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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	The ancestral strain used in the evolutionary experiment was Bacillus amyloliquefaciens DSM7T, and the corresponding genomic information was downloaded from the NCBI database as the reference genome (https://www.ncbi.nlm.nih.gov/nuccore/NC_014551.1).
Data analysis	Whole-genome population sequencing analysis for evolutionary groups was performed using paired-end sequencing on the DNBSEQ-Tx platform (MGI Tech), followed by data preprocessing with SOAPnuke (v1.5.6) ,Bowtie2 and seqtk (v1.3-r107-dirty; https://github.com/lh3/seqtk), and mutation identification with breseq (v0.35.7). Evolutionary parallelism was quantified using Jaccard similarity coefficients between lineages, and mutational dynamics were visualized through Müller plots generated by the lolipop package (v0.6; https://github.com/cdeitrick/lolipop). Two-way generalized linear model (GLM) analysis was implemented using R packages.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The authors confirm that the data supporting the findings of this study are available within the article or from the corresponding authors upon request.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	In this study, Bacillus amyloliquefaciens DSM7T, a weakly root-colonizing strain, underwent in vitro evolution experiments using root exudates-supplemented liquid medium (cultured for 7 days) and in planta rhizosphere-based in vivo evolution (cultured for 2 days), with harvested strains repeatedly reinoculated into identical environments for 20 cycles across 5 parallel evolutionary lineages per experiment. Post-evolution populations were assessed for root colonization capacity, root exudate utilization, IAA production, and biofilm formation with 6 biological replicates. Population genomic resequencing were performed for analyzing 10 evolved groups (5 lineages per experiment), encompassing both evolutionary conditions.
Research sample	In this study, Bacillus amyloliquefaciens DSM7T, a weakly root-colonizing strain purchased from the German Collection of Microorganisms (DSMZ, strain DSM7), was subjected to two evolutionary groups: the M group (in vitro root exudate-evolved populations, five lineages designated M1-M5) and the R group (in planta rhizosphere-evolved populations, five lineages R1-R5).
Sampling strategy	The entire evolved populations from each experimental lineage (10 populations in total) were subjected to functional validation assays and population genomic resequencing analysis

Data collection	For the evolutionary experiments, strains were cyclically inoculated in root exudate-supplemented liquid medium or rhizosphere environments for 20 cycles, with growth properties or root colonization quantified via dilution plating after each cycle. Root colonization assays, exudate utilization capacity, IAA production, and biofilm formation measurements were normalized to ancestral strain performance. Genomic sequencing and analysis included population genome resequencing involved paired-end sequencing on DNBSEQ-Tx platform, quality control with SOAPnuke, reference genome alignment (ancestral strain) via Bowtie2, standardized analysis using seqtk, and mutation detection with breseq. Evolutionary parallelism was assessed through Jaccard similarity coefficients across lineages, while mutation dynamics were visualized using lollipop package-generated Muller plots.
Timing and spatial scale	The evolutionary experiment was conducted for 140 days (2023/9/3-2024/1/21), the biological function assay was conducted for 60 days (2024/1/29-2024/3/29), and the population genome resequencing analysis was conducted for 60 days (2024/3/31-2024/5/30).
Data exclusions	To accurately identify mutations that arise during evolution, mutations that are already present in the original strain and mutations that do not reach 5% frequency in all lineages, are excluded. In order to illustrate the dynamics of mutations, mutations that occur only once in a single lineage and mutations between genes are excluded from the Mueller plot.
Reproducibility	Repeated experiments were successful in the measurement of root colonization, the determination of root exudate utilization, the determination of IAA production, and the assessment of biofilm formation. Evolutionary experiments and resequencing analyses do not require repeat experiments.
Randomization	Strain DSM7T was inoculated as an ancestral strain into root exudate liquid medium culture or rhizosphere environment for 20 times (5 parallel evolutionary lineages were set up for each evolutionary experiment, a total of 10 evolutionary populations), and the entire population was subjected to subsequent functional assays and resequencing analysis after the evolutionary experiment, which excluded the influence of random sampling that might lead to abnormal data.
Blinding	Not applicable. All the interaction conditions are established and enclosed.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Plants

Seed stocks	Tomato (Lycopersicon esculentum) seeds were purchased from the Jiangsu Academy of Agricultural Sciences, P. R. China.
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*