.* and *Bacillus*) were found to be positively interacting with each other. *Agromyces sp.* was found to be present in both stages of plant growth and was also observed to be falling in the category of keystone species in seedling stage.

[0093] We rationalized that the potentially important co-occurring plant beneficial members can be identified based on the properties of co-occurrence networks of rhizosphere communities. Further, some subnetworks will be similar between different leafy vegetables grown in the same location, but these networks will differ across the plant growth stages. Three such members were identified based on their (i) position in the network; (ii) co-occurrence at

seedling and adult stages; and (iii) positive interactions between them.

[0094] A similar ecological correlation was established for the three bacteria, *Priestia aryabhatai*, *Pseudomonas sp.* and *Atlantibacter sp.* that were used to formulate the consortium PPA.

Example 4 - Metabolic relevance and consortium stabilization

[0095] This example describes the metabolic relevance and consortium stabilization of the microbial consortium of the present disclosure. The genome scale metabolic model-based simulation study of the microbes forming the consortium SAB has been conducted (FIG. 11B). The study predicted the exchange of some of the metabolites (L-Cysteine, D-Fructose, 2-oxoglutarate, Putrescine and Sucrose) between these microbes. The prominent one was the feeding of *Bacillus sp.* to *Agromyces sp.* and *Streptomyces sp.* It was also predicted that in oxygen deficient conditions, these microbes can exchange several simple carbon compounds, such as fructose and oxoglutarate to metabolically sustain each other. Moreover, it was also predicted that the rate of glucose consumption is faster in *Bacillus sp.* and *Streptomyces sp.* than *Agromyces sp.*, but it was compensated by getting fructose from the rest of the two. Overall, the results support that these three bacteria show syntrophic behaviour. Furthermore, *Agromyces sp.* and *Bacillus sp.* were found to be co-occurring together in culture form with similar colony morphological characteristics. The SEM image of the isolate shows that *Agromyces sp.* and *Bacillus subtilis* are physically associated, and nanotubes-like appendages attach *Bacillus subtilis* to long hyphae-shaped *Agromyces* cells (FIG. 14). In the same line *Pseudomonas* and *Atlantibacter* were found to be co-occurring together and metabolic model has shown the metabolic exchange occurring between them (FIG. 19).

[0096] FIGS. 12A and 12B also illustrate an approach to computationally design pre-biotics for selected microbial consortia. The genome scale metabolic model-based simulation study shows the cross-feeding of specific metabolites among the three bacteria forming the

consortium. The predicted growth curve from the study shows their nearly same growth rate (FIG. 12A) and some of the metabolites are found to be exchanged between these bacteria during the exponential phase of their growth suggesting a stable functional consortium (FIG. 12B). The limiting metabolites identified from computationally simulated growth experiment *in silico* allows selection of suitable pre-biotics to improve stability and growth of consortia.

[0097] *Metabolomic validation of the metabolites sharing among consortium SAB members*

[0098] The present disclosure describes the experimental validation of metabolic cross-feeding among the members of consortium SAB. Validation study by LC-MS further confirmed the presence of these five metabolites in the culture filtrate of the consortium when cultured in the minimal medium (FIGs. 13B – 13F). Among all the metabolites, D-fructose was present in highest concentration which correlated with simulation study where it has shown the highest flux when compared to others.

Example 5 - Consortium boosting plant growth

[0099] By the pot experiments, it was found that consortium SAB treated choy sum seeds grow well and after 20 days show a significant increase in both shoot and root biomass (FIGS. 2A, 2B, 3A and 3B). An increase in both shoot and root biomass can be observed in the case of consortia treated seeds with just one-time application. The significant changes can be well observed phenotypically. Even the roots morphology changes with increase in root length and density. When compared with individual microbes, the standard deviation in both shoot and root biomass in the consortium was very less. This means that consortium give more consistent results by well establishing itself in the given niche and harmonizing with the plant much better. FIGS. 4A and 4B show the increase in both shoot and root biomass was higher in case of choy sum when the consortium was applied twice, where only consortium SAB was able to boost the growth of plants while individual isolate did not show any effect. It was also observed that the application of consortium SAB at both seed and seedling stage increases the plant biomass

significantly higher than one-time application at seed stage. Time series growth analysis also shows that consortium SAB increases the choy sum growth rate by 12% which can help in the reduction of its crop cycle leading to cost savings. Further, when consortium treated seeds were tested in non-autoclaved soil, it performs very well and shows 33% significant increase in both shoot and root fresh biomass. FIGS. 5A and 5B illustrate the two-time seed treatment of consortium SAB shows a significant increase in both shoot and root biomass over one-time seed treatment alone, however, the one-time seed treatment of consortia is also sufficient for biomass growth when compared with untreated. Finally, consortium SAB two-time treatment shows 74% increase in shoot fresh biomass under the greenhouse conditions with non-sterile soil (FIG. 6C). These results highlight that consortium designed based on its ecological relevance adapts quickly to varied environmental conditions with boosted desired effect. Comparison with single isolate effects also indicates that single member derived products may lack reliability and consistency.

[00100] A plant growth assay was performed using different soil and plant types in a greenhouse condition. A higher germination rate was observed in SAB-treated seeds in both peatmoss and garden soil, with even better performance in nutrient-poor garden soil (FIG. 7A).

[00101] Similar SAB-mediated plant growth benefits were observed in garden soil, with a clear and significant alteration of root volume and its architecture. The SAB-treated plants had higher biomass growth in peatmoss soil (FIGS. 7A to 7G), but the root architecture was not significantly altered (FIGS. 6D and 6E). In a similar fashion, the SAB-treated Arabidopsis and Kai-lan plants also showed higher biomass after 30 days compared to their respective untreated controls (FIGS. 9D-9E, and FIGS. 10D-10G).

[00102] The SAB-mediated growth benefit was perceivable from the early stages of growth and sustained to the later stages (FIGS. 8A, 8B, and 8C). We observed increased leaf photosynthetic efficiency (F_v/F_m) (FIG. 8D) and stomatal conductance in the SAB-treated

plants compared to the untreated ones (FIG. 8E).

[00103] The pot experiment of consortium SAB with *Arabidopsis* (FIGs. 9A to 9E) and kailan (FIGs. 10A to 10G) shows that consortium SAB promote both shoot and root growth in both plants, having a similar effect as compared with choy sum. Thus, it can be concluded that the consortium has a similar mechanism of boosting plant growth in *Brassicaceae* members and so can be adopted for wide variety of plants belonging to this family.

Example 6 - Ecological impact of Consortium SAB on choy sum microbiome

[00104] To investigate whether the introduction of consortium SAB treatment alters the native rhizospheric microbial communities and those within plants as a holobiont, the microbial communities in the rhizosphere, endosphere, and phyllosphere of consortium SAB-treated plants were studied. Consortium SAB-treated seeds were grown in peat-moss soil and the bacterial profile of the rhizosphere, endosphere, and phyllosphere sections of the plants were determined at both seedling and adult stages. Consortium SAB-untreated plants grown under the same conditions were used as a control to study the composition differences. At the seedling stage, there was no significant alteration of microbial alpha-diversity upon consortium SAB treatment in any section of the plants. However, at the adult stage, there was a significant increase and decrease of microbial alpha-diversity in the endosphere and phyllosphere of the plants, respectively, upon consortium SAB treatment compared to the untreated plants. Interestingly, there was no alteration of the microbial alpha-diversity in the rhizosphere of the adult stage plants upon consortium SAB treatment (FIGS. 15A and 15B).

[00105] Next, the differences in microbial community composition in the rhizosphere, endosphere, and phyllosphere sections of consortium SAB-treated and untreated plants were analyzed at both the seedling and adult stages, using permutational multivariate analysis of variance (PERMANOVA). As expected, the microbiome composition of each section was significantly different between seedling and adult stages (FIG. 16). However, at the seedling

stages, the rhizospheric and endospheric communities were significantly different, whereas at adult stages endospheric and phyllospheric communities were significantly different between consortium SAB-treated and untreated plants (FIGS. 15A, 15B and FIG. 16). The overall results revealed that although the rhizospheric community showed alterations at the seedling stage, it retained its original composition during adult stages even after consortium SAB treatment. The consortium SAB treatment mainly altered the endophytic community of the host plants.

Example 7 - Consortium providing drought tolerance capacity along with increase in biomass

[00106] Consortium PPA has shown promising result of imparting drought tolerance capacity to the choy sum plants under water limitation conditions (FIG.17). Moreover, in case of normal watering regime it also increases the plant biomass (around 30%) consistently in comparison to individual isolates (FIG.18). The drought tolerance capacity can be attributed to the consortia biofilm forming capacity.

[00107] Overall, both the ecologically derived consortia with their performance highlights that rather than single strain, bacteria in synergy with relevant partners deliver more consistent plant growth promoting activities. This strategy also depicts the power of genomics and metabolic modelling in rapid screening and selection of beneficial microbes from its large pool existing in the nature.

[00108] All examples described herein, whether of apparatus, methods, materials, or products, are presented for the purpose of illustration and to aid understanding and are not intended to be limiting or exhaustive. Modifications may be made by one of ordinary skill in the art without departing from the scope of the invention as claimed.

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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CLAIMS

1. An isolated microbial consortium for promoting plant growth, comprising:
 - a plurality of microbial strains, wherein one of the plurality of microbial strains contained in the isolated microbial consortium forms a syntrophic relationship with the remaining microbial strains contained in the isolated microbial consortium, and
 - wherein the isolated microbial consortium exhibits a plant-growth promoting effect and is selected from the group consisting of a microbial consortium deposited with NAIMCC under Accession No. NAIMCC-IDA-5 and a microbial consortium deposited with NAIMCC under Accession No. NAIMCC-IDA-6,
 - wherein the microbial consortium deposited with NAIMCC under Accession No. NAIMCC-IDA-5 comprises *Streptomyces sp.*, *Agromyces sp.*, and *Bacillus subtilis*; and
 - the microbial consortium deposited with NAIMCC under Accession No. NAIMCC-IDA-6 comprises *Priestia aryabhattai*, *Pseudomonas sp.*, and *Atlantibacter sp.*
2. A method for preparing an isolated microbial consortium for a target plant, comprising:
 - collecting a sample from rhizosphere communities of the target plant;
 - identifying microbial strains in the sample;
 - selecting microbial strains identified in the sample;
 - isolating the microbial strains from the sample;
 - predicting plant growth-promoting potential of each of the isolated microbial strains;and
 - validating the predicted plant growth-promoting potential with the target plant,
 - wherein the step of selecting the microbial strains comprises selecting microbial strains with each having at least a plant growth-promoting genetic element; and selecting at least one

microbial strain capable of forming a syntrophic relationship with the remaining microbial strains; and

wherein the selected microbial strains form an isolated microbial consortium exhibiting a plant growth-promoting effect, the isolated microbial consortium being selected from the group consisting of a microbial consortium deposited with NAIMCC under Accession No. NAIMCC-IDA-5 and a microbial consortium deposited with NAIMCC under Accession No. NAIMCC-IDA-6,

wherein the microbial consortium deposited with NAIMCC under Accession No. NAIMCC-IDA-5 comprises *Streptomyces sp.*, *Agromyces sp.*, and *Bacillus subtilis*; and

the microbial consortium deposited with NAIMCC under Accession No. NAIMCC-IDA-6 comprises *Priestia aryabhatai*, *Pseudomonas sp.*, and *Atlantibacter sp.*

3. The method of claim 2, wherein the step of predicting includes applying *in-silico* genomic and metabolic characterization and conducting a chemical assay on each of the isolated microbial strains.

4. A method of promoting growth of a target plant using the isolated microbial consortium according to claim 1, comprising:

contacting seeds of the target plant with an effective amount of the isolated microbial consortium to treat the seeds prior to sowing the seeds in soil.

5. Use of the isolated microbial consortium of claim 1 for treating a target plant to promote plant growth, comprising contacting seeds of the target plant with the isolated microbial consortium.

6. Use according to claim 5, wherein the target plant is a member of the *Brassicaceae* family.