Microbiological Research 279 (2024) 127546

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Exploiting the microbiome associated with normal and abnormal sprouting rice (*Oryza sativa* L.) seed phenotypes through a metabarcoding approach

Albert Dongmo Nanfack [a,d], Julienne Nguefack [a], Samson Musonerimana [b,c], Salvatore La China [d], Davide Giovanardi [d,*,1], Emilio Stefani [d,e]

[a] Department of Biochemistry, University of Yaoundé 1, Yaoundé, Cameroon
[b] International Centre for Genetic Engineering and Biotechnology, Padriciano, TS, Italy
[c] Burundi University, Faculty of Agronomy and Bio-Engineering 2, UNESCO Avenue, P.O. Box 2940, Bujumbura, Burundi
[d] Department of Life Sciences, University of Modena and Reggio Emilia, via Amendola 2, 42122 Reggio Emilia, Italy
[e] University Centre for International Cooperation and Development (CUSCOS), via Università 4, 41121 Modena, Italy

ARTICLE INFO

Keywords:
Oryza sativa
Rice seed
Microbiome
Bacterial endophytes
Seedlings development

ABSTRACT

Rice germination and seedlings' growth are crucial stages that influence crop establishment and productivity. These performances depend on several factors, including the abundance and diversity of seed microbial endophytes. Two popular rainfed rice varieties cultivated in Cameroon, NERICA 3 and NERICA 8, were used for investigating the seed-associated microbiome using the Illumina-based 16 S rRNA gene. Significant differences were observed in terms of richness index between normal and abnormal seedlings developed from sprouting seeds, although no significant species evenness index was assessed within either phenotype. Two hundred ninety-two bacterial amplicon sequence variants were identified in seed microbiome of the rice varieties, and principal coordinate analysis revealed that microbial communities formed two distinct clusters in normal and abnormal seedling phenotypes. Overall, 38 bacteria genera were identified, belonging to 6 main *phyla*. Furthermore, the core microbiome was defined, and the differential abundance of 28 bacteria genera was assessed. Based on the collected results, putative bacterial genera were directly correlated with the development of normal seedlings. For most genera that are recognised to include beneficial species, such as *Brevundimonas, Sphingomonas, Exiguobacterium, Luteibacter, Microbacterium* and *Streptomyces*, a significant increase of their relative abundance was found in normal seedlings. Additionally, in abnormal seedlings, we also observed an increased abundance of the genera *Kosakonia* and *Paenibacillus*, which might have controversial aspects (beneficial or pathogenic), together with the presence of some genera (*Clostridium sensu stricto*) that are commonly correlated to sick plants. The putative functional gene annotation revealed the higher abundance of genes related to the metabolic biosynthesis of soluble carbohydrates and starch, tryptophan, nucleotides and ABC transporters in normal seedlings. Data presented in this study may help in further understanding the importance of the seed endophyte microbiome for driving a correct development of rice plants at the early stages and to identify possible beneficial bacteria for technological applications aimed to increase seed quality and crop productivity.

1. Introduction

Seeds harbour a microcosm composed by microbial communities residing either on the seed surface or inside seeds (Porras-Alfaro and Bayman, 2011; Truyens et al., 2014). In its diversity and dynamics, the seed microbiota plays a significant role in plant health and productivity,

Abbreviations: NERICA, New Rice for Africa; A3, NERICA 3 abnormal seedling phenotypes; A8, NERICA 8 abnormal seedling phenotypes; N3, NERICA 3 normal seedling phenotypes; N8, NERICA 8 normal seedling phenotypes; Fastq, extension of the FASTA format; AVS, amplicon sequence variants; NCBI, National Centre for Biotechnology Information; ANCOM-BC, analysis of compositions of microbiomes with bias correction; package mvabund, R package for model-based analysis of multivariate abundance data; PICRUSt2, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; ACC deaminase, 1-aminocyclopropane-1-carboxylic acid deaminase; IAA, indole-3-acetic acid; MAPK, mitogen-activated protein kinase.

* Corresponding author.
E-mail address: davide.giovanardi@unimore.it (D. Giovanardi).
[1] https://orcid.org/0000–0001-7752–8944

https://doi.org/10.1016/j.micres.2023.127546
Received 29 August 2023; Received in revised form 16 October 2023; Accepted 7 November 2023
Available online 10 November 2023

modulating seed germination and seedling phenotypes, promoting root symbiosis, plant nutrition and growth, and inhibiting phytopathogens (Shade et al., 2017; Nelson, 2018; Ridout et al., 2019; Rodriguez et al., 2019). In fact, it has been reported that bacterial isolates from seeds can solubilize phosphorus, fix nitrogen, produce phytohormones and synthesize antimicrobial compounds (Weyens et al., 2009; da Silveira et al., 2019). Therefore, the exploration and knowledge of seed endophytes through their taxonomic identification and characterisation can be of great help in implementing new options to improve seeds quality, plant health and productivity: this is particularly required to ensure food security, considering that seed quality alone can contribute to 5–20% of higher yield in rice crop (Afzal et al., 2019). For instance, seed germination is affected by a range of abiotic (*i.e.*, water and nutrient availability, temperature, and other environmental factors, such as the nature of the soil) and biotic factors (*i.e.,* soil microbes, including soil-borne pathogens, soil-borne pests, weeds, granivores, herbivores and microbial endophytes, including seed-borne pathogens) (Lamichhane et al., 2018). Among biotic factors, bacterial endophytes, including microbes living in the rhizosphere and penetrating the germinating seed (Hardoim et al., 2012), may be crucial to influence germination and share a mutualistic association with the host seedlings (Ahumada et al., 2022). Endophytic bacteria help to promote plant development through different mechanisms: they improve plant nutrition through several mechanisms such as nitrogen fixation, phosphate and potassium solubilization, siderophore production, mechanisms allowing several nutrients to be assimilated for plants (Compant et el., 2019). Moreover, they facilitate plant development by producing different phytohormones such as auxins, which were found to be involved in coleoptile elongation under submergence (Nghi et al., 2021); gibberellins, which are growth regulators involved in several physiological processes such as germination or stem growth (Shahzad et al., 2016); IAA, which positively contribute to rice seedling establishment under submergence (Ahumada et al., 2022) or even by improving plants resistance to biotic stresses (L'Hoir and Duponnois, 2021). For example, seed endophytes can produce subtilomycin, which binds with flagella to affect Flagellin peptide flg22-induced plant defence or accumulate the PR1 protein and activates mitogen activated protein kinases (MAPKs) signalling and WRKY53 gene expression by the jasmonic acid and ethylene signalling pathway (Mengistu, 2020). Microorganisms, through their properties of synthesizing secondary metabolites with direct biocontrol activities or by inducing plant systemic resistance (Berg and Koskella, 2018; de Vrieze et al., 2018; Matsumoto and Takahashi, 2017), can complement and reduce the use of agrochemicals by ensuring more efficient plant protection and, therefore, contributing to a healthier environment (Naher et al., 2015).

Globally, rice (*Oryza sativa* L.) is the most important food crop in large part of the world, and its cultivation extends over five continents (FAOSTAT, 2019): therefore, a very high number of cultivars are available worldwide to ensure that any cropping area might be cultivated with the most suitable variety. However, considering the pan-tropical cultivation of rice, knowledge of its microbial communities and their role in plant health and productivity is still poor, especially for African regions. Despite several studies focused on the isolation, identification and characterisation of rice bacterial endophytes from different locations and varieties (Eyre et al., 2019; Kim et al., 2020c; Matsumoto et al., 2021; Ahumada et al., 2022), most of such research focused on endophytes associated with growing plants, and only a limited number of studies focused on seeds (Zhang et al., 2019; Zhang et al., 2022a; 2022b). This because most research considered the binary interaction between microbes and their host plants in an attempt to identify microorganisms beneficial to plants (Pereira et al., 2023). Conversely, studies on the seed microbial community may lead to the identification of the core microbiota intimately associated to the host plant, therefore those microbes that, together with the developing plant, have a role in recruiting and diversify the endophytic communities. Finally, seed microbiota possesses, in general, peculiar traits that might be remarkably interesting for technological applications: for instance, they tolerate extreme conditions, like high osmotic pressure or dehydration, or they may switch into the Viable But Not Culturable (VBNC) state as a survival strategy (Oliver, 2005; L'Hoir and Duponnois, 2021).

Here, we have explored the endophytic bacterial communities associated with normal and abnormal seedling phenotypes of two rainfed rice seed varieties, *i.e.,* NERICA 3 and NERICA 8. By the International Seed Testing Association (ISTA), a rice seedling phenotype is categorized as normal if root system and coleoptile are both well developed, complete, in proportion and healthy. This normal seedling has the potential for continued development into satisfactory plants. On the contrary, a seedling phenotype is considered abnormal if it has not the potential to develop into a normal plant, showing essential structures (*i.e.,* root system and coleoptile) missing, malformed or irreparably damaged that balanced development cannot be expected (ISTA, 2021).

NERICA (New Rice for Africa – NERICA) is a group of interspecific rice varieties and lines between *Oryza sativa* L. and *Oryza glaberrima* Stued, with tolerance to main pests affecting rice in sub-Saharan Africa (Kaneda, 2007; Somado et al., 2008). The two high-yielding interspecific hybrid varieties NERICA 3 and NERICA 8 were introduced in 2008 by the Africa Rice Centre, with the support of the Food and Agriculture Organization (FAO) and Japan International Cooperation Agency (Sagakami, 2022). These two rice varieties are extensively cultivated in Central and Western areas of Cameroon for their agronomical performances in terms of plant height, yield, grain shape and organoleptic characteristics (Malaa et al., 2016). Nevertheless, Cameroonian farmers reported seed germination problems, also as an effect of the development of malformed seedlings, which are crucial factors that result in poor crop yield (Tang and Ngome, 2015). Since it is well established that seed associated microbial communities may have a pivotal role in supporting seedling development and plant growth, the aim of our 16 S rRNA amplicon sequencing study was to assess the relationship between endophytic bacterial taxa and seed health and quality. In addition, putative functional gene annotation was performed to analyse biological function changes associated with differences in microbial communities. The beneficial bacterial species residing in rice seeds and identified in this study can be used as microbial inoculants applied *via* seed coating to improve germination performances and yield.

2. Materials and methods

2.1. Plant material

Two rainfed rice cultivars, NERICA 3 and NERICA 8, were grown according to the best agricultural practices in the fields of the Agricultural Research Institute for the Development of Cameroon (IRAD), Yaoundé (Cameroon) and harvested in July and September 2018, respectively. Seeds were collected, dried and stored at 25 °C in woven polypropylene bags. After two months of storage, seed lots of the two varieties were sampled for further analysis. To prevent the influence on germination performances and seedling development of unfilled or partially filled seeds, deformed or damaged seeds or the presence of inert material, both NERICA 3 and NERICA 8 rice seed samples (sample = 5.000 seeds per variety) were preliminarily cleaned by using a Laboratory Seed Blower SBL-100 (Seed Processing BV Holland, Enkhuizen, The Netherland) to select good (*i.e.,* heavy) seeds from waste (*e.g.,* dust, husks, straw, and empty seeds), and manually sorted and sieved to eliminate grain defects, and to uniform seed length. Then, the graded seeds were surface sterilized by soaking in 2% NaOCl for 20 min and then rinsed several times with sterile distilled water to not affect germination or seedling growth (Piernas and Guiraud, 1997). To verify the effectiveness of sterilization, 100 μL from the final rinse was spread on Nutrient Sucrose Agar (NSA) (Crosse, 1959) plates and incubated at 27 °C for 5 days to check that no bacterial colony grew on such plates.

2.2. Germination test and sample collection

The surface sterilized seed samples NERICA 3 and NERICA 8 were singularly germinated in a capped, sterile 30-mL test tube containing cheese cloth saturated with sterile deionized distilled water and then kept in a climatic chamber for 7 days at 25 °C, with 12 h light / 12 h dark. A sample of 500 seeds (*sample* = 50 seeds × 10 technical replicates) for both NERICA 3 and NERICA 8 was considered as a biological sample for germination testing. After 7 days of incubation, germinated seeds were counted: the seed was considered germinated when the radicle had protruded through the seed coat. Additionally, seedlings were assessed and categorized as normal or abnormal phenotype for both NERICA 3 and NERICA 8, according to the International Rules for Seed Testing (ISTA, 2021) (Supplementary Material Fig. 1). Subsequently, a biological sample was generated by collecting 1 g of seedling (*sample* = approx. 34 seedlings) for both normal and abnormal phenotypes. Then, hulls of all seedlings were aseptically removed using decontaminated forceps. This experiment was repeated three times. By using this criterion, a total of six samples of normal seedling phenotypes (3 replicate samples of NERICA 3 and 3 replicate samples of NERICA 8) and six samples of abnormal seedling phenotypes (3 replicate samples of NERICA 3 and 3 replicate samples of NERICA 8) were generated. In total 12 samples of 1 g each (*sample* = approx. 34 seedlings) were collected for microbiome studies.

The germination and seedling phenotype results for NERICA 3 and NERICA 8 seeds, respectively, were subject to paired samples t-tests at $P \leq 0.05$ using SPSS 15.0 for Windows R (SPSS Inc., Chicago, IL).

2.3. DNA extraction and 16 S rRNA gene amplicon library preparation

Both NERICA 3 and NERICA 8 seedlings belonging to normal and abnormal phenotypes, respectively, were subject to total genomic DNA extraction according to Johnston-Monje and Raizada (2011) with minor modifications. For each replicate sample, 1 g of seedlings (sample = approx. 34 seedlings) was ground in a sterile mortar with a pestle. Then, 1 mL of sterilized 50 mM Na_2HPO_4 buffer per gram of plant tissue was added. Finally, 450 µL of the shredded material was used for DNA extraction. Total DNA was extracted by using DNeasy Plant Mini Kit (Qiagen, Germany), according to the manufacturer's instructions. The quality and the quantity of DNA were spectrophotometrically checked using a ND 1000 Spectrophotometer (NanoDrop by Thermo Fischer, Waltham, USA) and by standard electrophoresis on 1.2% agarose gel. The 16 S rRNA gene amplicon library was performed as described in the Illumina MiSeq System kit. The targeted specific gene sequences amplified in our procedure were the 16 S rRNA gene V3 and V4 regions, according to Klindworth et al. (2013).

The 16 S rRNA gene amplicon library was prepared by using the Follow-length primer sequences: 16 S amplicon PCR forward primer 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGA-CAGCCTACGGGNGGCWGCAG-3' and 16 S amplicon PCR reverse primer 5'-GTCTCGTGGGGCTCGGAGATGTGTATAAGAGA-CAGGACTACHVGGGGTATCTAATCC-3'. A mix of 2.5 µL (5 ng/µL) microbial DNA, 5 µL (1 µM) of each primer, and 12.5 µL of 2 × KAPA HiFi Hot Start Ready Mix in the final volume of 25 µL were used for the first PCR to amplify the V3 and V4 regions of the16S rRNA gene by following this program: initial denaturation of 95 °C for 3 min followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s; a final 5 min of extension at 72 °C and hold at 4 °C.

The PCR products were cleaned according to the Illumina protocol, as described by Illumina (2013), and a second PCR for adding the Illumina index was set. A mix of 5 µL (PCR products), 5 µL of each Nextera XT Index Primer (N7xx and S5xx), 25 µL of 2xKAPA HiFi Hot Start Ready Mix, and 10 µL PCR-grade water in a final volume of 50 µL was prepared and the following program was used for the second PCR: initial denaturation of 95 °C for 3 min, followed by 8 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and a final 5 min extension at 72 °C, followed by a hold at 4 °C. The second cleaning was done as recommended in the protocol by using AMPure XP beads (ThermoFisher Scientific, Rodano, Italy).

The cleaned 16 S rRNA gene amplicon library from the second amplification was quantified using the Qubit Kit (Invitrogen, Massachusetts, USA) and the quality (integrity and presence of a unique band) was assessed by standard electrophoresis on 1.2% agarose gel. After quantification and normalization, all PCR products were diluted at 4 nM, and 5 µL aliquots of diluted DNA from each library were mixed for pooling with unique indices. Paired-end (2 × 250 bp) sequencing was performed on the HiSeq platform (Illumina, San Diego, CA, USA) by ARGO Open Lab Platform for Genome sequencing (AREA Science Park,

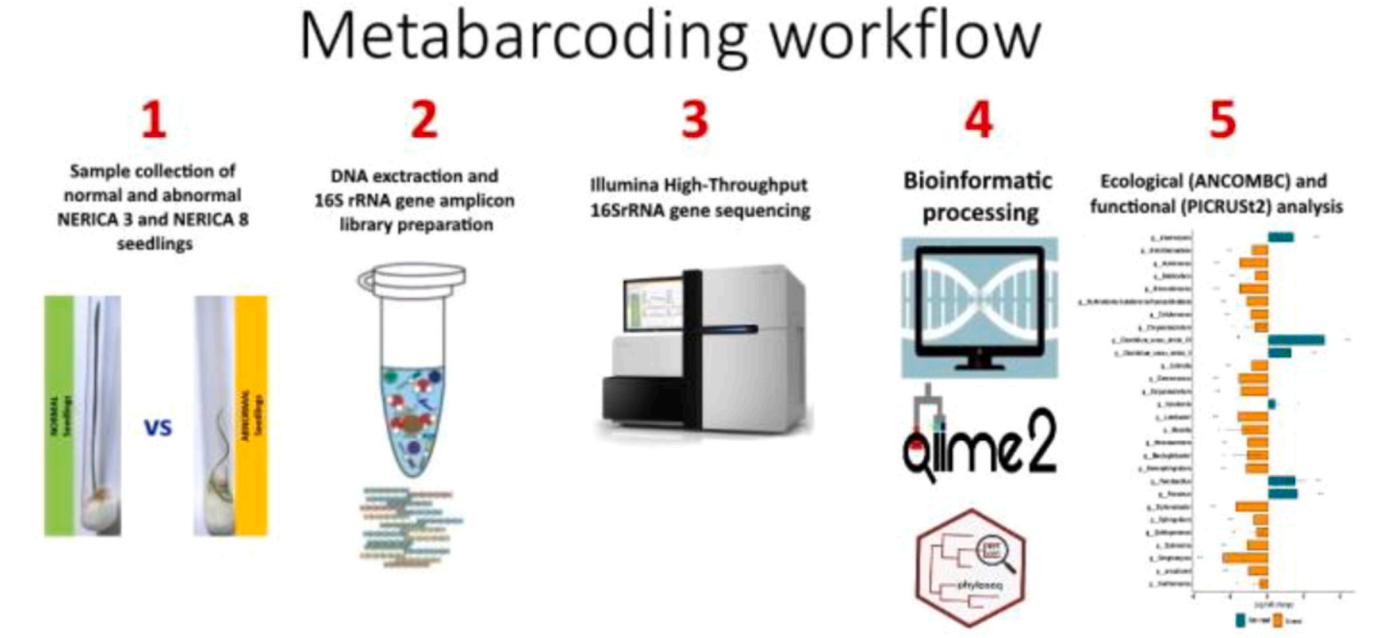

Fig. 1. Graphical representation of workflow chart for the metabarcoding process on seed microbiome.

Padriciano, 34149, Trieste, Italy).

2.4. Sequence data processing

Fastq files were analyzed using QIIME2 v2022.8.3 (Bolyen et al., 2019). Briefly, the integrated plugin cutadapt in QIIME2 was used to trim bases with low quality and remove index and primer sequences. After primer and index trimming, sequences were then filtered, denoised, merged and chimera removed using the DADA2 plugin integrated in QIIME2 (Callahan et al., 2016), and selecting the syntax –p-trunc-left 250 and –p-trunc-right 190. The resulting high quality amplicon sequence variants (ASVs Chao1 and Simpson indexes) were estimated with the function alpha in the package Microbiome. To describe the beta-diversity among the conditions, the Bray-Curtis distances were calculated using the function adonis2 from Vegan. The principal coordinate analysis (PcoA) was performed using the function ordinate from Phyloseq. The core taxa were inferred using the function core from Microbiome and were defined as the ASV with an occurrence in 90% of the samples and a minimum detection threshold of 1%. Differential abundance of ASVs was evaluated among the different groups using analysis of compositions of microbiomes with bias correction (ANCOM-BC) (Lin and Peddada, 2020). Bacterial genes were inferred from the 16 S derived ASVs using the PICRUSt2 v2.5.2 (Douglas et al., 2020). The inferred genes were annotated using the KEGG orthology database. The differential abundance analysis was performed with ANCOMBC software v3.17, by applying the Benjamini-Hochberg method for p-value adjustment. The workflow chart for the metabarcoding process used in the study is graphically represented in Fig. 1. The raw reads and raw data generated for this study can be found in the NCBI Sequence Read Archive as BioProject PRJNA97857.

2.5. Statistical analysis

The statistical differences among the thesis in the alpha-diversity metrics were inferred according to the Kruskal-Wallis test by using the function *kruskal* from the package agricolae and applying the Bejamini-Hockberg correction to control the false discovery rate (FDR) (Benjamini and Hochberg, 1995). The community dissimilarity estimated by the Bray-Curtis distance was assayed using the unweighted Unifrac metric by using the function ordinate from phyloseq and 1000 permutations (Andersson et al., 2008). Multivariate generalized linear model analysis was performed on the compositional abundance table in R by using manyglm function from the package mvabund (Wang et al., 2012). P-values from differential abundance testing *via* these approaches were adjusted using the method of Benjamini and Hochberg (1995). All statistical analysis and visualization were performed in R v4.2.2 with the required package ggplot2.

3. Results

3.1. Germination assessment and seedling categorization

NERICA 3 and NERICA 8 seed samples were checked during the germination assay to assess their viability and quality; counts and evaluation were done after 7 days from sowing and results are shown in Table 1. For both NERICA 3 and NERICA 8, the calculated germination rates (G%) were 83.67% and 86.42%, respectively, with no significant differences between the two rice varieties ($P > 0.05$). The germinated seeds produced 73.92% and 74.25% seedlings with normal phenotype and 9.75% and 12.17% seedlings with abnormal phenotype, for NERICA 3 and NERICA 8, respectively, and no significant difference between the two varieties ($P > 0.05$) was observed for the abnormal and normal seedlings development.

3.2. Metagenomic identification by Illumina High-Throughput 16SrRNA gene sequencing analyses

High throughput sequencing finally generated a total of 97.073 sequences (30.544 for normal seedlings NERICA 3; 21.946 for normal seedlings NERICA 8; 17.824 for abnormal seedlings NERICA 3; and 26.759 for abnormal seedlings NERICA 8). After quality trimming and removal of chimeric and plant reads, a total of 294 ASVs were used to represent the bacterial microbiome associated with rice seedling samples.

3.3. Taxonomic composition and diversity of seed-associated bacterial endophytes

To gain insight into the microbial structure of the bacterial communities associated with the two different rice varieties and the putative association with normal and abnormal phenotypes, the taxonomic assignment at *phylum* level was performed and alpha and beta diversity indexes were determined. The seed microbial communities of the two NERICA 3 and NERICA 8 varieties were composed of 6 main *phyla*: *Proteobacteria* (syn. *Pseudomonadota), Firmicutes* (syn. *Bacillota*), *Bdellovibrionota, Bacteroidota, Armatimonadota and Actinobacteriota* (Fig. 2A). Multivariate linear mixed model analysis highlighted differences in the microbial composition, mainly associated with the two seedling phenotypes (*i.e.*, normal and abnormal), explaining 29.6% of the observed variation (p-value < 0.001) and between the two cultivars (NERICA 3 and NERICA 8, p-value $= 0.001$). Principal Coordinates Analysis (PCoA = classical multidimensional scale) highlighted that the microbial community clustered in two groups based on the associated phenotypes (Fig. 2B). In detail, 99.6% of the variance was explained by the appearance of abnormal phenotypes ($R^2 = 0.205$, p-values $= 0.044$). A diversity in the microbial communities among the two seedling phenotypes was also highlighted by alpha-diversity indexes. When abnormal seedlings developed, a significant increase of the Chao1 index (p-value < 0.001) was observed (88.52), compared to normal seedlings (69.30) (Fig. 2C). No differences between the two conditions were detected by the Simpson index (Fig. 2D): this implies that the diversity in the microbial community lies in the abundance of determined species and not in the occurrence of different ones.

Normal and abnormal seedlings phenotypes of two rice cultivars NERICA 3 and NERICA 8 were used to create a Venn diagram (Supplementary Material, Fig. 2), which was used to investigate whether shared endophytic ASVs exist. At a 97% similarity level, the numbers of ASVs for each sample ranged from 61 to 87, with an average of 73.50. Among them, 205 endophytic ASVs (69.73%) coexisted in the four rice samples. In addition, only normal NERICA 3 and NERICA 8 phenotypes have

Table 1
Assessment of rice seed viability and quality during a germination test at the 7th day: classification and score of abnormal and normal seedlings developed from germinated NERICA 3 and NERICA 8 seed. Results are the average of three independent experiments, each one with four replicates. Same letters within columns indicate no significant differences according to the t-test ($P > 0.05$).

Sample	Mean germinated seeds[1] (%)	SD[2]	Mean abnormal seedling phenotype[3] (%)	SD[2]	Mean Normal seedling phenotype[4] (%)	SD[2]
NERICA 3	83.67[a]	± 1.53	9.75[a]	± 0.75	73.92[a]	± 2.27
NERICA 8	86.42[a]	± 2.13	12.17[a]	± 2.02	74.25[a]	± 0.25

[1] mean percentage of germinated rice seeds during the 7th day assessment; [2] standard deviations; [3] mean percentage of abnormal seedling rice phenotype developed; [4] mean percentage of normal seedling rice phenotype developed.

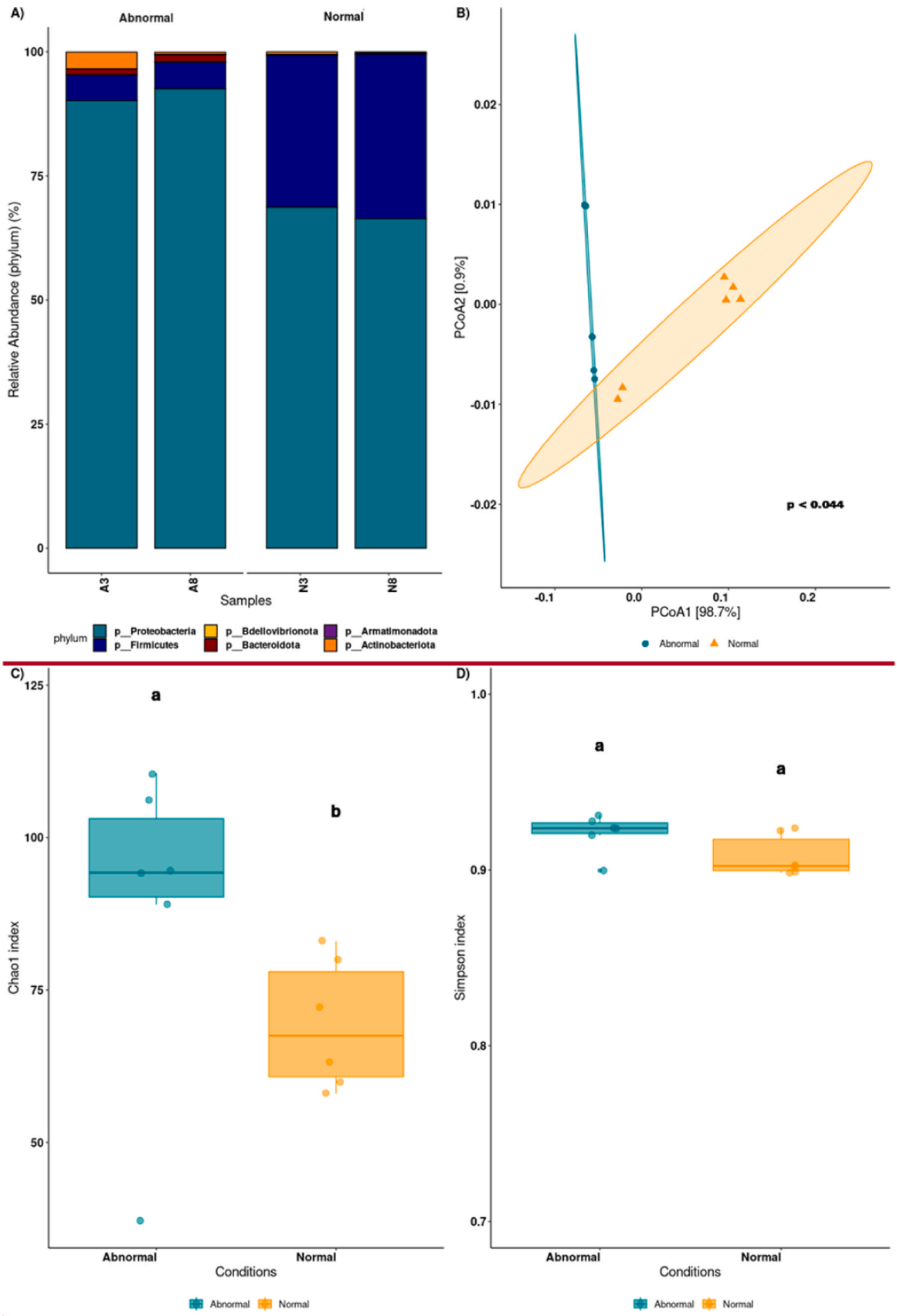

Fig. 2. Bacterial community diversity across the two cultivars NERICA 3 and NERICA 8 in two conditions: normal and abnormal seedling phenotypes. A) Bacterial microbiota composition across the two cultivars, in normal or abnormal phenotypes: relative abundance of the main phyla (relative abundance >0. 1%) is reported as mean of replicates (n = 3) for each category. B) Ordination of Principal components analysis (PCoA) based on Bray-Curtis dissimilarity (n = 12). C-D) Alpha-diversity indices Chao1 and Simpson for the two conditions of normal and abnormal seedling phenotypes.

unique ASVs, with a proportion of unique ASVs of 2.04% and 3.06%, respectively.

Differences in the abundance of bacterial *phyla* were illustrated in Fig. 2A and reported in Table 2. The main differences in term of relative abundance were observed in the *phyla Proteobacteria* and *Firmicutes.* For the bacterial members belonging to *phylum Proteobacteria*, a significant increase of 23.8% (p-value < 0.001) in abnormal seedling was observed. In contrast, the *Firmicutes* abundance decreased significantly by 26.60% (p-value < 0.001) in abnormal phenotypes, compared to the normal ones. In addition, differences were observed in other two *phyla* in both phenotypes. The *Actinobacteriota* resulted to be more abundant in NERICA 3 (3.42%) compared to NERICA 8 (0.48%) and a significant reduction in both cultivars was observed for normal seedlings (NERICA 3 = 2.92%; NERICA 8 = 0.20%) (p-value < 0.005). Again, considering the *phylum Bacteroidota* a significant reduction of 1.27% and 1.00% (p-value < 0.005) was observed for normal phenotypes of NERICA 8 and NERICA 3, respectively. Finally, other minor *phyla*, such as *Armatimonadota* and *Bdellovibrionota,* were detected with an abundance lower than 0.1%.

3.4. Definition of the seed core microbiota of NERICA cultivars and bacteria differential abundance in normal and abnormal phenotypes

A consistent core microbiota shared by normal and abnormal seedlings was found in both NERICA cultivars, accounting for 39.63% and 49.69% of microbial taxa in NERICA 3 and NERICA 8, respectively. Considering normal phenotypes of both cultivars, the core microbiota resulted increased, by 26.87% and 29.29% for NERICA 3 and NERICA 8, respectively. The core microbiota consisted of 14 genera (Fig. 3A) that were detected in at least 90% of the samples. The top-4 most abundant genera observed are represented by members of the *phylum Proteobacteria*, which includes the genera *Pantoea*, *Pseudomonas*, *Kosakonia* and *Xanthomonas*, detected in all samples, with a relative abundance of 9.5%, 3%, 3% and 1% respectively. Other members belonging to *Proteobacteria* were detected, such as *Sphingomonas*, *Rizhobium*, *Acidovorax*, *Azospirillum*, *Stenotrophomonas*, *Herbaspirillum*, with relative abundance of 1% or lower.

Two members belonging to *Firmicutes* were detected in the core microbiome, represented by *Paenibacillus* and *Bacillus. Paenibacillus* was detected with a relative abundance of 1% in all samples. The abundance of *Paenibacillus* increased considering 50% of samples (relative abundance = 3%) and 25% of samples (relative abundance = 9.5%). Besides, *Bacillus* displayed a relative abundance of 0.3% in all samples; its abundance increased considering 50% of samples (relative abundance = 1%) and 25% of samples (relative abundance = 3%). *Actinobacteriota* is another *phylum* that was observed in the core microbiome, represented by one genus: *Curtobacterium*. The relative abundance of *Curtobacterium* resulted to be 0.65% in the core microbiome, when considering 100% of samples. Decreasing the number of samples by 50%, the relative abundance of *Curtobacterium* increased to 1%. Thus, the latter two genera (*i.e.*, *Bacillus* and *Curtobacterium*) clearly highlighted a high standard deviation among the samples, irrespectively of phenotype or genotype. The *phylum Bacteroidota* was also represented in the core microbiome with one member only: *Chryseobacterium.* The relative abundance of *Chryseobacterium* resulted to be lower 1% in the core microbiome, even considering 100%, 50% or 25% of samples.

Table 2
Composition of bacterial communities (phyla) and ASV/phylum in normal and abnormal phenotype seedlings of two Cameroonian rice cultivars NERICA 3 and NERICA 8.

Taxa level	Taxa name	ASVs			
		NERICA 3 Seedlings		NERICA 8 Seedlings	
		Abnormal	Normal	Abnormal	Normal
Phylum	*Actinobacterota*	4	9	3	8
	Armatimonadota	0	0	0	3
	Bacteroidota	7	9	5	12
	Bdellovibrionota	1	0	0	3
	Firmicutes	16	12	16	9
	Proteobacteria	42	46	37	52
Total		70	76	61	87

Furthermore, we used ANCOM-BC to identify bacterial taxa, whose abundance in normal and abnormal seedlings was significantly different (Fig. 3B). Four genera were detected in abnormal phenotypes only, with different level of abundance; they were: *Anaerospora* and *Pelosinus*, belonging to the *Sporomusaceae* family ($2 <$ fold change < 3), and two members of the family *Clostridiaceae* represented by *Clostridium sensu stricto* 3 ($1 <$ fold change < 2) and *Clostridium sensu stricto* 10 (fold change > 4). Additionally, the relative abundance of *Paenibacillus* (*Paenibacillaceae*) ($2 <$ fold change < 3) and *Kosakonia* (*Enterobacteriaceae*) (fold change < 1) was significantly higher in abnormal seedlings compared to normal ones. Conversely, in normal seedlings, a significant increase in abundance was observed for the genus *Streptomyces* (fold change > 3), followed by the genera *Massilia, Exiguobacterium, Aureomonas, Brevundimonas, Enterococcus, Luteibacter, Siphonobacter* ($2 <$ fold change <3) and by the taxa *Bdellovibrio, Chryseobacterium, Sphingobium, Armatimonadales, Cohnella, Cellulomonas, Microbacterium, Novosphingobium, Mucilaginibacter, Spirosoma, Burkholderia* ($1 <$ fold change <2). Again, other taxa such as *Sphingomonas* and *Xanthomonas* showed a slight increase in the relative abundance (fold change < 1).

3.5. Bacterial functional profile in normal and abnormal seedling phenotypes

The taxonomic changes associated with normal and abnormal seedling phenotype corresponded to changes in the predicted function of seed endosphere microbiota. As predicted by PICRUSt2, we observed functional changes, mainly associated with metabolic functions (Fig. 4). Most of the predicted pathways (*i.e.,* 14 out of 26) resulted to be overexpressed in both varieties of NERICA seedlings with abnormal phenotypes. A massive overexpression of catabolic pathways, such as degradation of aromatic hydrocarbons, compounds of essential oils (volatile organic compounds) and flavonoid, resulted to be predominant in abnormal phenotypes, differently from the normal ones, in which the most overexpressed predicted pathways were related to the biosynthetic pathways, including starch, sucrose, fructose and mannose, tryptophane and glycosphingolipids. In addition, normal seedling phenotypes were associated with an enrichment of the predicted pathways involved in nucleotide, asamycins and ABC transporter metabolism. In abnormal seedlings the predicted pathways related to the basal transcription factors resulted to be overexpressed compared to normal phenotypes.

4. Discussion

In the field of microbial ecology and/or "functional microbiology", the study and the determination of microbial taxa intimately associated with a given host species and, in particular, the so called "core microbiome", may assist in addressing a range of questions related to clarify the role of such microbes in the "wellbeing" of the hosting eukaryote (Neu et al., 2021). Recently, the concept of "symbiotic agriculture" has been conceptualized and developed to highlight the possibility to fostering plant productivity and health through the knowledge of the microbial communities associated to crop plants and unravelling their functional role in the agro-environment (Vurukonda et al., 2017).

Rice seeds contain a rich microbial community that plays key roles in plant development, health, and productivity (Schlaeppi and Bulgarelli, 2015). A thorough study of the microbiota of sprouting rice seeds contributes to gaining deeper insight into their relationships with plant development at the very early stages and prior to the involvement of possible interactions with soil microbes. In our research, we used a metabarcoding approach to study the bacterial communities and their

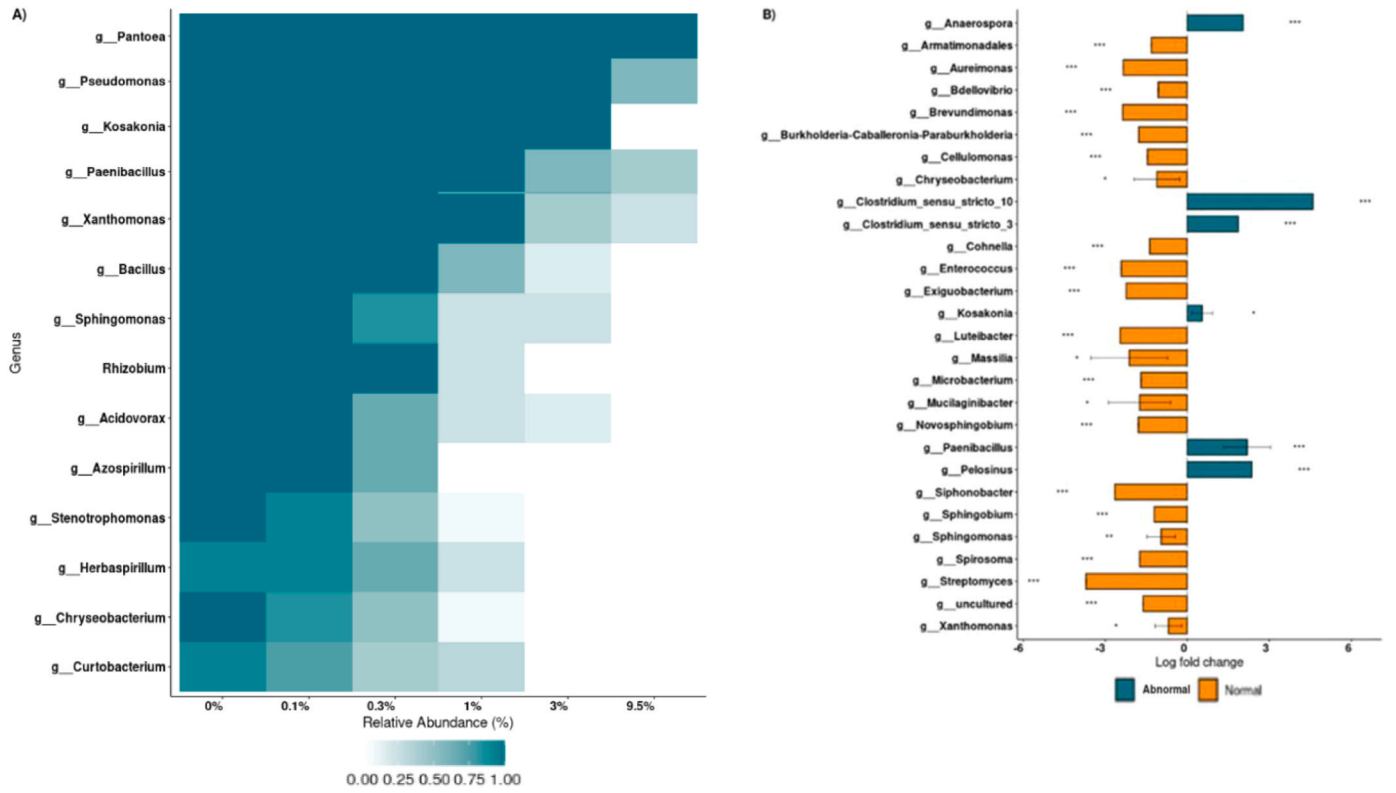

Fig. 3. Shared taxa and composition changes with the occurrence of abnormal phenotypes in NERICA seeds. **A)** Relative abundance and composition of the core microbiota in NERICA seeds. The frequency (in which 1 indicate the 100% of the sample) was represented in different colour gradient. **B)** Fold changes in bacterial abundance in NERICA normal seedling sample. Fold changes are log2 transformed and were calculated using the default (two-sided) ANCOM-BC model for two-group comparisons. Taxa with $P < 0.05$ after adjustment for multiple comparisons (Benjamini and Hochberg, 1995) are shown.

possible diversity within healthy and "sick" seeds generating, in turn, normal and abnormal rice seedling phenotypes for two popular Cameroonian rice varieties, such as NERICA 3 and NERICA 8. Our germination test resulted in the production of abnormal seedlings by 9–12% of the total germinating seeds, confirming the impact of seed quality for an optimal seedling establishment. In fact, these same abnormal seedlings, when under the more stressful field conditions, do not have abilities to grow adult plants due to their abnormalities, thus resulting in reduced yields. Based on the ASVs analysis of normal and abnormal rice seedlings, the diversity in the microbial community was mainly explained by the differential abundance of the identified genera and not by the appearance of a new genus. Those microbial communities derive from seeds, thus establishing a close association with roots and shoots (Wang et al., 2020). Our study targeted seed endophytes solely, without taking into consideration all the epiphytes, due to the lack of knowledge whether the bacteria epiphytically colonising the seeds are all able to internalise into the developing seedlings, thus playing a possible additional role in plant development.

In our research, we found that *Proteobacteria, Firmicutes*, *Bacteroidetes,* and *Actinobacteria* were the most dominant *phyla* detected and identified in both rice varieties (Fig. 2A). Changes in seed microbial community compositions using the Bray–Curtis dissimilarity and the principal coordinate analysis (PCoA) highlighted that endophytic bacterial taxa clustered in two groups based on seedling phenotypes, generated by healthy and "sick" seeds, respectively, in both NERICA varieties (Fig. 2B). These results confirmed previous findings, where the rice genotype has limited effects on the diversity and richness of seed endophytes (Zhang et al., 2019). Moreover, although no specific study was ever done on the influence of soil type on the rice seed bacterial communities, research on the wheat rhizosphere microbiome evidenced a core microbiome across several African soils (Simonin et al., 2020). Still, both rice seed lots of NERICA 3 and NERICA 8 were produced in the same area during one cropping season.

Our results showed that rice seeds contain 38 taxonomically distinct bacterial endophytes (Supplementary material Table 1) and that the microbial diversity in terms of taxa abundance between healthy and "sick" seeds is the main characteristic associated with the development of normal and abnormal seedlings, respectively (Fig. 2C). Additionally, the presence of a few unique bacterial genera in the "sick" seeds was highlighted, even if their role in the development of abnormal seedlings was not supported by a significant difference (Fig. 2D). This evenness in microbial composition can be explained by the fact that seed represents a relatively stable and niche-specific microhabitat, exhibiting a more severe and stronger selective pressure when compared to other plant microhabitats, such as the rhizosphere or the plant endosphere (Shade et al., 2017; Zhang et al., 2019).

The main *phylum* detected in both NERICA 3 and NERICA 8 seeds was *Proteobacteria*, whose bacterial genera displayed a significantly higher abundance in abnormal seedlings (Fig. 2A). The 4 dominant genera in the core microbiome belonging to *Protobacteria* were represented by *Pantoea* (9.5%), *Pseudomonas* (3%), *Kosakonia* (3%) and *Xanthomonas* (1%) (Fig. 3A). This result is consistent with previous findings reporting *Pantoea* as the dominant genus in the rice seed microbiome, also considering other geographical areas (Zhang et al., 2019). Interestingly, several *Pantoea* species have been reported as phytopathogens of at least 31 crop plants, including rice (Azizi et al., 2020). Phytopathogenic *Pantoea* species exhibited virulence to rice tissue with the symptoms such as grain discoloration (Egorova et al., 2015), leaf blight (Lee et al., 2010; Mondal et al., 2011), reduced germination of seeds (Carrer Filho et al., 2018), stem necrosis (Cother et al., 2004), palea browning (Cortesi, Pizzatti, 2007) and sheath rot (Choi et al., 2012). On the contrary, commensal rice *Pantoea* spp. could enhance the growth of leaf, stem, root hair (Banik et al., 2016) and yield (Yu et al., 2022) through phosphate solubilization, biosynthesis of siderophores and IAA, AAC

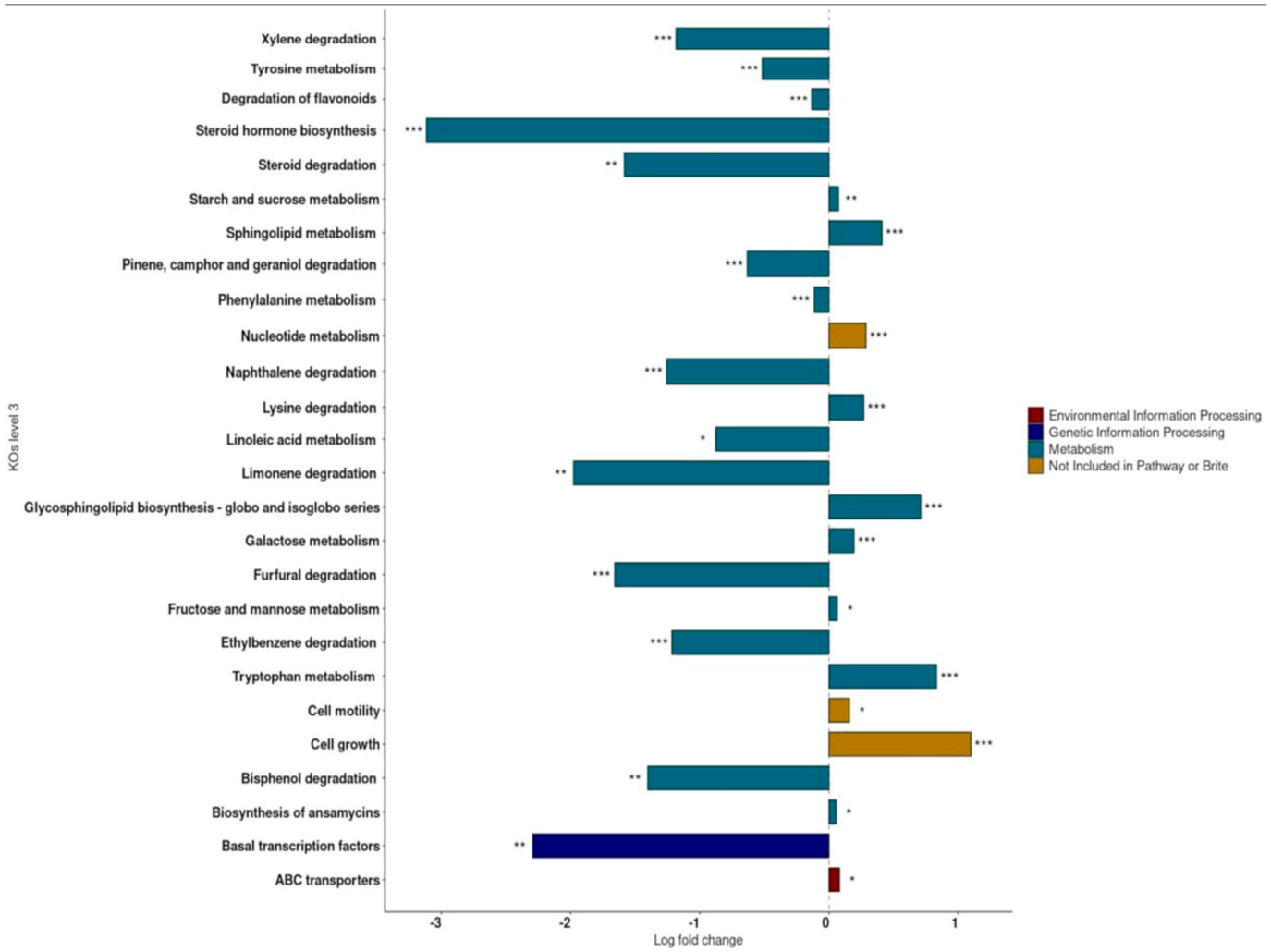

Fig. 4. Inferred functional pathways significantly different in normal NERICA seedling phenotypes compared to abnormal phenotypes. Bars were coloured based on the KO level 1 and represent the fold-change within 1 unit. Functional pathways were reported by using the KOs at level 3. Stars indicate the significance of each pathway (BH adjusted p-values) as follows: ≤ 0.05 (*), ≤ 0.01 (**), ≤ 0.001 (***).

deaminase, auxins, abscisic acid and gibberellic acid (Feng et al., 2006; Ghosh et al., 2021; Megías et al., 2017; Zhang et al., 2022a; 2022b). Again, several studies indicated that *Pantoea* spp. has a great potential in controlling various rice bacterial and fungal diseases such as rice leaf blight and rice blast, (*i*) by secreting extracellular hydrolytic enzymes and antibiotics (David et al., 2017; Williams, Stavrinides, 2021); (*ii*) through nutrient and spatial competition (Liu et al., 2020) or (*iii*) enhancing induced resistance by the secretion of extracellular polysaccharide and mediated defence response through the signalling pathways of both jasmonic acid and ethylene (Ortmann et al., 2006; Spence et al., 2014). Interestingly, *Pantoea* genus includes multifaceted species, such as *P. ananatis* (EFSA, 2023) and *P. agglomerans* (Lv et al., 2022) which can be either beneficial or pathogenic, indicating that the role of *Pantoea* in rice plants is very complex. The second most represented genus in the rice core microbiome was *Pseudomonas*, as also reported by Guo et al. (2021). *Pseudomonas* species are characterized by their great adaptability to different environmental conditions and their multiplicity of virulence factors that thwart the host's defences, thus driving to infections in predisposed areas (Baltrus et al., 2017; Passera et al., 2019). Conversely, several *Pseudomonas* species have been reported as beneficial microbes, able to control pathogens and improve host plant growth, including rice (Ngalimat et al., 2021). In the current study, the genus *Kosakonia* was found for the first time in the rice seed microbiome (Fig. 3A), with a significantly higher relative abundance in "sick" seeds (Fig. 3B). *Kosakonia* (*Enterobacteriaceae*) is a novel bacterial genus that includes diazotrophic beneficial species also associated with rice, like *Enterobacter oryzae* (Peng et al., 2009). Within this genus, however, several species were found to be phytopathogenic, causing a wide range of plant diseases with symptoms similar to those of bacterial wilt (Zhang et al., 2022b). The genus *Xanthomonas*, as already reported in other recent studies (Zhang et al., 2019), was represented in the rice core microbiome with a higher relative abundance in healthy seeds. Even if most *Xanthomonas* are well-known plant pathogens, such as the bacterial leaf blight of rice, caused by *Xanthomonas oryzae* pv. *oryzae*, Zhang et al. (2022a) found that *Xanthomonas* strains isolated from rice seed could antagonize the major pathogens of rice diseases, such as *Magnaporthe oryzae* and *X. oryzae* pv. *oryzae* itself. Moreover, apart from having significant phosphate solubilising activity, most of the *Xanthomonas* isolates by Zhang et al. (2022a) produced plant growth promoting substances such as auxins and ACC deaminase. In addition to these four dominant genera, *Rhizobium, Sphingomonas* and *Acidovorax* were represented as well in the core microbiome. Some species belonging to the genera *Rhizobium* and *Sphingomonas* have been found to play a role in promoting rice growth (Pan et al., 2016; Nguyen et al., 2022) and the latter displayed a significantly higher relative abundance in healthy seeds. In recent studies, *Acidovorax* has also been found as a dominant genus in the rice seed microbiota (Zhang et al., 2022a). Most of the species belonging to the genus *Acidovorax* are phytopathogenic,

including the agent of the bacterial brown stripe of rice (Masum et al., 2017). Therefore, based on the increase of the relative abundance of *Pantoea*, *Pseudomonas*, *Acidovorax* in the core microbiome of "sick" seeds described in the previous section, it would be very interesting to study and understand if those genera contain species that are somehow involved in abnormal seedling development. Additionally, other bacterial genera belonging to *Protobacteria* with growth-promoting properties and the ability to produce antibiotics were also present, with a significantly higher relative abundance in healthy seeds: *Brevundimonas* (Naqqash et al., 2020), *Luteibacter* (Kim et al., 2020b), *Massilia* (Li et al., 2021), *Novosphingobium* (Rangjaroen et al., 2017; Dhondge et al., 2022), *Sphingobium* (Boss et al., 2022).

The second most represented *phylum* in terms of relative abundance was *Firmicutes*, with a significantly higher abundance in healthy seeds (Fig. 2A). This *phylum* was extensively studied as a taxon that includes beneficial microorganisms with various applications in agroecology (*i.e.,* biofertilizers, biocontrol agent and metal uptake enhancers) (Hashmi et al., 2020). Indeed, the *Firmicutes* are known as a major fraction of the plant microbiome residing in the endophytic compartment (Lundberg et al., 2012). In the present study, *Firmicutes* is represented by the genus *Paenibacillus*, as the third most dominant species in the rice core microbiota of NERICA 3 and NERICA 8. Many *Paenibacillus* species can directly promote plant growth through biological nitrogen fixation, phosphate solubilization, production of the phytohormone indole-3–-acetic acid (IAA), and release of siderophores that enable iron uptake (Grady et al., 2016). Intriguingly, *Paenibacillus* showed a significantly higher abundance in "sick" seeds compared to healthy seeds. Among *Firmicutes*, the genus *Bacillus* ranked as the seventh most represented genus in the core microbiome, with a higher relative abundance (fold change = 0.74) in normal seedlings, compared to abnormal ones, even if this increase was not significant. To date, *Bacillus* spp. are the most prominent beneficial bacteria studied (Tsotetsi et al., 2022). In rice, *Bacillus* has been successfully used as a growth promoter and for its ability to biocontrol pathogens by suppressing plant immunity or secreting antimicrobial metabolites (Chithrashree et al., 2011; Jin et al., 2020). In this study, the higher relative abundance of *Firmicutes* in healthy seed may confirm a role for *Bacillus* in normal seedling development. Regarding *Firmicutes*, it is also worth noting the significant increase in the relative abundance of *Exiguobacterium* in healthy seeds: for this genus, several isolates were recently reported to enhance plant growth in horticultural plants (Marfetán et al., 2023) and to have antagonistic effects against various plant pathogens (Kasana, Pandey, 2018). Conversely, *Clostridium sensu stricto* 3 and *Clostridium sensu stricto* 10 were only found in "sick" seeds among the microbiome of both cultivars NERICA 3 and NERICA 8. The genus *Clostridium sensu stricto* includes species that can produce exotoxins in the environment, affect crop growth, and cause plant disease (Jin et al., 2018; Masum et al., 2018; Spigaglia et al., 2020).

As shown in the previous section, the *phylum Actinobacteriota* ranked third in terms of abundance, with a marked reduction in healthy seeds. Several species of *Curtobacterium* have been associated with plant diseases (Vidaver, 1982) or, as reported by Yang et al. (2020a), (2020b), (2020c), their presence was significantly enriched in diseased rice leaves by *Xanthomonas oryzae* pv. *oryzae*, although the role of *Curtobacterium* spp. in disease occurrence remains unclear. Among this *phylum*, *Streptomyces* and *Microbacterium* showed a significantly higher abundance in healthy seeds. Both genera are beneficial species that can enhance plant growth (Passera et al., 2019; Vurukonda et al., 2018). In addition, *Streptomyces* spp. have antagonistic activities against several plant pathogens, including the causal agents of major rice diseases (Suárez-Moreno et al., 2019; Ngalimat et al., 2021). In this study, six bacteria isolated from rice seeds and belonging to *Streptomyces* spp. confirmed their ability to solubilize inorganic phosphorus, synthetise siderophores, produce ammonia and have strong antagonistic activities against fungal pathogens, including *Bipolaris oryzae* (teleomorph: *Cochliobolus miyabeanus*) the causal agent of the brown spot of rice (data not shown). This suggests that among the *phylum Actinobacteriota*, *Curtobacterium* spp. are not beneficial endophytes, like *Streptomyces* and *Microbacterium*, but may play a role, directly or indirectly, in hindering the plant growth and development.

Finally, the fourth *phylum* represented with a relative abundance higher the 1% was *Bacteroidota*. Among this *phylum*, the relative abundance of *Chryseobacterium* was significantly higher in healthy seeds. Indeed, this genus has been reported to be associated with plant growth promoting bacteria (Dardanelli et al., 2010; Montero-Calasanz et al., 2014).

Trought the functional metagenomic analysis predicted by PICRUSt2, the genes for carbohydrates, tryptophan, nucleotides and ATP-binding cassette (ABC) transporters metabolisms were found to be significantly more abundant in microbial communities' resident into normal seedlings. These metabolic pathways are strictly associated with a correct germination and plant development at the early stage and higher concentrations of carbohydrates in plant tissue can support the germination process, where energy metabolism plays a dominant role in regulating seed development (Chaturvedi et al., 1996; Dkhil and Denden, 2010; Höftberger et al., 2022). Therefore, the higher abundance of such metabolic pathways may be positively correlated with the higher relative abundance of several beneficial bacteria present in normal seedlings, which can facilitate the uptake of certain nutrients from the seed environment.

The genes for sphingolipids and glycosphingolipids metabolisms were also significantly more abundant in bacterial communities of normal phenotypes: sphingolipids are essential components of the plasma membrane of plant cells, involved in development and the responses to various abiotic and biotic stresses (Markham et al., 2013; Huby et al., 2020; Liu et al., 2007). Here, a positive correlation between these metabolic pathways and the microbiome may be explained by a significantly higher abundance, in normal seedling phenotypes, of *Sphingomonas* spp: indeed, sphingolipids metabolism is a defining characteristic of this genus (Kim et al., 2020a). Again, for the biosynthesis of ansamycins, an important class of antibiotics produced by plant endophytic *Streptomyces* spp., a positive correlation was found by gene overexpression and higher abundance of this genus in normal phenotypes.

In abnormal bacterial communities, most of significantly overexpressed genes were related to the metabolic degradation of aromatic hydrocarbons such as xylene, naphthalene, ethylbenzene, and volatile organic compounds, such as limonene, pinene, camphor and geraniol. These secondary metabolites, if present in high abundance, can be phytotoxic and cause significant changes in root and shoot development (Aina et al., 2006; Anokhina et al., 2020; Zheng et al., 2015; Wang et al., 2015). Bacterial genera involved in such catabolic pathways mainly belong to *Proteobacteria* and *Actinobacteria* (Fu et al., 2023), which are significantly more abundant in abnormal phenotypes: this suggests the co-association between the microbes sheltering these genes and abnormal phenotypes. Furthermore, overexpressed genes related to the flavonoid's degradation pathway were also associated to the abnormal phenotype microbiota. Flavonoids are essential molecules involved in the determination of patterns of root growth and development, as well as acting on plant defence mechanisms against abiotic factors (Tan et al., 2019). Here, the characteristic of *Clostridium sensu stricto* genus to degrade flavonoids (Zhou et al., 2023) may be associated to its unique presence in abnormal seedling microbiome.

Additionally, genes for tyrosine, phenylalanine and linoleic metabolism were found to be overexpressed in the abnormal phenotype associated microbiome: these are metabolic pathways involved in the biosynthesis of secondary metabolites and play important roles in plant (Xu et al., 2019; Feduraev et al., 2020; Razzaq et al., 2020). Our results are in line with those of Mashabela et al. (2022), who showed that beneficial *Paenibacillus* spp. are involved in such metabolic pathways. Indeed, the higher abundance of *Paenibacillus* genus may be associated to the overexpression of tyrosine, phenylalanine and linoleic genes in

abnormal phenotypes microbiota.

Although results obtained by analysing healthy and sick seed microbial communities and their putative metabolic capacities highlighted the correlation between seedling phenotype and a set of endophytic bacterial taxa, culture experiments are necessary to obtain better insights into the roles and function of the enriched bacterial taxa and their potential role as growth promoter.

Differences in rice seed microbiota can be summarised as follows: *i*) rice seed microbial communities are clustered into two groups, based on normal and abnormal seedling phenotypes generated by healthy and "sick" seeds, respectively (Fig. 2B); *ii*) a remarkable difference in relative abundance is associated to the phyla *Proteobacteria* and *Firmicutes*: *Proteobacteria* abundance was significantly higher in "sick" seeds and, by contrast, *Firmicutes* abundance was significantly higher in healthy seeds (Fig. 2A); *iii*) the majority of bacterial genera known to promote plant growth or to have antimicrobial activity against phytopathogens (13 out of 16) were significantly more abundant in healthy rice seeds (Fig. 3B); *iv*) healthy seeds were devoid of *Clostridium sensu stricto* species, which are known to be harmful to plants; *v*) higher abundance of bacterial genes related to the metabolic biosynthesis of soluble carbohydrates and starch, tryptophan, nucleotides and ABC transporters was correlated with normal seedling developments (Fig. 4).

We showed and confirmed that, as any other plant species, rice harbours a highly diversified microbial community, also considering the seed solely as the plant compartment hosting the core microbiome. As several other authors, we attempted to highlight their possible interactions with the host plant, in a kind of binary interactions that may influence the plant wellbeing as, *e.g.*, excellently reviewed by Pereira et al. (2023). More issues now require to be elucidated, especially the interactions or crosstalk among microbes, which may influence microbial dynamics endophytically and may shape, from time to time, the phytobiome. A deeper insight and knowledge on such aspect may represent a possible direction in future research.

5. Conclusion

Our results provide a better understanding of the complex bacterial composition of the rice microbiota in relation to seed germination and seedling development. In fact, the current studies clearly highlight the correlation between seed health and a set of endophytic bacterial taxa, such as *Brevundimonas, Sphingomonas, Exiguobacterium, Luteibacter, Microbacterium* and *Streptomyces*, which can improve seed quality and health. The improved knowledge on the taxonomic identity of rice seeds endophytes allows us to carry forward our studies on a selected number of them, their role, and mechanism in driving a correct development of rice plants at the early stage, in order to select growth promoting microbes and microbial antagonists. Indeed, these specific microbes may be used to design plant-beneficial microbial consortia for more sustainable agricultural practices, *e.g.*, developing a seed-coating product containing selected microbes, as very recently suggested (Paravar et al., 2023). This appears particularly important since: *i*) such microbes belong to the natural rice microcosm and are well adapted to the local agro-environmental conditions; *ii*) the rural communities in Cameroonian regions may take advantage by a technological development of commercial products based on well-studied beneficial microbes, thus diminishing their need to import costly fertilizers or pesticides; *iii*) cost reduction and increased yield will support farmers' remunerability, thus supporting social stability in rural Cameroon.

Funding

The European Commission financially supported the early career scientists' mobility between the University of Yaoundé I (Cameroon) and the University of Modena and Reggio Emilia (Italy), in the framework of the Erasmus ICM KA 107 project.

CRediT authorship contribution statement

Albert Dongmo Nanfack: Conceptualization, Data curation, Methodology, Writing – original draft. **Julienne Nguefack:** Conceptualization. **Samson Musonerimana:** Methodology, Writing – original draft. **Salvatore La China:** Data curation, Formal analysis, Software, Writing – original draft. **Davide Giovanardi:** Conceptualization, Methodology, Data curation, Writing – original draft, supervision, Writing – review & editing. **Emilio Stefani:** Conceptualization, Methodology, Funding acquisition, Writing – original draft, supervision, Writing – review & editing.

Declaration of Competing Interest

None.

Data Availability

The raw reads and raw data generated for this study can be found in the NCBI Sequence Read Archive as BioProject PRJNA978571 [https://www.ncbi.nlm.nih.gov/sra/PRJNA978571].

Acknowledgements

The authors thank Stefano Cassanelli, University of Modena and Reggio Emilia (Italy), for his valuable technical suggestions and for a critical revision of the manuscript. We are also grateful to Vittorio Venturi and ICGEB, Trieste (Italy), for supervising Dongmo Nanfack Albert during his stay in their lab.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.micres.2023.127546.

References

Afzal, I., Shabir, R., Rauf, S., 2019. Seed Production Technologies of Some Major Field Crops. Agronomic crops. Springer, Singapore, pp. 655–678. https://doi.org/10.1007/978-981-32-9151-5_28.

Ahumada, G.D., Gómez-Álvarez, E.M., Dell'Acqua, M., Bertani, I., Venturi, V., Perata, P., Pucciariello, C., 2022. Bacterial endophytes contribute to rice seedling establishment under submergence. Front. Plant Sci. 29 (13), 908349.

Aina, R., Palin, L., Citterio, S., 2006. Molecular evidence for benzo [a] pyrene and naphthalene genotoxicity in Trifolium repens L. Chemosphere 65 (4), 666–673.

Andersson, A.F., Lindberg, M., Jakobsson, H., Bäckhed, F., Nyrén, P., Engstrand, L., 2008. Comparative analysis of human gut microbiota by barcoded pyrosequencing. PLoS ONE 3 (7), e2836. https://doi.org/10.1371/journal.pone.0002836.

Anokhina, T.O., Esikova, T.Z., Gafarov, A.B., et al., 2020. Alternative naphthalene metabolic pathway includes formation of *ortho*-phthalic acid and cinnamic acid derivatives in the *Rhodococcus opacus* strain 3D. Biochem. Mosc. 85, 355–368. https://doi.org/10.1134/S0006297920030116.

Azizi, M.M.F., Ismail, S.I., Ina-Salwany, M.Y., Mohd Hata, E., Zulperi, D., 2020. The emergence of Pantoea species as a future threat to global rice production. J. Plant Prot. Res. 60 (4), 327–335. https://doi.org/10.24425/jppr.2020.133958.

Baltrus, D.A., McCann, H.C., Guttman, D.S., 2017. Evolution, genomics and epidemiology of *Pseudomonas syringae*. Mol. Plant Pathol. 18, 152–168.

Banik, A., Mukhopadhaya, S.K., Dangar, T.K., 2016. Characterization of N2-fixing plant growth promoting endophytic and epiphytic bacterial community of Indian cultivated and wild rice (*Oryza* spp.) genotypes. Planta 243, 799–812. https://doi.org/10.1007/s00425-015-2444-8.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc.: Ser. B. Methodol. 289–300.

Berg, M., Koskella, B., 2018. Nutrient- and dose-dependent microbiome-mediated protection against a plant pathogen. Curr. Biol. 28 (15), 2487–2492 e3.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37 (8), 852–857.

Boss, B.L., Wanees, A.E., Zaslow, S.J., Normile, T.G., Izquierdo, J.A., 2022. Comparative genomics of the plant-growth promoting bacterium *Sphingobium* sp. strain AEW4 isolated from the rhizosphere of the beachgrass *Ammophila breviligulata*. BMC Genom. 23 (1), 13, 508.

Bragard, C., Baptista, P., Chatzivassiliou, E., Di Serio, F., Gonthier, P., Jaques Miret, J.A., Justesen, A.F., MacLeod, A., Magnusson, C.S., Milonas, P., Navas-Cortes, J.A., Parnell, S., Potting, R., Stefani, E., Thulke, H.H., Van der Werf, W., Civera, A.V.,

Yuen, J., Zappala, L., Migheli, Q., Vloutoglou, I., Maiorano, A., Streissl, F., Reignault, P.L., EFSA (E.F.S.A. Panel on Plant Health), 2023. Scientific opinion on the pest categorisation of *Pantoea ananatis*. EFSA J. 2023 21 (3), 7849, 33 pp.
Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Natural. Methods 13, 581–583.
Carrer Filho, R., Rodrigues, L., Abreu, A., Souza, R., Rangel, P., Mello, R., Rocha, G., Cunha, M., 2018. Detection of Pantoea agglomerans in germplasm rice accessions (Oryza sativa) in Brazil. Plant Dis. 102, 237. https://doi.org/10.1094/PDIS-06-17-0905-PDN.
Chaturvedi, G.S., Ram, P.C., Singh, A.K., Ram, P., Ingram, K.T., Singh, B.B., Singh, R.K., Singh, V.P., 1996. Carbohydrate status of rainfed lowland rice in relation to submergence, drought and saline shade tolerance. International Rice Research Institute. Manila 108.
Chithrashree, Udayashankar, Chandra, A.C., Nayaka, S., Reddy, M.S., Srinivas, C., 2011. Plant growth-promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas* oryzae pv. oryzae. Biol. Control. 59, 114–122.
Choi, O.-H., Kim, H.-Y., Lee, Y.-S., Kim, J.-W., Moon, J.-S., Hwang, I.-G., 2012. First report of sheath rot of rice caused by *Pantoea ananatis* in Korea. Plant Pathol. J. 28, 331. https://doi.org/10.5423/PPJ.DR.08.2011.0150.
Compant, S., Samad, A., Faist, H., Sessitsch, A., 2019. A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. J. Adv. Res. 20 (19), 29–37. https://doi.org/10.1016/j.jare.2019.03.004.
Cortesi, P., Pizzatti, C., 2007. Palea browning, a new disease of rice in Italy caused by Pantoea ananatis. J. Gen. Plant Pathol. 89, S69–S76.
Cother, E.J., Reinke, R., McKenzie, C., Lanoiselet, V., Noble, D., 2004. An unusual stem necrosis of rice caused by Pantoea ananas and the first record of this pathogen on rice in Australia. Australas. Plant Pathol. 33, 495–503. https://doi.org/10.1071/AP04053.
Crosse, J.E., 1959. Bacterial canker of stone fruits. IV. Investigation of a method for measuring the inoculum potential of cherry trees. Ann. Appl. Biol. 47, 306–317.
da Silveira, A.P.D., Iorio, R.P.F., Marcos, F.C.C., Fernandes, A., de Souza, S., Kuramae, E. E., Cipriano, M.A.P., 2019. Exploitation of new endophytic bacteria and their ability to promote sugarcane growth and nitrogen nutrition. Antonie Van. Leeuwenhoek 112 (2), 283–295.
Dardanelli, M.S., Manyani, H., González-Barroso, S., Rodríguez-Carvajal, M.A., Gil-Serrano, A.M., Espuny, M.R., López-Baena, F.J., Bellogín, R.A., Megías, M., Ollero, F. J., 2010. Effect of the presence of the plant growth promoting rhizobacterium (PGPR) *Chryseobacterium balustinun* Aur9 and salt stress in the pattern of flavoids exuded by soybean roots. Plant Soil 328, 483–493.
David, P.T., Hongwei, L., John, T.N., Zipporah, P., Zogbo, L., Charles, F.K., Melissa, S.S., Victor, M.V., 2017. Biological control of potential antagonistic bacteria isolates to restrict *Magnaporthe grisea* infection on rice. Afr. J. Microbiol. Res.. 11, 1108–1119. https://doi.org/10.5897/AJMR2017.8562.
de Vrieze, J., Pinto, A.J., Sloan, W.T., Ijaz, U.Z., 2018. The active microbial community more accurately reflects the anaerobic digestion process: 16S rRNA (gene) sequencing as a predictive tool. Microbiome 6, 1–13.
Dhondge, H.V., Barvkar, V.T., Syed, D.P., Dastager, G., Pable, A.A., Nadaf, A.B., 2022. Exploring the core microbiota in scented rice (*Oryza sativa* L.) rhizosphere through metagenomics approach. Microbiol. Res. 263. https://doi.org/10.1016/j.micres.2022.127157.
Dkhil, B.B., Denden, M., 2010. Salt stress induced changes in germination, sugars, starch and enzyme of carbohydrate metabolism in Abelmoschus esculentus (L.) Moench seeds. Afr. J. Agric. Res. 5, 408–415.
Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M., et al., 2020. PICRUSt2 for Prediction of Metagenome Functions. In: Nat Biotechnol, 38. Nature Publishing Group, pp. 685–688.
Egorova, M., Mazurin, E., Ignatov, A., 2015. First report of Pantoea ananatis causing grain discolouration and leaf blight of rice in Russia. N. Dis. Rep. 32, 21. https://doi.org/10.5197/j.2044-0588.2015.032.021.
Eyre, A.W., Wang, M., Oh, Y., Dean, R.A., 2019. Identification and characterization of the core rice seed microbiome. Phytobiomes J. 3 (2), 148–157.
FAOSTAT. 2019. Food and Agriculture Organisation of the United Nations Database. Séries principales de statistiques mondiales.
Feduraev, P., Skrypnik, L., Riabova, A., Pungin, A., Tokupova, E., Maslennikov, P., Chupakhina, G., 2020. Phenylalanine and tyrosine as exogenous precursors of wheat (*Triticum aestivum* L.) secondary metabolism through PAL-associated pathways. Plants 9 (4), 476. https://doi.org/10.3390/plants9040476.
Feng, Y., Shen, D., Song, W., 2006. Rice endophyte *Pantoea agglomerans* YS19 promotes host plant growth and affects allocations of host photosynthates. J. Appl. Microbiol.. 100, 938–945. https://doi.org/10.1111/j.1365-2672.2006.02843.x.
Fu, W., Zheng, X., Chen, X., Wang, W., Liu, A., Ji, J., Guan, C., 2023. The potential roles of carotenoids in enhancing phytoremediation of bisphenol A contaminated soil by promoting plant physiology and modulating rhizobacterial community of tobacco. Chemosphere 316, 137807.
Ghosh, A., Pramanik, K., Bhattacharya, S., Mondal, S., Ghosh, S.K., Ghosh, P.K., Maiti, T. K., 2021. Abatement of arsenic-induced phytotoxic effects in rice seedlings by an arsenic-resistant *Pantoea dispersa* strain. Environ. Sci. Pollut. Res.. 8, 21633–21649. https://doi.org/10.1007/s11356-020-11816-7.
Grady, E.N., MacDonald, J., Liu, L., Richman, A., Yuan, Z.C., 2016. Current knowledge and perspectives of *Paenibacillus*: a review. Microb. Cell Factor. 15, 203.
Guo, J., Ling, N., L,i, Y., Li, K., Ning, H., Shen, Q., Guo, S., Vandenkoornhuyse, P., 2021. Seed-borne, endospheric and rhizospheric core microbiota as predictors of plant functional traits across rice cultivars are dominated by deterministic processes. N. Phytol. 230 (5), 2047–2060.
Hardoim, P.R., Hardoim, C.C.P., Overbeekl, L.S.V., Elsas, J.D.V., 2012. Dynamics of seed-borne rice endophytes on early plant growth stages. PLoS ONE 7 (2), e30438.
Hashmi, I., Bindschedler, S., Junier, P. 2020. Firmicutes. Beneficial Microbes in Agro-Ecology, Academic Press: 363–396.
Höftberger, M., Althammer, M., Foissner, I., Tenhaken, R., 2022. Galactose induces formation of cell wall stubs and cell death in *Arabidopsis* roots. Planta 256 (2), 26.
Huby, E., Napier, J.A., Baillieul, F., Michaelson, L.V., Dhondt-Cordelier, S., 2020. Sphingolipids: towards an integrated view of metabolism during the plant stress response. N. Phytol. 225 (2), 659–670 https://doi.org/10.1111/nph.15997.
Illumina (2013). 16S Metagenomic sequencing library preparation. Part #15044223 Rev.B https://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html.
ISTA. 2021. International Rules for Seed Testing 2021. International Seed Testing Association, Bassersdorf, Switzerland. Available at: ⟨https://www.seedtest.org/en/publications/international-rules-seed-testing-1168.html⟩.
Jin, P., Wang, Y., Tan, Z., Liu, W., Miao, W., 2020. Antibacterial activity and rice-induced resistance, mediated by C15surfactin A, in controlling rice disease caused by Xanthomonas oryzae pv. oryzae. Pestic. Biochem. Physiol. 169, 104669.
Jin, S.L., Hu, Z.J., Huang, Y.Z., Pan, H.H., Hu, Y., 2018. Effects of rice straw, rice straw ash, and bone charcoal on uptake and accumulation of rare earth elements in rice plants. BioResoures 13, 8593–8613.
Johnston-Monje, D., Raizada, M.N., 2011. Conservation and diversity of seed associated endophytes in zea across boundaries of evolution, ethnography and ecology. PLoS ONE 6 (6), e20396.
Kaneda, C., 2007. Breeding and dissemination efforts of NERICA (1) Breeding of upland rice. Jpn. J. Trop. Agr. 51 (1), 1–4.
Kasana, R.C., Pandey, C.B., 2018. *Exiguobacterium*: an overview of a versatile genus with potential in industry and agriculture. Crit. Rev. Biotechnol. 38 (1), 141–156.
Kim, H., Lee, K.K., Jeon, J., Harris, W.A., Lee, Y.H., 2020a. Domestication of *Oryza* species eco-evolutionarily shapes bacterial and fungal communities in rice seed. Microbiome 8, 20.
Kim, N., Kim, J.J., Kim, I., Mannaa, M., Park, J., Kim, J., et al., 2020b. Type VI secretion systems of plant-pathogenic *Burkholderia glumae* BGR1 play a functionally distinct role in interspecies interactions and virulence. Mol. Plant. Pathol. 218, 1055–1069.
Kim, Y.J., Park, J.Y., Balusamy, S.R., Huo, Y., Nong, L.K., Thi Le, H., Kim, D., 2020c. Comprehensive genome analysis on the novel species *Sphingomonas panac*is DCY99T reveals insights into iron tolerance of ginseng. Int. J. Mol. Sci. 21 (6), 2019.
Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Oliver, G.F., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. Vol. 41 (No. 1), 11p.
L'Hoir, M., Duponnois, R., 2021. Combining the seed endophytic bacteria and the back to the future approaches for plant holonbiont breeding. Front. Agron. 3, 724450 https://doi.org/10.3389/fagro.2021.724450.
Lamichhane, J.R., Debaeke, P., Steinberg, C., You, M.P., Barbetti, M.J., Aubertot, J.N., 2018. Abiotic and biotic factors affecting crop seed germination and seedling emergence: a conceptual framework. Plant Soil 432, 1–28.
Lee, H.B., Hong, J.P., Kim, S.B., 2010. First report of leaf blight caused by *Pantoea agglomerans* on rice in Korea. Plant Dis. 94 (11), 1372.
Li, C., Cao, P., Du, C., Zhang, X., Bing, H., Li, L., Sun, P., Xiang, W., Zhao, J., Wang, X., 2021. *Massilia rhizosphaerae* sp. nov., a rice-associated rhizobacterium with antibacterial activity against *Ralstonia solanacearum*. Int. J. Syst. Evolut. Microbiol. 71 (9), 005009.
Lin, H., Peddada, S.D., 2020. Analysis of compositions of microbiomes with bias correction. Nat. Commun. 11, 3514.
Liu, H., Ravichandran, S., The, O.K., McVey, S., Lilley, C., Teresinski, H.J., et al., 2007. The RING-Type E3 ligase XBAT35.2 is involved in cell death induction and pathogen response. Plant Physiol. 175 (3), 1469–1483. https://doi.org/10.1104/pp.17.01071.
Liu, X., Gao, Y., Yang, H., Li, L., Jiang, Y., Li, Y., Zheng, J., 2020. *Pichia kudriavzevii* retards fungal decay by influencing the fungal community succession during cherry tomato fruit storage. Food Microbiol. 88, 103404 https://doi.org/10.1016/j.fm.2019.103404.
Lundberg, D.S., Lebeis, S.L., Paredes, S.H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., Del Rio, T.G., Edgar, R.C., Eickhorst, T., Ley, R.E., Hugenholtz, P., Tringe, S.G., Dangl, J.L., 2012. Defining the core *Arabidopsis thaliana* root microbiome. Nature 488, 86–90.
Lv, L., Luo, J., Ahmed, T., Zaki, H.E.M., Tian, Y., Shahid, M.S., Chen, J., Li, B., 2022. Beneficial effect and potential risk of *Pantoea* on rice production. Plants, 4 11 (19), 2608. https://doi.org/10.3390/plants11192608.
Malaa, D.K., Jaff, A.B., Agboh-Noameshie, A.R., Ngonkeu, E.L.M., Bebom, N.K.T., Woin, N., 2016. Technology transfer through participatory approach: the case of NERICA introduction in Cameroon. J. Econ. Dev. Stud. 4, 71–80. https://doi.org/10.15640/jeds.v4n4a7.
Marfetán, J.A., Gallo, A.L., Farias, M.E., Vélez, M.L., Pescuma, M., Ordoñez, O.F., 2023. *Exiguobacterium* sp. as a bioinoculant for plant-growth promotion and selenium biofortification strategies in horticultural plants. World J. Microbiol. Biotechnol. 39, 134.
Markham, J.E., Lynch, D.V., Napier, J.A., Dunn, T.M., Cahoon, E.B., 2013. Plant sphingolipids: function follows form. Curr. Opin. Plant Biol. 16 (3), 350–357. https://doi.org/10.1016/j.pbi.2013.02.009.
Mashabela, M.D., Tugizimana, F., Steenkamp, P.A., Piater, L.A., Dubery, I.A., Mhlongo, M.I., 2022. Untargeted metabolite profiling to elucidate rhizosphere and leaf metabolome changes of wheat cultivars (*Triticum aestivum* L.) treated with the plant growth-promoting rhizobacteria *Paenibacillus alvei* (T22) and *Bacillus subtilis*. Front. Microbiol. 13, 971836 https://doi.org/10.3389/fmicb.2022.971836.

Masum, M.M.I., Yang, Y., Li, B., Olaitan, O.S., Chen, J., Zhang, Y., Fang, Y., Qiu, W., Wang, Y., Sun, G., 2017. Role of the genes of type VI secretion system in virulence of rice bacterial brown stripe pathogen *Acidovorax avenae* subsp. *avenae* Strain RS-2. Int. J. Mol. Sci. 18. E2024.

Masum, M.M.I., Liu, L., Yang, M., Masum, M.M., Siddiqa, M.M., Supty, M.E., Ogunyemi, S.O., Hossain, A., An, Q., Li, B., 2018. Halotolerant bacteria belonging to operational group *Bacillus amyloliquefaciens* in biocontrol of the rice brown stripe pathogen *Acidovorax oryzae*. J. Appl. Microbiol. 125, 1852–1867.

Matsumoto, A., Takahashi, Y., 2017. Endophytic actinomycetes: promising source of novel bioactive compounds. The journal of antibiotics 70 (5), 514–519.

Matsumoto, H., Fan, X., Wang, Y., Kusstatscher, P., Duan, J., Wu, S., Chen, S., Qiao, K., Wang, Y., Ma, B., Zhu, G., Hasidoko, Y., Berg, G., Cernava, T., Wang, M., 2021. Bacterial seed endophyte shapes disease resistance in rice. Nat. Plant 7, 60–72.

Megías, E., dos Reis Junior, F.B., Ribeiro, R.A., Ollero, F.J., Megías, M., Hungria, M., 2017. Draft genome sequence of *Pantoea ananatis* strain 1.38, a bacterium isolated from the rhizosphere of *Oryza sativa* var. *Puntal* that shows biotechnological potential as an inoculant. Genome Announc 6. https://doi.org/10.1128/genomeA.01547-1.

Mengistu, A.A., 2020. Endophytes: Colonization, Behaviour, and Their Role in Defense Mechanism. Int. J. Microbiol. 30, 6927219. https://doi.org/10.1155/2020/6927219.

Mondal, K.K., Mani, C., Singh, J., Kim, J.G., Mudgett, M.B., 2011. A new leaf blight of rice caused by *Pantoea ananatis* in India. Plant Dis. 95 (12), 1582.

Montero-Calasanz, M.C., Göker, M., Rohde, M., Spröer, C., Schumann, P., Busse, H.J., Schmid, M., Klenk, H.P., Tindall, B.J., Camacho, M., 2014. *Chryseobacterium oleae* sp. nov., an efficient plant growth promoting bacterium in the rooting induction of olive tree (Olea europaea L.) cuttings and emended descriptions of the genus *Chryseobacterium*, *C. daecheongense, C. gambrini, C. gleum, C. joostei, C. jejuense, C. luteum, C. shigense, C. taiwanense, C. ureilyticum* and *C. vrystaatense*. Syst. Appl. Microbiol. 37 (5), 342–350.

Naher, U.A., Othman, R., Panhwar, Q.A., Ismail, M.R., 2015. Biofertilizer for sustainable rice production and reduction of environmental pollution. Crop Production and Global Environmental Issues. Springer, Berlin, Germany, pp. 283–291.

Naqqash, T., Imran, A., Hameed, S., Shahid, M., Majeed, A., Iqbal, J., Hanif, M.K., Ejaz, S., Malik, K.A., 2020. First report of diazotrophic *Brevundimonas* spp. as growth enhancer and root colonizer of potato. Sci. Rep. 10 (1), 30, 12893.

Nelson, E.B., 2018. The seed microbiome: origins, interactions, and impacts. Plant Soil 422, 7–34.

Neu, A.T., Allen, E.E., Roy, K., 2021. Defining and quantifying the core microbiome: challenges and prospects. PNAS Vol. 118 (No. 51) e2104429118.

Ngalimat, M.S., Mohd Hata, E., Zulperi, D., Ismail, S.I., Ismail, M.R., Mohd Zainudin, N. A.I., Saidi, N.B., Yusof, M.T., 2021. Plant growth-promoting bacteria as an emerging tool to manage bacterial rice pathogens. Microorganisms 9, 682.

Nghi, K.N., Tagliani, A., Mariotti, L., Weits, D.A., Perata, P., Pucciariello, C., 2021. Auxin is required for the long coleoptile trait in *japonica* rice under submergence. New Phytol. 229, 85–93.

Nguyen, P.M., Nguyen, H.T., Le, H.T.T., Nguyen, L.B., Tran, P.H., Dinh, Y.B., Nguyen, T. K.N., Nguyen, M.H., 2022. The effects of *Rhizobium* inoculation on the growth of rice (*Oryza sativa* L.) and white radish (*Raphanus sativus* L.). IOP Conf. Ser.: Earth Environ. Sci. 995, 012053.

Oliver, J.D., 2005. The viable but non-culturable state in bacteria. J. Microbiol. 43, 93–100.

Ortmann, I., Conrath, U., Moerschbacher, B.M., 2006. Exopolysaccharides of *Pantoea agglomerans* have different priming and eliciting activities in suspension-cultured cells of monocots and dicots. FEBS Lett. 580, 4491–4494. https://doi.org/10.1016/j.febslet.2006.07.025.

Pan, F., Meng, Q., Wang, Q., Luo, S., Chen, B., Khan, K.Y., Yang, X., Feng, Y., 2016. Endophytic bacterium *Sphingomonas* SaMR12 promotes cadmium accumulation by increasing glutathione biosynthesis in *Sedum alfredii* Hance. Chemosphere 154, 358–366.

Paravar, A., Piri, R., Balouchi, H., Ma, Y., 2023. Microbial seed coating: an attractive tool for sustainable agriculture. Biotechnol. Rep. 37, e00781.

Passera, A., Compant, S., Casati, P., Maturo, M.G., Battelli, G., Quaglino, F., Antonielli, L., Salerno, D., Brasca, M., Toffolatti, S.L., Mantegazza, F., Delledonne, M., Mitter, B., 2019. Not just a pathogen? Description of a plant-beneficial *Pseudomonas syringae* strain. Front. Microbiol. 10, 1409.

Peng, G., Zhang, W., Luo, H., Xie, H., Lai, W., Tan, Z., 2009. Enterobacter oryzae sp. nov., a nitrogen-fixing bacterium isolated from the wild rice species *Oryza latifolia*. Int. J. Syst. Evol. Microbiol 59, 1650–1655.

Pereira, L.B., Thomazella, D.P.T., Teixeira, P.J.L., 2023. Plant-microbiome crosstalk and disease development. Curr. Opin. Plant Biol. 72 https://doi.org/10.1016/j.pbi.2023.102351.

Piernas, V., Guiraud, J.P., 1997. Disinfection of rice seeds prior to sprouting. J. Food Sci. 62 (3), 611–615.

Porras-Alfaro, A.-, Bayman, P., 2011. Hidden fungi, emergent properties: endophytes and microbiomes. Annu. Rev. Phytopathol. 49, 291–315.

Rangjaroen, C., Sungthong, R., Rerkasem, B., Teaumroong, N., Noisangiam, R., Lumyong, S., 2017. Untapped endophytic colonization and plant growth-promoting potential of the genus *Novosphingobium* to optimize rice cultivation. Microbes Environ. 32 (1), 84–87.

Razzaq, A., Ali, A., Safdar, L.B., Zafar, M.M., Rui, Y., Shakeel, A., Yuan, Y., 2020. Salt stress induces physiochemical alterations in rice grain composition and quality. J. Food Sci. 85 (1), 14–20.

Ridout, M.E., Schroeder, K.L., Hunter, S.S., Styer, J., Newcombe, G., 2019. Priority effects of wheat seed endophytes on a rhizosphere symbiosis. Symbiosis 78, 19–31.

Rodriguez, C.E., Antonielli, L., Mitter, B., Trognitz, F., Sessitsch, A., 2019. Heritability and functional importance of the *Setaria viridis* bacterial seed microbiome. Phytobiomes J. 4, 40–52.

Sagakami, J.I., 2022. Prospect of rice development in Africa. J. Int. Coop. Agric. Dev. 20, 13–25.

Schlaeppi, K., Bulgarelli, D., 2015. The plant microbiome at work. Mol. Plant-Microbe Inter. 28, 212–217.

Shade, A., Jacques, M.A., Barret, M., 2017. Ecological patterns of seed microbiome diversity, transmission, and assembly. Curr. Opin. Microbiol. 37, 15–22.

Shahzad, R., Waqas, M., Khan, A.L., Asaf, S., Khan, M.A., Kang, S.M., Byung-Wook, Y., Lee, I.J., 2016. Seed-borne endophytic *Bacillus amyloliquefaciens* RWL-1 produces gibberellins and regulates endogenous phytohormones of Oryza sativa. Plant Physiol. Biochem. 106, 236–243.

Simonin, M., da Silva, C., Terzi, V., Ngonkeu, E.L.M., Diouf, D., Kane, A., Béna, G., Moulin, L., 2020. Influence of plant genotype and soil on the wheat rhizosphere microbiome: evidences for a core microbiome across eight African and European soils. FEMS Microbiol. Ecol. 96 (6), fiaa067.

Somado, E.A., Guei, R.G., Keya, S.O. 2008. NERICA: the New Rice for Africa – a Compendium, 2008 edition, Africa Rice Center (WARDA).

Spence, C., Alff, E., Johnson, C., Ramos, C., Donofrio, N., Sundaresan, V., Bais, H., 2014. Natural rice rhizospheric microbes suppress rice blast infections. BMC Plant Biol. 14, 1–17. https://doi.org/10.1186/1471-2229-14-130.

Spigaglia, P., Barbanti, F., Marocchi, F., Mastroleo, M., Baretta, M., Ferrante, P., Caboni, E., Lucioli, S., Scortichini, M., 2020. *Clostridium bifermentans* and *C. subterminale* are associated with kiwifruit vine decline, known as moria, in Italy. Plant Pathol. 69, 765–774.

Suárez-Moreno, Z.R., Vinchira-Villarraga, D.M., Vergara-Morales, D., Castellanos, L., Ramos, F.A., Guarnaccia, C., Degrassi, G., Venturi, V., Moreno-Sarmiento, N., 2019. Plant-growth promotion and biocontrol properties of three *Streptomyces* spp. isolates to control bacterial rice pathogens. Front. Microbiol. 10, 290.

Tan, H., Man, C., Xie, Y., Yan, J., Chu, J., Huang, J., 2019. A crucial role of GA-regulated flavonol biosynthesis in root growth of arabidopsis. Mol. Plant 12 (4), 521–537.

Tang, E.N., Ngome, A.F., 2015. Rice seed quality as influenced by storage duration and package type in Cameroon. Int. J. Biol. Chem. Sci. 9 (3), 1229–1242.

Truyens, S.N., Weyens, A., Cuypers, J.V., 2014. Bacterial seed endophytes: genera, vertical transmission and interaction with plants. Environ. Microbiol. Rep. 7, 40–50.

Tsotetsi, T., Nephali, L., Malebe, M., Tugizimana, F., 2022. *Bacillus* for plant growth promotion and stress resilience: what have we learned? Plants 11 (19), 2482.

Vidaver, A.K., 1982. The plant pathogenic corynebacteria. Rev. Microbiol. 36, 495–517.

Vurukonda, S.S., Giovanardi, D., Stefani, E., 2017. Symbiotic agriculture: plant growth promotion and biocontrol activity of beneficial microorganisms. J. Plant Pathol. 99 (Supplement), S49.

Vurukonda, S.S., Giovanardi, D., Stefani, E., 2018. Plant growth promoting and biocontrol activity of streptomyces spp. as endophytes. Int. J. Mol. Sci. 19, 952.

Wang, M., Eyre, A.W., Thon, M.R., Oh, Y., Dean, R.A., 2020. Dynamic changes in the microbiome of rice during shoot and root growth derived from seeds. Front. Microbiol. 11, 559728.

Wang, Y., Naumann, U., Wright, S.T., Warton, D.I., 2012. mvabund - an R package for model-based analysis of multivariate abundance data. Methods Ecol. Evol. 3, 471–474.

Wang, Y., Wang, S., Luo, C., Xu, Y., Pan, S., Li, J., Li, X., 2015. Influence of rice growth on the fate of polycyclic aromatic hydrocarbons in a subtropical paddy field: a life cycle study. Chemosphere 119, 1233–1239.

Weyens, N., van der Lelie, D., Taghavi, S., Newman, L., Vangronsveld, J., 2009. Exploiting plant-microbe partnerships to improve biomass production and remediation. Trends Biotechnol. 27 (10), 591–598.

Williams, A.N., Stavrinides, J., 2021. Genetic and evolutionary characterization of the major facilitator superfamily transporters of the antibacterial, pantoea natural product 3. Res. Microbiol. 173, 103899 https://doi.org/10.1016/j.resmic.2021.103899.

Xu, J.J., Fang, X., Li, C.Y., Yang, L., Chen, X.Y., 2019. General and specialized tyrosine metabolism pathways in plants. aBIOTECH 1 (2), 97–105. https://doi.org/10.1007/s42994-019-00006-w.

Yang, F., Zhang, J., Zhang, H., Ji, G., Zeng, L., Li, Y., Yu, C., Fernando, W.G.D., Chen, W., 2020a. Bacterial blight induced shifts in endophytic microbiome of rice leaves and the enrichment of specific bacterial strains with pathogen antagonism. Front. Plant. Sci. 11, 963.

Yang, Q., Zhao, D., Liu, Q., 2020b. Connections between amino acid metabolisms in plants: lysine as an example. Front. Plant Sci. 11, 928. https://doi.org/10.3389/fpls.2020.00928.

Yang, Q., Zhao, D., Liu, Q., 2020c. Connections between amino acid metabolisms in plants: lysine as an example. Front. Plant Sci. 11, 928 https://doi.org/10.3389/fpls.2020.00928.

Yu, L., Yang, C., Ji, Z., Zeng, Y., Liang, Y., Hou, Y., 2022. First report of new bacterial leaf Blight of rice caused by *Pantoea ananatis* in Southeast China. Plant Dis. 106, 310. https://doi.org/10.1094/PDIS-05-21-0988-PDN.

Zhang, J., ·Zhang, C., ·Yang, J., ·Zhang, R., Gao, ·J., Zhao, X., ·Zhao, ·J., Zhao, D., Zhang, X., 2019. Insights into endophytic bacterial community structures of seeds among various *Oryza sativa* L. rice genotypes. J. Plant. Growth Regul. 38, 93–102.

Zhang, X., Ma, Y.N., Wang, X., Liao, K., He, S., Zhao, X., Guo, H., Zhao, D., Wei, H.L., 2022a. Dynamics of rice microbiomes reveal core vertically transmitted seed endophytes. Microbiome 10 (1), 216.

Zhang, Y., Wang, B., Li, Q., Huang, D., Zhang, Y., Li, G., He, H., 2022b. Isolation and complete genome sequence analysis of *Kosakonia cowanii* Pa82, a novel pathogen causing Bacterial Wilt on Patchouli. Front. Microbiol. 12, 818228.

Zheng, D., Bao, J., Lu, J., et al., 2015. Isolation and characterization of a furfural-degrading bacterium *Bacillus cereus* sp. strain DS1. Curr. Microbiol 70, 199–205. https://doi.org/10.1007/s00284-014-0702-9.

Zhou, Y., Wei, Y., Jiang, L., Jiao, X., Zhang, Y., 2023. Anaerobic phloroglucinol degradation by *Clostridium scatologenes*. Mbio e01099-23.