BMC Research Notes

RESEARCH NOTE Open Access

Optimising RNA extraction for paddy bulk soil samples for metatranscriptome sequencing

Sumitra Sivaprakasam[1], Tan Yee Fan[1], Yukgehnaish Kumarasan[1], Erneeza Mohd Hata[2], Ganesan Vadamalai[2,3], Bent Petersen[1,4], Thomas Sicheritz-Pontén[1,4], Sivachandran Parimannan[1,4] and Heera Rajandas[1,4*]

Abstract

Objective Extraction of high-quality RNA is crucial for understanding the molecular dynamics of microbiomes in the growth and development of paddy plants. However, paddy soil poses challenges due to contaminants such as humic substances and its clayish nature, which lead to RNA adsorption and reduced yield. This study aimed to improve existing RNA extraction methods for bulk soil samples collected from a paddy field in Perak, Malaysia. We first evaluated different published protocols, selected the best based on RNA yield and quality, and further optimized it for highly pigmented soil samples. The resulting RNA was subjected to metatranscriptome sequencing, *de novo* assembly and annotation.

Results Upon evaluation, the RNA extraction protocol by Peng et al., 2018 (method B3) was optimized by incorporating 20% and 30% PEG-based precipitation to remove carry-over pigmentation. Comparative testing showed that 20% PEG produced the highest quality RNA, yielding pigment-free RNA (> 100 ng/µl, integrity > 7, and A260/A280 of 2.02 ± 0.02). Metatranscriptome sequencing and analysis with Trinity, BUSCO, and Kraken2 confirmed superior quality and higher bacterial read assignment for RNA extracted with 20% PEG, highlighting its effectiveness for downstream microbial transcriptomic applications.

Keywords RNA extraction, Paddy, Bulk soil, Metatranscriptome sequencing

Introduction

Microbial inoculants offer a promising solution for enhancing paddy growth, meeting rising demands, improving crop quality, and promoting sustainable agriculture [1, 2]. As rice fields harbour a vast number of microbial communities that drive key ecological processes and biological functions influencing soil fertility and enhancing productivity, a molecular-level understanding of the paddy soil microbiome is crucial [3]. This could be achieved through high-throughput sequencing which also paves the path to the discovery of novel molecular markers, regulatory sequences and paddy-associated microbiomes [4, 5].

Advancement in sequencing technology have driven meta-omics studies, including soil metagenomics and

*Correspondence:
Heera Rajandas
heera@aimst.edu.my
[1]Centre of Excellence for Omics-Driven Computational Biodiscovery (COMBio), AIMST University, Bedong, Kedah 08100, Malaysia
[2]Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor 43400, Malaysia
[3]Laboratory of Sustainable Agronomy and Crop Protection, Institute of Plantation Studies, Universiti Putra Malaysia, Serdang, Selangor 43400, Malaysia
[4]Center for Evolutionary Hologenomics, Globe Institute, University of Copenhagen, Copenhagen, Denmark

metatranscriptomics [6]. Extraction of high-quality, inhibitor-free RNA from soil samples remains challenging due to RNA's instability and presence of inhibitors like tannins and humic acids that complicates RNA recovery and can negatively impact sequencing [6, 7]. Unlike DNA, RNA is more prone to degradation and thus, there is a need to optimize existing protocols to not only improve the RNA yield but also to enhance the removal of inhibitory compounds.

Literature review shows limited studies on manual (phenol-chloroform-based) RNA extraction methods from paddy soil [8–13]. While kit-based methods are faster and have demonstrated the potential to yield high-quality RNA, the proprietary components limit optimization and up-scaling. Manual methods, in contrast are more cost-effective, making them especially suitable for large-scale or resource-limited studies. Hence, this study aims to compare and evaluate various total RNA extraction methods from inhibitor-rich paddy bulk soil for metatranscriptome sequencing. It also highlights major challenges encountered during RNA extraction and the strategies used to overcome them. The optimized method is manual, transparent, and adaptable, allowing for stepwise optimization as needed.

Method

Sample collection and processing

Bulk soil samples were collected during the ripening stage of paddy plants from a drained field in Kampung Gajah, Perak, Malaysia (4.1841° N, 100.9389° E). All necessary permissions for sampling were obtained from the respective paddy field owners through the local authority, Integrated Agriculture Development Area (IADA), Seberang Perak, Malaysia. Using a Dutch auger, soil was sampled at a 10 cm depth, and approximately 50 g of soil was transferred into a sterile 50 ml Falcon tube. Three volumes of RNAlater solution (Invitrogen, Thermo Fisher Scientific, US) were added to the soil samples, placed on dry ice and transported to the laboratory under the same conditions, where they were stored at -80 °C until further processing.

Total RNA extraction

Total RNA was initially extracted from paddy bulk soil using five different protocols, each performed with three independent replications. These protocols included Methods B1 [14], B2 [15], B3 [16], B4 (RNeasy PowerSoil Total RNA Kit by Qiagen, Germany), and B5 [17]. A summary of these protocols, adapted from the cited references is provided in Supplementary Table 1. Methods B1, B2, B3, and B5 involved phenol-chloroform extraction and were classified as manual extraction methods, while Method B4 utilized a commercial kit and was categorized as a kit-based method.

RNA quality and quantity analysis

The purity of the RNA was measured based on A260/A280 nm and A260/A230 nm absorbance ratios using a UV-Vis spectrophotometer (Implen NanoPhotometer), while the RNA yield and integrity were quantified using a Qubit 4 fluorometer and Qubit RNA HS Assay Kit (Thermo Scientific, USA). RNA integrity was assessed based on the presence of 23 S and 16 S bands on 1% agarose gel electrophoresis stained with ViSafe red gel stain (Vivantis, USA) using a 1 kb Vivantis ladder. Upon quantification, all the data are expressed as mean ± SD values unless otherwise stated. All statistical analysis was conducted using IBM SPSS Statistics 22.

Optimized method for total RNA extraction from paddy bulk soil

Upon evaluating five RNA extraction methods, Method B3 showed relatively better performance by showing distinct intact band on gel electrophoresis, indicating satisfactory RNA integrity. Therefore, several modifications were made to Method B3, detailed in Supplementary Data S1. For the precipitation step, the following combinations were tested; 2.5 volumes of pre-cooled absolute ethanol with 1/10 volume of 3 M sodium acetate [18, 19], 30% PEG (Polyethylene glycol 6000) with 5 M NaCl, and 20% PEG with 5 M NaCl [20, 21].

RNA sequencing

RNA extracts were treated with DNase I and 1 µl of SUPERase RNase Inhibitor (Thermo Scientific, USA) and purified using the RNA Clean & Concentrator-5 kit (Zymo Research) according to manufacturer's protocol. The cDNA libraries were then constructed using the NEBNext Ultra II RNA Library Prep kit for Illumina (NEB, USA) and sequenced on the Illumina NovaSeq 6000 platform (2 × 150 bp) by Nanjing Novogene Bio-Technology Co., Ltd.

Bioinformatic analysis

The Illumina paired-end reads were quality filtered again by trimming off low-quality bases using Trimmomatic v0.38, with a sliding window of 4:25 and a minimum sequence length of 80 bp [22]. The quality of the trimmed sequences was assessed using FastQC v0.11.9 through visual inspection [23]. Trinity (v2.13.2) was then used to perform *de novo* metatranscriptome assembly of all individual clean reads [24]. Assembly and contig quality analysis was performed using the built-in Trinity script to assess the Gene contig Nx statistics [25]. The assembled sequences were then analyzed with BUSCOv5.2.2 using the lineage dataset bacteria_odb10 dataset (prokaryota, 2020-03-06) to evaluate the genome completion by identifying the number of markers that mapped against the bacteria_odb10 database [26]. Following that, the

assembled sequences were also subjected to taxonomic classification using Kraken2 v2.0.8 (PlusPF database, 2023-03-14) to identify the proportion of reads classified as Bacteria at the domain level [27].

Results

Evaluation of RNA quality and quantity across existing extraction methods

The RNA extracted using previously published protocols was evaluated and deemed suitable for sequencing based on four quality criteria: RNA concentration ≥ 50 ng/µL, A260/A280 ratio of 1.8–2.0, A260/A230 ratio of 1.8–2.2, and RNA integrity number (RIN) of ≥ 6 [28–32]. Results indicated that all the tested methods did not result in satisfactory output (Table 1; Fig. 1).

The evaluated methods yielded low RNA concentrations, with some methods below the Qubit detection limit (≤ 4 ng) and failing to produce bands during gel electrophoresis [23]. Method B4 (RNeasy PowerSoil Total RNA Kit, Qiagen) gave the highest RNA concentration but showed inconsistent results across replicates. Manual extraction methods (B1, B2, B3, B5) produced highly pigmented RNA (brown to black), severely affecting purity, as indicated by absorbance readings [24, 25] (Fig. 2). Interestingly, despite method B3's RNA concentration being undetectable by Qubit, distinct ribosomal bands appeared on gel electrophoresis, suggesting contaminants including pigmentation may have interfered with quantification.

Further optimization for total RNA extraction from paddy bulk soil

Overall, RNA recovery was poor across all methods, with severe pigmentation observed in manual extractions. Method B3 showed clear rRNA bands but exhibited severe pigmentation, while Method B4 produced pigment-free RNA but with low yield and purity, despite using 2 g of starting material. Additionally, B4, a spin-column kit, incurred higher costs compared to traditional phenol-chloroform methods, making it less cost-effective for routine use. Therefore, Method B3 was selected for further optimization. Originally employing isopropanol for precipitation, Method B3 was subsequently tested with a combination of different precipitating reagents to identify the most effective approach for removing the carry-over pigmentation (Table 2; Fig. 3).

Replacing isopropanol with PEG-based precipitation in method B3 improved RNA recovery, yielding high-purity RNA without carry-over pigmentation (Table 2; Fig. 3). Both 30% and 20% PEG removed pigmentation, but 20% PEG yielded higher RIN values and concentration. RNA extracted using method mB3a and mB3b met the predefined quality criteria for sequencing and thus, was subsequently subjected to further purification and metatranscriptome sequencing.

Post-sequencing quality check and assembly statistics

Upon sequencing, the paired-end raw reads were pre-processed by removing the adapters, reads containing $N > 10\%$ (N represents the base cannot be determined) and reads containing low quality (Qscore < = 5) base. After pre-processing, 28,832,018 and 55,210,358 clean reads were obtained from the sequenced library of total RNA extracted using methods mB3a and mB3b, respectively. A quality assessment of the resulting assemblies was performed following *de novo* assembly with Trinity. The Nx gene statistics were computed using the longest isoform per gene, showing higher total transcripts, genes, and assembled bases for the modified method with 20% PEG (mB3b) compared to 30% PEG (mB3a). (Table 3).

A higher percentage of complete BUSCOs were identified for the assembled sequence obtained from modified methods mB3b, indicating superior assembly quality based on the percentage of sequences that mapped to the bacteria_odb10 dataset (Seppey et al., 2019) (Table 4). As expected, a higher duplication rate was detected, which can be attributed to the inclusion of all transcripts generated by Trinity in the analysis, rather than limiting it to unigenes (i.e., the longest isoform per gene) [33].

In addition, to get a better idea of the taxonomic compositions associated with the bacterial domain, the assembled sequences were also subjected to a fast taxonomic application using Kraken2 which quickly determines the number of reads assigned to the domain Bacteria [27]. The analysis indicated that a total of 36.31% and 38.20% of the assembled reads from methods mB3a and mB3b, respectively, were assigned to Bacteria, indicating slightly higher bacterial representation in mB3b.

Table 1 Average RNA concentration and purity from paddy bulk soil samples using five published methods

Extraction method	Concentration (ng/µl)	A260/A280	A260/A230
B1	N/A	1.64 ± 0.07	0.78 ± 0.02
B2	5.34 ± 0.14	1.33 ± 0.07	0.83 ± 0.01
B3	N/A	1.55 ± 0.08	0.99 ± 0.08
B4	30.6^{M}	1.70 ± 0.03	0.89 ± 0.45
B5	N/A	1.50 ± 0.05	0.77 ± 0.03

N/A indicates RNA concentration that is below the detection limit of the Qubit fluorometer. "M" corresponds to the RNA concentration obtained from a single replicate out of the three. RIN was not reported in the table as all methods had values below the detection limit of 4 ng

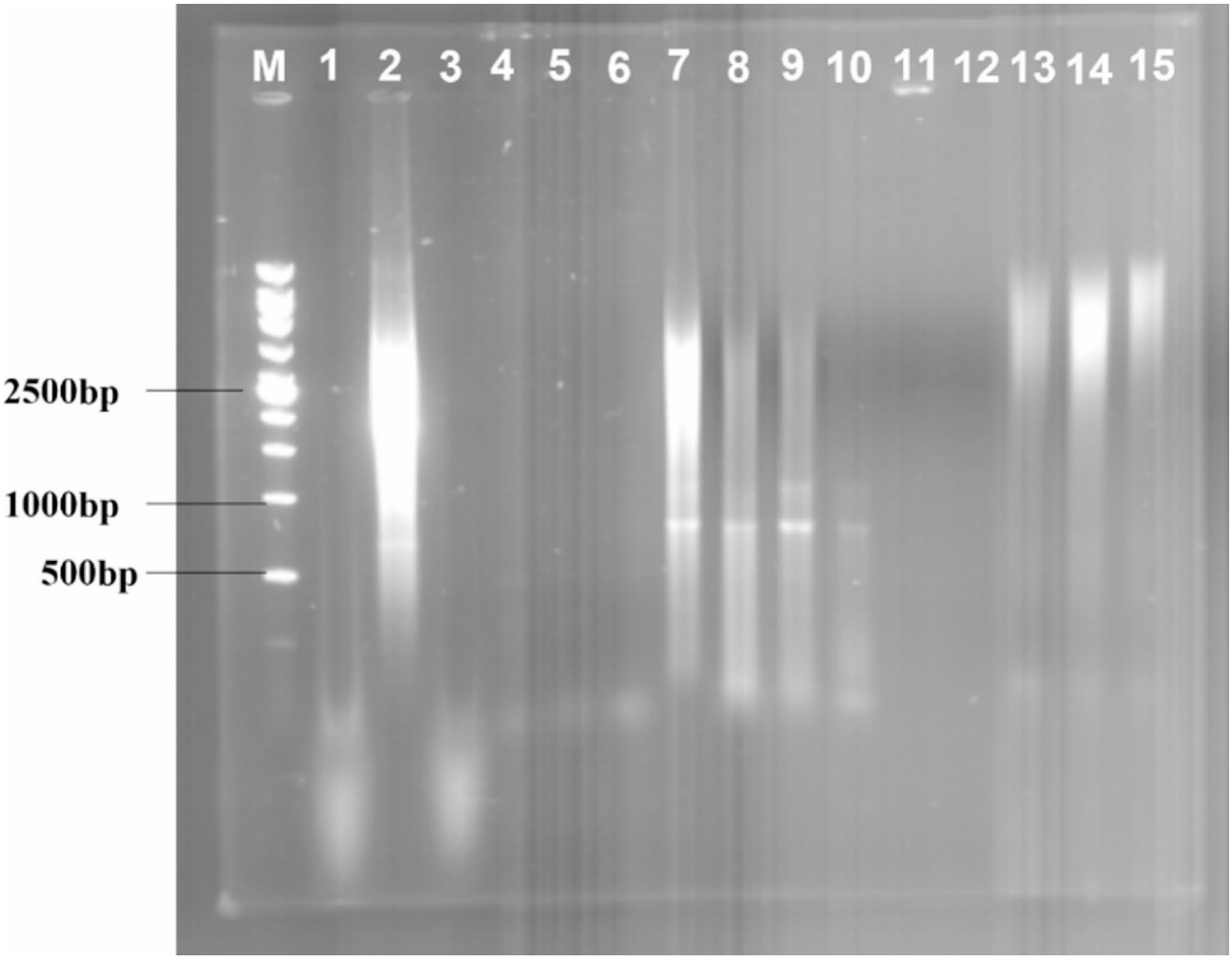

Fig. 1 Agarose gel electrophoresis of total RNA extracted from paddy bulk soil samples using published methods. M is VC 1 kb DNA ladder (Vivantis). Lanes 1–3 represent the triplicates of Method B1, lanes 4–6 represent method B2, lanes 7–9 represent method B3, lanes 10–12 represent method B4 and lanes 13–15 represent method B5. Full-length gel is presented in Supplementary Fig. 1

Discussion

This study evaluated existing RNA extraction methods to obtain high-quality RNA from paddy bulk soil for metatranscriptome sequencing. Paddy bulk soil was collected from a drained paddy field at a depth of 10 cm. The sample was clay-rich with high organic matter and humic substances, which contributed to the dark brown to black pigmentation observed during RNA extraction. After testing 5 different existing methods, method B3, a phenol-chloroform-based method, was chosen for optimization due to its consistent, intact rRNA band visibility in gel electrophoresis. A major challenge in RNA extraction from bulk soil samples was the low RNA yield and carry-over pigmentation leading to dark-coloured eluates that negatively impacted RNA recovery. Low RNA yields from soil are commonly reported, with only tens of nanograms to a few micrograms recovered per gram of soil [11, 34]. RNA extraction from soil is more challenging than from pure cultures due to strong RNA adsorption to soil particles, especially in clay-rich soils which are common in paddy fields. Additionally, humic substances, which are dark-coloured heterogeneous organic compounds often co-extract with RNA, interfering with enzymatic reactions and further complicating the recovery of high-quality RNA [25].

Dark pellets and eluates formed during RNA extraction from bulk soil samples (Fig. 2) indicated the presence of humic substances, known to interfere with RNA purity and downstream applications [11, 35–37]. To address this, several modifications were made to Method

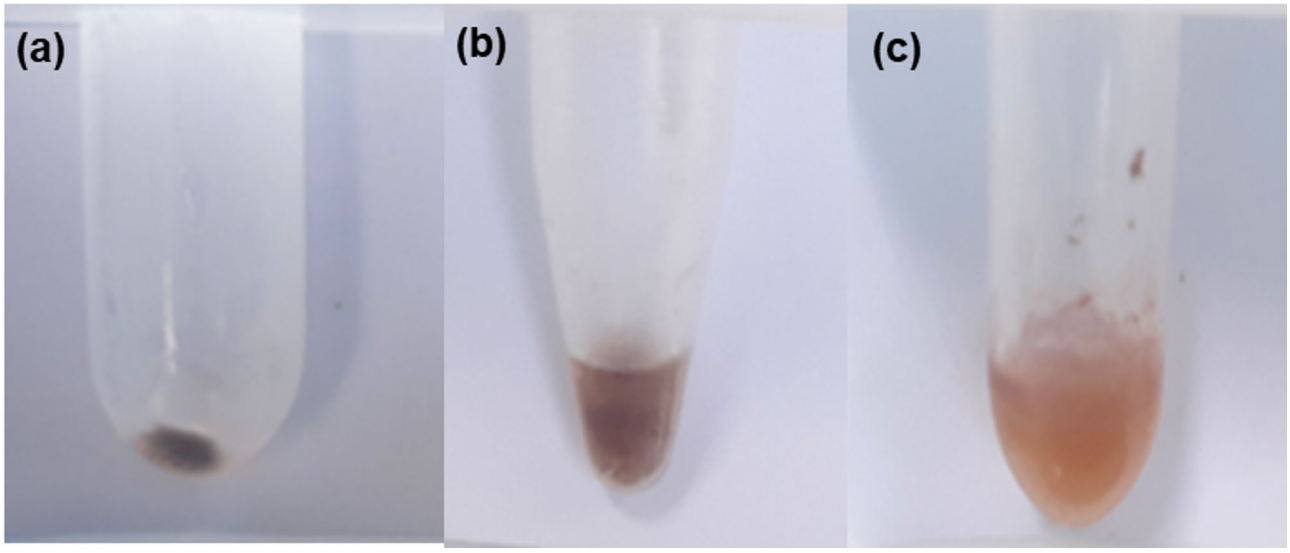

Fig. 2 Pigmented RNA extracted from soil samples from methods B2 and B5. (**a**) The pigmented pellet obtained from method B2 after the precipitation step. (**b**) Pigmented elute obtained from method B2 after eluting the pellet into 50 µl of TE buffer. (**c**) Pigmented and gel-like sediment in the elute obtained from method B5

Table 2 RNA purity and concentration using optimized method B3 with different precipitating agents

Precipitation method	Concentration (ng/µl)	A260/A280	A260/A230	RIN
mB3a (30% PEG + 5 M NaCl)	36.9 ± 1.5	1.84 ± 0.03	1.89 ± 0.18	6.0
mB3b (20% PEG + 5 M NaCl)	104.5 ± 0.5	2.02 ± 0.02	2.14 ± 0.04	7.55 ± 0.25
mB3c (Sodium acetate + Abs Ethanol).	N/A	1.70 ± 0.04	0.79 ± 0.03	N/A

The A260/A280 and A260/A230 absorbance ratios reflect the RNA purity. N/A indicates that the RNA concentration is below the detection limit of the Qubit fluorometer

B3. The addition of BME to the lysis buffer was essential for removal of polyphenolic compounds and inhibition of RNase activity that compromise the quality and yield of RNA [38–40]. Thorough physical lysis and the use of lysozyme were also incorporated to enhance the bacterial cell lysis. Samples were subsequently subjected to two rounds of Phenol: Chloroform: Isoamyl alcohol (P: C:I) treatment with higher ratio of phenol content to purify RNA from the contaminants [11]. An additional chloroform: isoamyl alcohol extraction step was performed to remove residual phenol, ensuring RNA of high purity [41]. Additionally, PEG-based precipitation was introduced to deal with residual pigments. Although both 20% and 30% PEG effectively removed pigmentation, RNA extracted with 20% PEG showed higher RIN values and overall better quality. Subsequent ethanol washing and column-based purification are still recommended to remove residual PEG and other inhibitors.

Due to financial constraints, sequencing was limited to one sample per method without biological replicates. Despite this limitation, comparative evaluation of sequencing outputs including assembled read counts, gene Nx statistics, BUSCO analysis, and taxonomic classification indicated that mB3b (20% PEG) outperformed mB3a (30% PEG). While these differences cannot be statistically validated, the results highlight mB3b's overall improved RNA quality, supported by gel electrophoresis profiles, higher RNA yield, and integrity. Based on these findings, mB3b was selected for large-scale RNA extraction from 32 bulk paddy soil samples, representing 80% of all collected samples. These samples were collected from multiple paddy plots in Kampung Gajah, Perak, across three growing seasons (March 2021–June 2022) and successfully sequenced on the Illumina NovaSeq 6000 platform, with data deposited in NCBI SRA (BioProject PRJNA770166) (Supplementary Table 2).

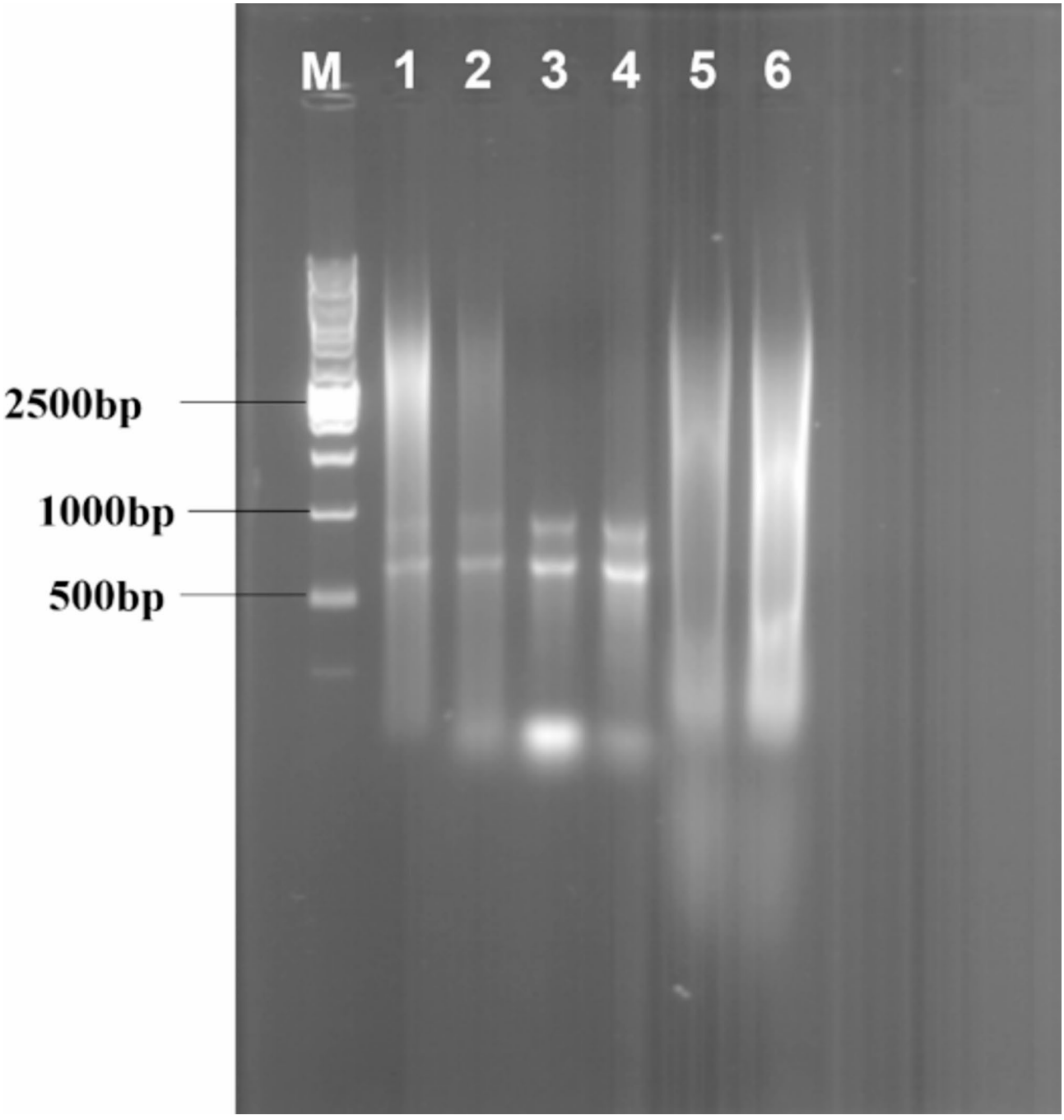

Fig. 3 Gel electrophoresis of total RNA extracts using optimized B3 methods with different precipitation reagents. Lanes 1–2 represent the duplicates of method mB3a (30% PEG and 5 M NaCl), lanes 3–4 represent method mB3b (20% PEG and 5 M NaCl), and lanes 5–6 represent method mB3c (Sodium acetate and absolute ethanol). Full-length gel is presented in Supplementary Fig. 2

Table 3 Summary of post-assembly statistics

Method	Total Trinity genes	Total Trinity transcripts	Total assembled bases
mB3b	616,740	691,902	225,083,288
mB3a	553,743	577,531	185,313,454

Table 4 BUSCO output using the lineage dataset bacteria_odb

Method	Complete (%)	Duplicated (%)	Fragmented (%)	Missing (%)
mB3b	33.9	21.0	27.4	38.7
mB3a	23.4	14.5	33.9	42.7

The recovered matches were classified as 'complete' if the query lengths fell within the expected length of the BUSCO group length. If they were found more than once then they were classified as 'duplicated'. Partially recovered matches were classified as 'fragmented' and those that passed the orthology test without any matches were classified as 'missing'

Limitations

This study is limited by its small sample size and its focus on paddy soil from one geographical region. Due to budget constraints, sequencing was performed on only one sample per method, preventing statistical validation of differences between methods. While method mB3b demonstrated better RNA quality and sequencing output, further studies with biological replicates are needed to confirm its reproducibility and applicability. Nonetheless, the successful sequencing of 32 paddy bulk soil samples using the optimized protocol (mB3b) highlights its efficiency for metatranscriptomic applications.

Abbreviations

PEG Polyethylene glycol 6000
RIN RNA integrity number
N/A Not available
P: C:I Phenol: Chloroform: Isoamylalcohol

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-025-07342-9.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Acknowledgements

This research was carried out as a part of the project "Deciphering Paddy Probiotics: A Precision Agricultural 'Moonshoot' for Rice Cultivation" funded by CREST, Malaysia. We would also like to sincerely thank IADA Seberang Perak and the farmers for allowing and assisting us with the sampling work at the paddy field; the Globe Institute, University of Copenhagen for providing us access to their computational infrastructure, and the curators of the biological samples in NCBI SRA where the samples examined were deposited, for their assistance.

Author contributions

All authors contributed to the study's conception and design. Material preparation and data collection were performed by T.Y.F., Y.K., E.M.H., G.V., B.P. and T.S.P. Analysis was performed by S.S. The main manuscript was written by S.S. and H.R. and all authors commented on previous versions of the manuscript. All authors reviewed the final manuscript.

Funding

This work was supported by the Collaborative Research in Engineering, Science and Technology (CREST), Malaysia (CREST/1/2021/T09C2-19/AIMST/01).

Data availability

Data is available at NCBI SRA data: PRJNA770166; mB3a (SRR20028842); mB3b (SRR25337253). All other related data not presented in the manuscript are made available in the supplementary documents.

Declarations

Ethics approval and consent to participate

The current study did not involve human subjects, human material, human data or animals. However, permission for soil sampling was obtained from the paddy field owners through the local authority, Integrated Agriculture Development Area (IADA), Seberang Perak, Malaysia.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 1 April 2025 / Accepted: 24 June 2025
Published online: 05 July 2025

References

1. Shahwar D, Mushtaq Z, Mushtaq H, Alqarawi AA, Park Y, Alshahrani TS, et al. Role of microbial inoculants as bio fertilizers for improving crop productivity: A review. Heliyon. 2023;9(6):e16134.
2. Doni F, Suhaimi NSM, Mispan MS, Fathurrahman F, Marzuki BM, Kusmoro J, et al. Microbial contributions for rice production: from conventional crop management to the use of 'omics' technologies. Int J Mol Sci. 2022;23(2):737.
3. Joos L, De Tender C. Soil under stress: the importance of soil life and how it is influenced by (micro)plastic pollution. Comput Struct Biotechnol J. 2022;20:1554–66.
4. Ahmar S, Gill RA, Jung KH, Faheem A, Qasim MU, Mubeen M, et al. Conventional and molecular techniques from simple breeding to speed breeding in crop plants: recent advances and future outlook. Int J Mol Sci. 2020;21(7):2590.
5. Trivedi P, Schenk PM, Wallenstein MD, Singh BK. Tiny microbes, big yields: enhancing food crop production with biological solutions. Microb Biotechnol. 2017;10(5):999–1003.
6. Lim NYN, Roco CA, Frostegård Å, Transparent. DNA/RNA Co-extraction Workflow Protocol Suitable for Inhibitor-Rich Environmental Samples That Focuses on Complete DNA Removal for Transcriptomic Analyses. Front Microbiol. 2016 [cited 2023 Apr 18];7. Available from: https://www.frontiersin.org/articles/https://doi.org/10.3389/fmicb.2016.01588
7. Yockteng R, Almeida AMR, Yee S, Andre T, Hill C, Specht CD. A method for extracting high-quality RNA from diverse plants for next-generation sequencing and gene expression analyses1. Appl Plant Sci. 2013;1(12):apps1300070.
8. Itoh H, Ishii S, Shiratori Y, Oshima K, Otsuka S, Hattori M, et al. Seasonal transition of active bacterial and archaeal communities in relation to water management in paddy soils. Microbes Environ. 2013;28(3):370–80.
9. Knauth S, Schmidt H, Tippkötter R. Comparison of commercial kits for the extraction of DNA from paddy soils. Lett Appl Microbiol. 2013;56(3):222–8.
10. Masuda Y, Itoh H, Shiratori Y, Senoo K. Metatranscriptomic insights into microbial consortia driving methane metabolism in paddy soils. Soil Sci Plant Nutr. 2018;64(4):455–64.
11. Wang Y, Hayatsu M, Fujii T. Extraction of bacterial RNA from soil: challenges and solutions. Microbes Environ. 2012;27(2):111–21.

12. Turner TR, Ramakrishnan K, Walshaw J, Heavens D, Alston M, Swarbreck D, et al. Comparative metatranscriptomics reveals Kingdom level changes in the rhizosphere Microbiome of plants. ISME J. 2013;7(12):2248–58.
13. Ito K, Kuramata M, Tanikawa H, Suda A, Yamaguchi N, Ishikawa S. Diversity and transcription of genes involved in respiratory As(V) reduction and As(III) methylation in Japanese paddy soils. BMC Microbiol. 2024;24(1):396.
14. Fang C, Xu T, Ye C, Huang L, Wang Q, Lin W. Method for RNA extraction and cDNA library construction from microbes in crop rhizosphere soil. World J Microbiol Biotechnol. 2014;30(2):783–9.
15. Qin H, Chen X, Tang Y, Hou H, Sheng R, Shen J. Modified method for the extraction of mRNA from paddy soils. Biotechnol Lett. 2016;38(12):2163–7.
16. Peng J, Wegner CE, Bei Q, Liu P, Liesack W. Metatranscriptomics reveals a differential temperature effect on the structural and functional organization of the anaerobic food web in rice field soil. Microbiome. 2018;6(1):169.
17. Costa R, Gomes NCM, Milling A, Smalla K. An optmized protocol for simultaneous extraction of DNA and RNA from soils. Braz J Microbiol. 2004;35:230–4.
18. Azizi P, Rafii MY, Mahmood M, Abdullah SNA, Hanafi MM, Latif MA, et al. Evaluation of RNA extraction methods in rice and their application in expression analysis of resistance genes against Magnaporthe oryzae. Biotechnol Biotechnol Equip. 2017;31(1):75–84.
19. Chan KL, Ho CL, Namasivayam P, Napis S. A simple and rapid method for RNA isolation from plant tissues with high phenolic compounds and polysaccharides. Research Square; 2007 [cited 2025 Mar 12]. Available from: https://www.researchsquare.com/article/nprot-208/v1
20. Lever MA, Torti A, Eickenbusch P, Michaud AB, Šantl-Temkiv T, Jørgensen BB. A modular method for the extraction of DNA and RNA, and the separation of DNA pools from diverse environmental sample types. Front Microbiol. 2015 [cited 2023 May 10];6. Available from: https://www.frontiersin.org/articles/https://doi.org/10.3389/fmicb.2015.00476
21. Guerra V, Beule L, Lehtsaar E, Liao HL, Karlovsky P. Improved protocol for DNA extraction from subsoils using phosphate Lysis buffer. Microorganisms. 2020;8(4):532.
22. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for illumina sequence data. Bioinformatics. 2014;30(15):2114–20.
23. Andrews S. FastQC. 2023 [cited 2023 Jul 13]. Available from: https://github.com/s-andrews/FastQC
24. Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, et al. De Novo transcript sequence reconstruction from RNA-seq using the trinity platform for reference generation and analysis. Nat Protoc. 2013;8(8):1494–512.
25. Langmead B, Salzberg SL. Fast gapped-read alignment with bowtie 2. Nat Methods. 2012;9(4):357–9.
26. Seppey M, Manni M, Zdobnov EM. BUSCO: assessing genome assembly and annotation completeness. Methods Mol Biol Clifton NJ. 2019;1962:227–45.
27. Wood DE, Lu J, Langmead B. Improved metagenomic analysis with kraken 2. Genome Biol. 2019;20:257.
28. Chong GOh, Han HS, Lee SD, Lee YH. Improvement in RNA quantity and quality in cervico-vaginal cytology. Virol J. 2020;17:8.
29. Jakše J, Wang Y, Matoušek J. Chapter 14 - Transcriptomic analyses provide insights into plant-viroid interactions. In: Adkar-Purushothama CR, Sano T, Perreault JP, Yanjarappa SM, Di Serio F, Daròs JA, editors. Fundamentals of Viroid Biology. Academic Press; 2024 [cited 2024 Apr 26]. pp. 255–74. Available from: https://www.sciencedirect.com/science/article/pii/B9780323996884000109
30. Luca F, Pai AA. Chapter 9 - Guidelines and important considerations for 'omics-level studies. In: Dluzen DF, Schmidt MHM, editors. Rigor and Reproducibility in Genetics and Genomics. Academic Press; 2024 [cited 2024 Apr 26]. pp. 189–209. (Translational and Applied Genomics). Available from: https://www.sciencedirect.com/science/article/pii/B9780128172186000139
31. Nath O, Fletcher SJ, Hayward A, Shaw LM, Agarwal R, Furtado A, et al. A comprehensive High-Quality DNA and RNA extraction protocol for a range of cultivars and tissue types of the Woody crop avocado. Plants. 2022;11(3):242.
32. Sheng Q, Vickers K, Zhao S, Wang J, Samuels DC, Koues O, et al. Multi-perspective quality control of illumina RNA sequencing data analysis. Brief Funct Genomics. 2017;16(4):194–204.
33. Yadav C, Smith ML, Yack JE. Transcriptome analysis of a social caterpillar, Drepana arcuata: de Novo assembly, functional annotation and developmental analysis. PLoS ONE. 2020;15(6):e0234903.
34. Sessitsch A, Reiter B, Pfeifer U, Wilhelm E. Cultivation-independent population analysis of bacterial endophytes in three potato varieties based on eubacterial and Actinomycetes-specific PCR of 16S rRNA genes. FEMS Microbiol Ecol. 2002;39(1):23–32.
35. Persoh D, Theuerl S, Buscot F, Rambold G. Towards a universally adaptable method for quantitative extraction of high-purity nucleic acids from soil. J Microbiol Methods. 2008;75(1):19–24.
36. Hurt RA, Qiu X, Wu L, Roh Y, Palumbo AV, Tiedje JM, et al. Simultaneous recovery of RNA and DNA from soils and sediments. Appl Environ Microbiol. 2001;67(10):4495–503.
37. Wnuk E, Waśko A, Walkiewicz A, Bartmiński P, Bejger R, Mielnik L, et al. The effects of humic substances on DNA isolation from soils. PeerJ. 2020;8:e9378.
38. Barman P, Choudhary AK, Geeta R. A modified protocol yields high-quality RNA from highly mucilaginous Dioscorea tubers. 3 Biotech. 2017;7(2):150.
39. Khairul-Anuar MA, Mazumdar P, Lau SE, Tan TT, Harikrishna JA. High-quality RNA isolation from pigment-rich Dendrobium flowers. 3 Biotech. 2019;9(10):371.
40. Toju H, Kurokawa H, Kenta T. Factors Influencing Leaf- and Root-Associated Communities of Bacteria and Fungi Across 33 Plant Orders in a Grassland. Front Microbiol. 2019 [cited 2023 Apr 26];10. Available from: https://www.frontiersin.org/articles/https://doi.org/10.3389/fmicb.2019.00241
41. Toni LS, Garcia AM, Jeffrey DA, Jiang X, Stauffer BL, Miyamoto SD, et al. Optimization of phenol-chloroform RNA extraction. MethodsX. 2018;5:599–608.

Publisher's note