Clay chips and beads capture in situ barley root microbiota and facilitate in vitro long-term preservation of microbial strains

Mohamed R. Abdelfadil, Manar H. Taha, Mohamed El-Hadidi, Mervat A. Hamza, Hanan H. Youssef, Mohab Khalil, Ahmed R. Henawy, Rahma A. Nemr, Hend Elsayewy, Gylaine Vanissa, Tchisuisseu Tchakounte, Mohamed Abbas, Gehan H. Youssef, Katja Witzel, Mohamed Essam Shawky, Mohamed Fayez, Steffen Kolb, Nabil A. Hegazi, Silke Ruppel

1 Thaer-Institute, Faculty of Life Sciences, Humboldt University of Berlin, 10115 Berlin, Germany
2 Department of Microbiology, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt
3 Bioinformatics Group, Center of Informatics Sciences (CIS), Nile University, 12677 Giza, Egypt
4 Department of Plant Microbe Systems, Leibniz Institute of Vegetable and Ornamental Crops, 14979 Großbeeren, Germany
5 Department of Microbiology, Faculty of Agriculture and Natural Resources, Aswan University, 81528 Aswan, Egypt
6 Department of Soil Chemistry and Physics, Soil, Water and Environment Research Institute, Agricultural Research Centre (ARC), 12112 Giza, Egypt
7 Department of Soil Science, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt
8 Faculty of Organic Agriculture, Heliopolis University, 11785 Cairo, Egypt
9 RA Landscape Functioning, Leibniz Centre for Agricultural Landscape Research (ZALF), Eberswalder Str. 84, D-15374 Müncheberg, Germany

*Corresponding author: Department of Plant Microbe Systems, Leibniz Institute of Vegetable and Ornamental Crops, Großbeeren 14979, Germany. E-mail: abdelfadil@igzev.de

One sentence summary: This study evaluates the ability of clay chips and beads as a practical and cheap methodology to capture the microbiota from barley roots and preserve pure isolates up to 11 months.

Editor: Angela Sessitsch

Abstract

Capturing the diverse microbiota from healthy and/or stress resilient plants for further preservation and transfer to unproductive and pathogen overloaded soils, might be a tool to restore disturbed plant–microbe interactions. Here, we introduce Aswan Pink Clay as a low-cost technology for capturing and storing the living root microbiota. Clay chips were incorporated into the growth milieu of barley plants and developed under gnotobiotic conditions, to capture and host the rhizospheric microbiota. Afterward, it was tested by both a culture-independent (16S rRNA gene metabarcoding) and -dependent approach. Both methods revealed no significant differences between roots and adjacent clay chips in regard total abundance and structure of the present microbiota. Clay shaped as beads adequately supported the long-term preservation of viable pure isolates of typical rhizospheric microbes, i.e. Bacillus circulans, Klebsiella oxytoca, Sinorhizobium meliloti, and Saccharomyces sp., up to 11 months stored at −20°C, 4°C, and ambient temperature. The used clay chips and beads have the capacity to capture the root microbiota and to long-term preserve pure isolates. Hence, the developed approach is qualified to build on it a comprehensive strategy to transfer and store complex and living environmental microbiota of rhizosphere toward biotechnological application in sustainable plant production and environmental rehabilitation.

Keywords: clay beads, clay chips, long-term microbial preservation, microbiota capturing, microbiota transfer, plant microbiota

Introduction

Microbiota play an essential role in many ecosystem functions, e.g. enhancing the stability, resilience, and well-being of their hosts; plants, animals, and humans (Trivedi et al. 2020, Joseph and Curtis 2021). Management of these microbiota can offer an opportunity to improve plant resilience against biotic and abiotic stresses, including ecological restoration via approaches such as synthetic microbial community (SynCom) and microbiome engineering (Castrillo et al. 2017, Valliere et al. 2020, Ke et al. 2021). For instance, the transplantation of tomato rhizosphere microbiota from a resistant variety to a pathogen susceptible variety successfully suppressed the soil-borne pathogen Ralstonia solanacearum (Kwak et al. 2018). However, transfer and recruitment of the desirable microbiome and SynCom to restore microbiome functions requires suitable delivery systems for living microbe assemblages (Compant et al. 2019). Such systems should be able not only to capture the diverse microbiota, but also to maintain them during their transfer into the target ecosystems (Sessitsch et al. 2019). In spite of their importance, there is only a limited number of studies available focusing on developing effective microbiota delivery strategies (Qiu et al. 2019). Methods such as magnetic beads and nano-filters are used to capture and trap microbial isolates; however, these methods are limited to their use in clinical diagnosis so far (Sande et al. 2020).

Clay beads have shown to be more efficient in capturing the most significant parts of bacterial diversity in water, i.e. groundwater, surface water, and wastewater, when compared with gravel particles and glass beads (Voisin et al. 2016). Also, clay beads succeeded to act as trapping matrices for capturing the representative bacterial communities developing in stormwater infiltration systems (SIS) to assess the microbial quality of aquifers (Mermillod-Blondin et al. 2019). Such approaches inspired us to
use clay as a natural matrix to capture and host the plant root-associated microbiota. A clay, known as ‘Pink Kaolinite Clay’, is tentatively connected to kaolinitic sediments (Rodziewicz 1992).

Most microbial product delivery methods have been developed as liquid formulations for soil applications, foliar sprays, seed and pellet coatings, or granules and powders that meet challenges such as limited shelf life, and depend on farmers’ storage capacity and handling skills (Sessitsch et al. 2019). In fact, the effective microbial delivery strategy should support the long-term survival of microbes and persistence in the environment to prevent the rapid decline of introduced inoculants (Qiu et al. 2019, Batista and Singh 2021). Moreover, different studies refer to the importance to preserve the microbial community’s diverse and beneficial microbiota from undergoing changes (Bello et al. 2018, West et al. 2019).

We aimed to develop a practical and low-cost methodology for comprehensively capturing and hosting the root microbiota. The Egyptian local type of clay used for pottery making, as an inexpensive and available natural material, is presented as an efficient trapping material, in the form of clay chips. To prove if clay chips, together mixed with quartz sand used as plant growth substrate, are able to capture the root-inhabiting microbiota, the tested barley roots with the adjacent clay chips. The second part of the study tested the ability of clay beads to preserve and long-term maintain microbial pure cultures. Such efficiency and handling skills (Sessitsch et al. 2019, Jackson 1958, Richards 1965, Table 1).

**Materials and methods**

**General structure and overview of the experiments**

The workflow in Fig. 1 illustrates the main experimental setup of the two experiments. In the first part of the study, plants were grown in glass tubes to test the capability of clay chips, as a growth milieu, to capture the microbiota associated with developed barley roots (Fig. 1A). Culture-independent (MiSeq 16S rRNA sequencing) and culture-dependent (CFU counts and MALDI-TOF) methods were used to compare the microbiota load and structure of barley roots with the adjacent clay chips. The second part of the study (Fig. 1B) aimed to test the efficiency of clay beads to preserve and long-term maintain microbial pure cultures. Such efficiency was assessed at three different storage temperatures (freezing at −20°C, cooling at 4°C, and ambient room temperature)

**Clay origin and physicochemical composition**

Samples of Aswan Pink/red Kaolinitic Clays pastes were obtained from a local pottery handcraft at Old Cairo, Egypt. This type of clay, known as ‘Pink Kaolinite Clay’ was historically, and since the Pharos and Greco-Roman time, used in the manufactures of different kinds of ceramic artifacts of table wares and vessels forms. It derives from the Modern Aswan region, which is synonymous with the ancient cities of Syene and Elephantine (Rembart and Betina 2021). Such ‘Pink Clay’ (Rodziewicz 1992) is the term used in literature for this type of Egyptian clay tentatively connected to kaolinitic sediments. The Aswan Pink Clay sediments/quarries, are supposed to derive from the decomposition of pinkish feldspar minerals found in the local rose granite, available abundantly in the entire broader area (Soliman 1985). The physico-chemical profiles of the tested ‘Aswan Pink Clay’ reveal its suitability as good quality and environment-friendly raw material. This specifically includes: clay size fractions, low-order kaolinite, and illite, absence of I/S minerals, low fluxing agents such as alkali oxides (Na2O and K2O) and alkaline earth oxides (CaO and MgO), low S and Cl contents, and low contents of toxic elements (As, Cd, Hg, and Pb) (Baoumy and Ismael 2014). This resulted in the extensive use of such clays along the Egyptian history in manufacturing table wares and vessels for safe human consumption, a strong argument and justification for its use for housing microorganisms as well.

The chemical analysis of major and trace elements of the tested clays was carried out using X-ray fluorescence and performed by the Central laboratories of the Egyptian Mineral Resources Authority (EMRA). Giza, Egypt. Further, physico-chemical properties, e.g. saturation point (SP), porosity, water holding capacity (WHC), CaCO3, pH, EC, and soluble cations and anions, were also carried out by the Soils, Water, and Environment Research Institute, Agricultural Research Center, ARC, Giza, Egypt, using standardized methods (Jackson 1958, Richards 1965, Table 1).

**Manual production of clay chips and clay beads**

The freshly prepared clay paste was used for the production of both clay chips and clay beads. Manually, the clay paste was shaped into beads of 5–8 mm diameter. On the other hand, a pastry wooden roller was used to prepare the fresh flattened sheets of clay paste (1–2 mm thick); then with the help of a sharp cutter, chips of ca. 4–6 mm × 4–6 mm were prepared. Both clay beads and chips were subjected to hard-firing at 800–900°C in an electrical furnace to increase their hardness and decrease shrink–swell capacity in aqueous solutions. Before use, the prepared clay chips and clay beads were packed into metal boxes and were dry heat sterilized in a volcano thermal heater at 300°C for 3 h.

**Clay chips as substrate to capture barley root microbiota under gnotobiotic conditions**

As growth substrate, clay chips and coarse sand particles/grains (sieved to 2–4 mm diameter and carefully washed several times with distilled water) were used. Aliquots of 30 g of a mixture (1:1, v/v) of clay chips and sand grains were filled into glass tubes (diameter 3 cm, height of 21 cm). Each tube was supplemented with 22 ml of semi-solid solution previously prepared from autoclaved barley-straw/distilled water infusion (0.1 g straw + 1.7 g agar L⁻¹ distilled water), and then covered with tubes bigger in size (4 cm diameter and 11 cm length). The prepared growth tubes were autoclaved, then thoroughly mixed by vortex mixer prior transfer of barley seedlings. The system was prepared and adjusted according to Youssif et al. (2004). Barley seeds (Hordeum vulgare L., Giza 127) were obtained from the Field Crops Research Institute, Agricultural Research Center (ARC), Giza. Healthy and intact seeds were germinated at room temperature (> 20–25°C) on water agar plates for 3 days. A total of three healthy seedlings were planted into the clay/sand mixture of each culture tube. A total of three sets, each of nine tubes, were prepared representing three biological repli-
Figure 1. Workflow of the experiments: (A) capturing bacterial community on clay chips and (B) long-term preservation of different microbial pure strains (*Bacillus circulans*, *Klebsiella oxytoca*, *Sinorhizobium melloti*, and *Saccharomyces* sp.) on clay beads.

cates. The growth tubes were kept in an environmentally controlled growth chamber having a day/night cycle of 8 h and 16 h, respectively, with a temperature of 20–22°C for 45 days.

A total of three tubes were randomly selected from each of the three sets of tested plant growth tubes. Contents of each of the tubes were aseptically sorted to obtain two composite samples representing roots and clay chips. To prepare the original suspension, the entire intact roots (ca. 0.1 g), and clay chips (ca. 40 g) were separately suspended into 20 ml saline solution, half strength basal salts of CCM, and shaken for 1 h at 120 rpm. Thereafter, the prepared original suspensions were further used for culture-dependent and culture-independent analyses.
Culturedependent bacterialcommunity characterization of barley root and clay chips

Original root and clay chips suspensions were further serial diluted (1:10) in the saline solution (half strength basal salts of CCM). Aliquots of 200 μl from suitable dilutions (10⁻⁴–10⁻⁷) were spread onto agar plates of tested culture media; R2A and plant (barley)-based culture medium (PM) in three replicates. CFUs developed on agar plates (Fig. 1A). We used bound water, which is the semi-solid water agar content, i.e. found between fresh and dry weights of root and clay chips. We used it as unit to compare the settlement of microorganisms of surface area. The simplified dry weights of root and clay chips. We used it as unit to compare the settlement of microorganisms of surface area.

MALDI-TOF MS analysis and protein profiling of bacterial isolates

The MALDI-TOF MS via MALDI Biotyper platform on intact cells was used for the construction of the phylproteomics of the secured bacterial isolates as described previously by Nembr et al. (2020). A total of 417 bacterial colonies, representing all emerged morphotypes of CFUs of each milieu was picked (Table S1, Supporting Information). They were inoculated into the respective agar plates, originating from root and clay chips and grown on two different culture media, PM and R2A (each in three replicates, 12 samples in total), were harvested and carefully washed using 7 ml of buffer solution (0.05 M NaCl). Then, cells were collected by centrifugation at 10,000 rpm for 15 min. DNA was extracted from the resulting pellets using QIAGEN DNeasy Plant Mini Kit (Qiagen).
Inc., Hilden, Germany) according to the manufacturer’s instructions.

For culture-independent measurements, total DNA was extracted from the original suspensions prepared for roots and adjacent clay chips (in three replicates each, six samples in total). DNA quality was assessed using NanoPhotometer (NanoPhotometer NP80 Touch, Implen GmbH, Munich, Germany). A total of 18 DNA samples was subjected to paired-end read Illumina MiSeq platform targeting the V4 region of the 16S rRNA gene using the 515f/806r primer set by ATLAS Biolabs GmbH, Berlin, Germany.

Amplicon sequence data analysis

Raw sequences from the bacterial 16S rRNA gene were processed using a script by a combination of tools including USEARCHv11.0.667 (Edgar 2010), and VSEARCH v2.15.2 (Rognes et al. 2016). The paired-end 16S reads of each sample were merged into a single sequence (2,166,570 merged sequences) using USEARCH (Edgar 2010). Subsequently, quality of merged sequences was filtered based on maximum expected error (maxee = 1) and sequences with maxee values higher than 1 discarded by VSEARCH (Rognes et al. 2016). In this step, 61,118 sequences were discarded and 2,105,452 quality-filtered sequences kept. Then, sequences were dereplicated using VSEARCH (Rognes et al. 2016) and assigned as amplicon sequence variants (ASVs) via the denoising method as well as removed chimeric sequences using USEARCH (Edgar 2010). This yielded 130 ASVs. All sequences were clustered at 99% similarity when mapping the merged sequences to ASVs for generating the count table (1,952,148 of 2,105,452 (92.72%)). This clustering step was performed with VSEARCH (Rognes et al. 2016). The ASVs were taxonomically classified using USEARCH with RDP classifier using RDP database (Edgar2010, Cole et al. 2016). The rarefaction curve was generated for all samples to demonstrate the sequencing depth of each sample (Figure S1, Supporting Information). Alpha diversity estimates were computed for observed ASVs and Shannon diversity. Adonis statistical test was applied to detect significant differences among the microbiota based on UniFrac distances.

Statistical analysis

All statistical analyses were performed with R v.4.1.1 (https://www.r-project.org/) and R-studio (https://www.rstudio.com/). The downstream analyses of the four generated files, i.e. ASVs count table, taxonomy table, phylogenetic tree, and metadata sheet, were carried out using phyloseq package (McMurdie and Holmes 2013).

Results

Growth of barley seedlings reported in the assembled gnotobiotic plant culture tubes

The physico-chemical properties of the used natural Aswan Pink Clay (Table 1) revealed its suitability as good quality and environment-friendly natural raw material. As a result, barley seedlings exhibited normal growth and differentiation of healthy roots and shoots (Figure S2, Supporting Information). As well, it appeared that the natural nutrients provided through the diluted infusion of barley straw further supported such normal growth.

Microbiota composition-comparison between barley roots and adjacent clay chips

The Adonis test based on Unifrac distance was used to investigate variance among the whole existing microbiota of barley roots and adjacent clay chips based on detected ASVs (Table 2). Across all samples, no significant differences were reported for overall microbial compositions between clay chips and barley roots (Adonis test, P-values > 0.05). In both analyses of total microbiota and the cultivable part of the community, no significant differences were reported between clay chips and roots, in both Observed and Shannon diversity index (Wilcoxon test, P-values > 0.05, Fig. 2). Microbiota composition on genus level is shown in (Figure S3, Supporting Information). The highest abundance of Pseudomonas and Sphingomonas was reported in all tested samples, both of cultured communities and total DNA analysis of barley roots and clay chips.
Table 2. Adonis test between ASVs observed of clay chips and barley roots microbiota, analyzed culture-independently from root (Root_M) and clay chips (Clay_M) or cultured on plant based medium (Root_PM, Clay_PM) or cultured on R2A medium (Root_R2A, Clay_R2A) based on UniFrac distances (Lozupone et al. 2011).

| Column_name   | Comparison_between                                                                 | Sample_counts | P-values |
|---------------|-------------------------------------------------------------------------------------|---------------|----------|
| Root vs. Clay | Root | Clay | 9 | 9 | 0.753 |
| Milieu        | Root_M | Clay_M | PM | R2A | 6 | 6 | 6 | 0.229 |
| All samples   | Root_M | Clay_M | Root_PM | Clay_PM | Clay_R2A | Root_R2A | 3 | 3 | 3 | 3 | 3 | 3 | 0.924 |

Figure 2. Alpha diversity indices to compare microbiota structure of barley roots and adjacent clay chips, using culture-independent analysis (Clay and Root), and cultured microbiota on R2A and plant-only-based (PM) culture media (Clay_PM, Root_PM, and Clay_R2A, Root_R2A). Alpha diversity boxplots were determined using observed ASVs and Shannon diversity index, using Amplicon 16S rRNA MiSeq sequencing. P-values between the different groups were shown using the Wilcoxon test.

Culture-dependent quantification of microbiota associated with barley roots and adjacent clay chips

Numbers of CFUs were quantified on two different culture media, the plant (barley)-teabags culture media and the synthetic R2A culture media. This is to evaluate if the same bacterial counts reported for barley roots could also be recovered from adjacent clay chips. Additionally, we wanted to test the efficiency of in vitro rhizobacteria cultivation when using a culture medium based on plant materials providing plant-adapted nutrient spectra (Sarhan et al. 2016) compared to a synthetic culture medium (R2A). No significant differences in CFU counts were observed between the root and adjacent clay chips when using the plant-only-based culture medium. This was not the case when using the synthetic R2A culture medium, where the differences were significant (Figure S4, Supporting Information).

Protein profiling of bacterial isolates associated with barley roots and clay chips on both culture media

The protein profile data from bacterial isolates were used to assess the differences between the cultured microbiota of barley roots and adjacent clay chips. The presented dendrogram (Figure S5, Supporting Information) has been constructed from the weighted matrix of all isolates proteotypes recovered from CFUs grown on plant-only-based (PM) and R2A culture media. These CFUs originated from the root and adjacent clay chips. Interestingly, the hierarchical clustering revealed that clay chips remarkably clustered together with plant roots. And, the two distinctive clusters of protein profiles indicated that the two culture media support growth of different bacterial communities (Figure S5, Supporting Information).

Assessment of clay beads for long-term microbial preservation of tested pure isolates

For achieving the potential aim to capture, preserve, and transfer microbial communities using clay material, in the first trial we checked three pure isolates of different bacterial families (B. circulans, K. oxytoca, and S. meliloti), and one yeast isolate (Saccharomyces sp.) for their long-term survival on clay beads stored on three different temperatures (−20°C, 4°C, and ambient). In general, all the tested microbes successfully survived at the three different storage temperatures in clay beads for more than 11 months. According to ANOVA analysis of CFU counts of B. circulans, there were no significant differences attributed either to the independent effect of storage temperature (P = 0.812) or time (P = 0.451), nor to the interaction between them (P = 0.980). The initial mean viable counts of the tested B. circulans were log 5.92 ± 0.76 CFU bead⁻¹. After 42 weeks of storage, still nearly identical numbers were recovered, log 5.56 ± 0.63, 5.78 ± 0.89, and 5.66 ± 0.71 at −20°C, 4°C, and ambient temperature, respectively (Fig. 3A).
Figure 3. Viable cell counts (CFUs) of the tested pure isolates of microorganisms, which were preserved on clay beads and kept at different storage temperatures (−20°C, 4°C, and ambient temperatures): (A) B. circulans, K. oxytoca, and Saccharomyces sp. as developed on standard CCM and plant-only-based (PM) culture media. Different letters indicate significant differences based on Tukey’s Honestly Significant Differences (HSD), $P < 0.05$, $n = 8$. (B) Sinorhizobium meliloti developed on agar plates of YEM and plant-only-based culture media. Student’s $t$-test was used in comparison and levels of significance are ns: $P > 0.05$, $^*P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$. The far-right panels show examples of CFUs morphologies developed.

As to K. oxytoca, and throughout 11 months of preservation, the viability was comparable at storage temperatures of −20°C and 4°C. According to ANOVA analysis and HSD test, there were no significant differences between initial viable counts (log 7.39 ± 0.26 bead$^{-1}$) and those reported after 42 weeks of storage (log 7.04 ± 0.95 and 6.95 ± 0.42 bead$^{-1}$, at −20°C and 4°C, respectively). For ambient room storage, the CFUs counts significantly decreased ($P < 0.05$) over time, down to log 4.79 ± 0.64 bead$^{-1}$ after 42 weeks (Fig. 3A).

The tested isolate of Saccharomyces sp. successfully maintained survivability after 42 weeks of preservation (Fig. 3A). At the three tested storage conditions, no significant reduction in viable counts was reported at −20°C and 4°C, compared with the initial counts of 6.72 ± 0.21 bead$^{-1}$. Whereas, at the ambient room temperature, the clay beads maintained Saccharomyces sp. counts comparable to the initials until 22 weeks of storage, with slight but significant decreases after 42 weeks (log 6.35 ± 0.12 bead$^{-1}$).
In the second trial, clay beads were efficient as well in the long-term preservation of *S. meliloti*. Irrespective of storage temperature and tested culture media, the decrease in the number of viable cell counts did not exceed a single log after 47 weeks of storage (Fig. 3B).

**Discussion**

Exploiting and manipulating the plant microbiome is an opportunity to improve plant growth and act against current ecosystem degradation (Toju et al. 2018). Although microbiome transfer has revealed the potential to restore the functionality of degraded environments, it faces challenges in terms of storage, handling, and delivery systems (Tosi et al. 2020). We proved the efficacy of clay chips to capture and host the living microbiota of barley roots. We also demonstrated the usability of clay for long-term preservation of rhizospheric bacterial and fungal pure strains at three different storage temperatures and even under ambient conditions.

The interaction of clay chips with barley roots during growth in a gnotobiotic plant growth system was successful in capturing and trapping most of microbiota associated with barley roots (Fig. 2). Correspondingly, Voisin et al. (2016) and Mermillod-Blondin et al. (2019) demonstrated that clay beads supported biofilm development, and were able to capture bacterial communities in different aqueous environments. This capability of clay chips is likely due to the roughness and porosity of their surface that supports microbial adhesion to the porous structure, which concurred with a number of reports (Yakub and Soboyejo 2012, Al-Amshawee et al. 2021). Tsoka et al. (2021) also revealed that pottery-shard (PS) of ceramic is capable of adhering lactic acid bacteria in its porous structure. Additionally, a number of studies showed the suitability of kaolinite for adhesion and biofilm formation of different types of microbes (Wu et al. 2014, Huang et al. 2015). An explanation for the ability of clay chips to capture barley root microbes is their naturality that allows intimate interaction with growing plant roots, thus served as an extended habitat for the dwelling rhizobacteria. Further, the heterogeneity of pores in the clay chips support the accumulation of nutrients and water, while at the same time maintain an airflow that well simulates the environmental conditions within the plant–soil system. In the present study and by employing culture-dependent and culture-independent techniques, we reported the indistinguishable communities of microbial genera and species, that allow them to persist for years. This has been demonstrated by Shoemaker et al. (2021) who reported that 21 bacterial taxa were able to survive for 1000 days in a closed system with zero resources. This is probably how microbes persisted on clay beads for such a long period of time in our study.

If the nearly intact bacterial community, which we captured from the barley rhizosphere under gnotobiotic conditions, will also survive on these clay chips for a long time has to be proven in the next step. Furthermore, following experiments will answer the question if these captured microorganisms can be transferred to a new donor plant by incorporating these clay chips into the rhizosphere.

**Conclusions**

Clay chips have the capacity to capture, host, and memorize microbiota. Such nature-inspired technology opens up new avenues for further progress in manipulating and delivery of environmental microbiomes toward soil rehabilitation. In addition, microbial preservation on clay beads is a low-cost, efficient methodology even suitable for less-equipped laboratories, and has the potential to easily transport and preserve microbes at various temperatures for several months, which in the present study was proven for microbial pure cultures. If such a long-term preservation will also be possible for complex microbial communities has to be attested in subsequent experiments.

**Supplementary data**

Supplementary data is available at FEMSEC online.

**Data availability statement**

Raw sequence data were deposited into the NCBI Sequence Read Archive (SRA) under BioProject accession PRJNA816545.

**Authors’ contributions**

Conceptualization: N.A.H., S.R., and M.R.A. Validation: M.R.A., M.A.H., H.H.Y., and M.A. M.E.S. and G.H.Y. supervised clay material analysis. M.E., M.H.T., and M.R.A. carried out the bioinformatics analysis. K.W. supervised the MALDI-TOF experiments. M.K., A.R.H., R.A.N., H.E., and G.V. provided the technical support. Writing original draft preparation: M.R.A., M.F., N.A.H., and S.R. Writing and editing: M.R.A., S.R., N.A.H., M.F., S.K., and M.A. All authors have read and agreed to the published version of the manuscript.

**Acknowledgments**

The authors are grateful to Sascha Patz for his bioinformatics training during the summer school ‘IGZ-ESRU, 2019’. Thanks are also extended to Kerstin Fischer and Mandy Heinze (IGZ) for excellent technical support. With gratitude, we acknowledge the lab support of Mohamed S. Sarhan, Mohamed Y. Saleh, Elhussein...
F. Mourad, Mennatuliah Abdou, Bishoy Sameh, Essam Adel, Saif Khodary, Abdul-Karim Noah, and Saad M. Abdelwakeel.

**Funding**

The work was supported by Alexander von Humboldt Foundation (AvH) through equipment subsidy and financial support, and the German Academic Exchange Service (DAAD) for funding the Summer School ‘IGZ-ESRU, 2019’. We acknowledge the financial support of Yousef Jameel Academic Program at the Humboldt-University of Berlin, Germany.

**Conflict of interest statement.** None declared.

**References**

Al-Amshawee S, Yunus MYB, Lynam JG et al. Roughness and wettability of biofilm carriers: a systematic review. *Environ Technol Innov* 2021;**21**:101233.

Baioomy HM, Ismael IS. Composition, origin and industrial suitability of the Aswan ball clays, Egypt. *Appl Clay Sci* 2014;**102**:202–12.

Batista BD, Singh BK. Realities and hopes in the application of microbial tools in agriculture. *Microb Biotechnol* 2021;**14**:1258–68.

Bello MGD, Knight R, Gilbert JA et al. Preserving microbial diversity. *Science* 2018;**362**:33–4.

Castrillo G, Teixeira FPJ, Paredes SH et al. Root microbiota drive direct integration of phosphate and immunity. *Nature* 2017;**543**:518–8.

Clark CM, Costa MS, Sanchez LM et al. Coupling MALDI-TOF mass spectrometry protein and specialized metabolite analyses to rapidly discriminate bacterial function. *Proc Natl Acad Sci* 2018;**115**:4981–6.

Cole JR, Wang Q, Fish JA et al. Ribosomal database project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res* 2014;**42**:D633–42.

Compart S, Samad A, Faist H et al. A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. *J Adv Res* 2019;**19**:29–37.

Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010;**26**:2460–1.

Hegazi N, Hamzza MA, Osman A et al. Modified combined carbon N-deficient medium for isolation, enumeration and biomass production of diazotrophs. In: *Nitrogen Fixation with Non-legumes*. Berlin: Springer, 1998, 247–53.

Huang Q, Wu H, Cai P et al. Atomic force microscopy measurements of bacterial adhesion and biofilm formation onto clay-sized particles. *Sci Rep* 2015;**5**:16857.

Jackson M. Soil Chemical Analysis. Vol. 498. Englewood Cliffs: Prentice Hall Inc, 1958, 183–204.

Joseph S, Curtis MA. Microbial transitions from health to disease. *Periodontal* 2000 2021;**86**:201–9.

Katoh K, Misawa K, Kuma K et al. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* 2002;**30**:3059–66.

Ke J, Wang B, Yashikuni Y. Microbiome engineering: synthetic biology of plant-associated microbiomes in sustainable agriculture. *Trends Biotechnol* 2021;**39**:244–61.

Kolari M, Schmidt U, Kuismanen E et al. Firm but slippery attachment of *Deinococcus geothermalis*. *J Bacteriol* 2002;**184**:2473–80.

Krumnow AA, Sorokulova IB, Olsen E et al. Preservation of bacteria in natural polymers. *J Microbiol Methods* 2009;**78**:189–94.

Kwak MJ, Kong HG, Choi K et al. Rhizosphere microbiome structure alters to enable wilt resistance in tomato. *Nat Biotechnol* 2018;**36**:1100–09. DOI: 10.1038/sbtt423.

Lozupone C, Lladser ME, Knights D et al. UniFrac: an effective distance metric for microbial community comparison. *ISME J* 2011;**5**:169–72.

McMurdie PJ, Holmes S. Phyloseq: an r package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* 2013;**8**:e61217.

Mermillod-Blondin F, Voisin J, Marjolet L et al. Clay beads as artificial trapping matrices for monitoring bacterial distribution among urban stormwater infiltration systems and their connected aquifers. *Environ Monit Assess* 2019;**191**:58.

Nemr RA, Khalil M, Sarhan MS et al. “In situ similis” culturing of plant microbiota: a novel simulated environmental method based on plant leaf blades as nutritional pads. *Front Microbiol* 2020;**11**:454.

Qiu Z, Egidi E, Liu H et al. New frontiers in agriculture productivity: optimised microbial inoculants and in situ microbiome engineering. *Biotechnol Adv* 2019;**37**:107371.

Reasoner DJ, Geldrich EE. A new medium for the enumeration and subculture of bacteria from potable water. *Appl Environ Microbiol* 1985;**49**:1–7.

Rembart L, Betina L. On the tracks of Aswan pink clay. New studies on the local clay deposits in the region of Aswan/Upper Egypt. *Interdisc Archaeol* 2021;**XII**:37–44.

Richards L. Physical condition of water in soil. In: *Methods of Soil Analysis: Part 1 Physical and Mineralogical Properties, Including Statistics of Measurement and Sampling*. Vol. 9. Madison: American Society of Agronomy, 1965, 128–52.

Rodziewicz M. Field notes from Elephantine on the early Aswan pink clay pottery. In: *Cahiers De La Céramique Égyptienne, Ateliers De Potiers Et Productions Céramiques En Egypte*. Vol. 3. Cairo: Institut Français d’archéologie Orientale, 1992, 103–7.

Rognes T, Fouri T, Nichols B et al. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 2016;**4**:e2584.

Sande MG, Caykara T, Silva CJ et al. New solutions to capture and enrich bacteria from complex samples. *Med Microbiol Immunol* 2020;**209**:335–41.

Sarhan MS, Mourad EF, Hamza MA et al. Plant powder teabags: a novel and practical approach to resolve culturability and diversity of rhizobacteria. *Physiol Plant* 2016;**157**:403–13.

Sessitsch A, Pfaffenbichler N, Mitter B. Microbiome applications from lab to field: facing complexity. *Trends Plant Sci* 2019;**24**:194–8.

Shoemaker WR, Jones SE, Muscarella ME et al. Microbial population dynamics and evolutionary outcomes under extreme energy limitation. *Proc Natl Acad Sci* 2021;**118**:e2101691118.

Soliman. Some textural patterns and their hearing on the origin of the granitic rocks of the Aswan region, south Egypt. *J Univ Kuwait* 1985;**12**:299–308.

Somasegaran P, Hoben HJ. Quantifying the growth of rhizobia. In: *Handbook for Rhizobia*. Berlin: Springer, 1994, 47–57.

Taoka Y, Sakai K, Kinoshita H et al. Evaluation of rate of adhesion of *Lactobacillus nanumensis* strain GYP-74 to porous fine ceramics. *Proc Natl Acad Sci* 2021;**118**:e2101691118.

Toju H, Peay KG, Yamamichi M et al. Preservation of bacteria from potable water. *Front Microbiol* 2020;**11**:58.

Trivedi P, Leach JE, Tringe SG et al. Plant-microbiome interactions: from community assembly to plant health. *Nat Rev Microbiol* 2020;**18**:607–21.
Valliere JM, Wong WS, Nevill PG et al. Preparing for the worst: utilizing stress-tolerant soil microbial communities to aid ecological restoration in the anthropocene. *Ecol Sol Evid* 2020;1:e12027.

Voisin J, Cournoyer B, Mermillod-Blondin F. Assessment of artificial substrates for evaluating groundwater microbial quality. *Ecol Indic* 2016;71:577–86.

West AG, Waite DW, Deines P et al. The microbiome in threatened species conservation. *Biol Conserv* 2019;229:85–98.

Wu HY, Chen WL, Rong XM et al. Adhesion of Pseudomonas putida onto kaolinite at different growth phases. *Chem Geol* 2014;390:1–8.

Yakub I, Soboyejo WO. Adhesion of E. coli to silver- or copper-coated porous clay ceramic surfaces. *J Appl Phys* 2012;111:124324.

Youssef HH, Fayez M, Monib M et al. *Gluconacetobacter diazotrophicus*: a natural endophytic diazotroph of Nile Delta sugarcane capable of establishing an endophytic association with wheat. *Biol Fertil Soils* 2004;39:391–7.