An optimized method for RNA extraction from the polyurethane oligomer degrading strain *Pseudomonas capeferrum* TDA1 growing on aromatic substrates such as phenol and 2,4-diaminotoluene

Maria José Cárdenas Espinosa¹, Tabea Schmidgall¹, Georg Wagner¹, Uwe Kappelmeyer¹, Stephan Schreiber², Hermann J. Heipieper¹, Christian Eberlein¹*

¹ Department of Environmental Biotechnology, Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany, ² Department Molecular Systems Biology, Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany

*christian.eberlein@ufz.de

**Abstract**

Bacterial degradation of xenobiotic compounds is an intense field of research already for decades. Lately, this research is complemented by downstream applications including Next Generation Sequencing (NGS), RT-PCR, qPCR, and RNA-seq. For most of these molecular applications, high-quality RNA is a fundamental necessity. However, during the degradation of aromatic substrates, phenolic or polyphenolic compounds such as polycatechols are formed and interact irreversibly with nucleic acids, making RNA extraction from these sources a major challenge. Therefore, we established a method for total RNA extraction from the polyurethane degrading *Pseudomonas capeferrum* TDA1 based on RNAzol® RT, glycogen and a final cleaning step. It yields a high-quality RNA from cells grown on TDA1 and on phenol compared to standard assays conducted in the study. To our knowledge, this is the first report tackling the problem of polyphenolic compound interference with total RNA isolation in bacteria. It might be considered as a guideline to improve total RNA extraction from other bacterial species.

**Introduction**

During several decades, various microorganisms have evolved metabolic pathways to degrade environmental pollutants derived from anthropogenic activities (e.g. agriculture, solid waste, untreated industrial effluents, oil and solvent industry, etc.) which are present in different habitats [1]. In nature, aromatic compounds belong to the most persistent and hazardous pollutants, causing deleterious effects on human and animal health [2,3]. Their biodegradation has been intensely studied, however the removal of organic compounds has been focused on the role of bacteria due to their quick adaptation, metabolic versatility and genetic plasticity.
allowing them to use aromatic substrates as their sole carbon and energy source [4,5]. Hundreds of bacterial species of several phylogenetic origins have been identified as being capable to aerobically degrade all different kinds of aromatic compounds. Among all these, *Pseudomonas* species are by far the best studied ones because of their unique properties to degrade and tolerate a wide variety of xenobiotic compounds [6,7].

The mayor challenge in bacterial degradation is to overcome the resonance energy that stabilizes the aromatic ring [8,9]. To do so, aerobic bacteria rely on the addition of either one or two atoms of molecular oxygen by mono or dioxygenases. They transform aromatic compounds into central intermediates of aromatics degradation such as catechol, protocatechuate and gentisate [10]. Then, ring-cleaving dioxygenases catalyze ring fission via the ortho- or meta-cleavage pathway. During ortho-cleavage pathway, the aromatic ring fission occurs between two hydroxyl groups while during meta-cleavage this is done between one hydroxylated carbon and other adjacent non-hydroxylated carbon. Both ways are catalyzed by intradiol and extradiol dioxygenases, respectively, using Fe$^{3+}$ and Fe$^{2+}$ at the active site [2,11,12]. Finally, ring cleavage products are transformed into aliphatic molecules that can be channeled to the central metabolism. Some central intermediates of the biodegradation of aromatics such as phenols and catechols can be easily oxidized to yield the corresponding quinones. This reaction is regulated by the activity of enzymes known as polyphenol oxidases (PPOs) which are the principal basis of the browning reactions in plant tissues and extracts [13–16]. Such oxidases were also observed in *Pseudomonas* and other bacterial species [17]. Several authors have suggested that enzymatic oxidation of phenolic compounds and the presence of polysaccharides and other secondary metabolites represent a major problem in molecular studies [18–21]. A modified and improved RNA isolated protocol have been described in a bacterial culture containing pyrene as a carbon source [22].

Nucleic acid isolation is regularly the starting point for all downstream applications. However, isolation of intact RNA can be a challenge due to several factors including hydrolysis susceptibility, enzymatic and heat degradation [23]. To overcome these problems, reliable extraction methods such as commercial RNA extraction kits and organic solvents yield high quality RNA from different types of samples including cell lines, plant and mammalian tissues, bacteria, virus, etc. Nevertheless, phenolic compounds, polysaccharides, proteins and other secondary metabolites interfere with nucleic acids tend to co-precipitate or degrade RNA, restricting its yield and quality [13,23–27].

In many follow up applications including cDNA library construction, gene expression studies and next generation sequencing, the reproducibility and validity of the data depend on the quality of the RNA extracted [26]. In addition, the accurate assessment of RNA integrity and the correct quantification are key elements for further molecular analysis.

In this study, total RNA extraction was conducted for the *Pseudomonas capeferrum* TDA1 growing on three different carbon sources including phenol, succinate and 2,4-diaminotoluene (2,4-TDA); an aromatic diamine and precursor for the production of polyurethane. In previous reports, this compound was degraded by *Pseudomonas capeferrum* TDA1 and a preliminary degradation pathway was suggested [28,29]. Regardless of the proposed pathway, mono- and dioxygenases are involved for sure, leading to polycatecholic/phenolic intermediates. Those are likely to be subjected to the activity of oxidases present in the strain’s genome and responsible for the formation of polyphenolic compounds observed as dark precipitation during growth on 2,4-TDA. In order to obtain high quality RNA, commercial kits and conventional methods were tested but all of them failed for the cells grown in 2,4-TDA media, most likely due to the presence of polyphenolic compounds that could interfere with the RNA [30–34]. In order to solve this problem, a simple and effective RNA extraction method from bacterial cultures grown on 2,4-TDA was developed. This procedure uses a mixture of guanidine
thiocyanate and phenol in a monophasic solution, which is a frequent protocol for some varieties of biological samples considered as a “challenge” due to several factors. It provides purified total RNA suitable for RT-PCR, qPCR and cDNA libraries.

Materials and methods

Bacterial strain and growth conditions

Prior to the experiment, *Pseudomonas capeferrum* TDA1 was cultivated in Hartman’s mineral salts medium [28] and succinate (4 g/L) as carbon source at 30˚C and 150 rpm overnight. Afterwards, two milliliters of each culture were centrifuged (7 minutes at 18,000 g) and the resulting cell pellets were washed with KNO₃ (10 mM) while the supernatant was discarded.

The pellets were added to mineral media containing only one carbon source (4 g/L succinate, 2 mM 2,4-TDA or 2 mM phenol) and incubated for 8 hours (succinate) and 7 days (2,4-TDA) until they reached the exponential phase (OD₅₆₀ = ~0.8). The cells grown on phenol were harvested after 5 days and added to a fresh medium, reaching the exponential phase 4 days later. After that, two milliliters of the culture of every single media were centrifuged (3 minutes at 18,000 g), re-suspended in RNA Later (Sigma-Aldrich, St. Louis, MO, USA) and stored at -80˚C.

RNA isolation

The following standard laboratory kits and methods were tested for RNA extraction from *Pseudomonas capeferrum* growing on succinate, phenol and 2,4-TDA following the manufacturer’s instructions: RNeasy, RNeasy PowerPlant (Qiagen, Düsseldorf, Germany), peqGOLD TriFast (VWR, Leuven, Belgium) and phenol chloroform protocol [35]. Additionally, the improved protocol was applied for all three carbon sources mentioned. This was done by using RNAzol™ RT (Sigma-Aldrich, St. Louis, USA) with the following modifications: Initially, the cell solution was centrifuged (5 minutes at 20,000 g) to collect cells and discard the supernatant. 0.5 milliliters of RNAzol™ RT were added to the pellets and re-suspended in the reagent. Each solution was transferred to the lysing matrix B tubes and homogenized using the FastPrep-24 (MP Biomedicals, Inc) during 35 seconds at 6.5 m/s. After homogenization, the samples were transferred to 1.5 mL micro-centrifuge tubes and 0.2 mL of RNase-free water were added for DNA, protein, and polysaccharide precipitation according to the protocol [36]. Afterwards, the supernatant was transferred to a new 1.5 mL low binding micro-centrifuge tube with an equal volume of isopropanol and 1 μL of glycogen (molecular biology grade, Thermo Fisher, Waltham, United States). The samples were incubated at −80˚C for 40 minutes and centrifuged at 12,000 g for 10 minutes at room temperature. The RNA pellets were washed twice with 0.4 mL of 70% ethanol (v/v) and centrifuged at 8,000 g during 2.5 minutes at room temperature. The supernatant was removed carefully and the pellets were solubilized by adding RNase free water (45 μL) (Fig 1). Finally, the samples were cleaned up using the RNA Clean & Concentrator™-5 kit (Zymo Research, California, USA) following the protocol suggested by the manufacturer for total RNA extraction. Due to the low RNA concentration (2.5–3.0 ng/μL) yielded from 2,4-TDA samples, a pooling step (2 or 3 samples per pool) was added to the protocol before the cleaning up process.

RNA quantification and RIN determination

Total RNA was quantified using a fluorescent RNA-binding dye Qubit Fluorometer (Thermo Fisher, Waltham, United States) according to the manufacturer’s instructions. A₂₆₀/₂₈₀ and A₂₆₀/₂₃₀ values for RNA samples were measured using Nanodrop ND-1000.
Spectrophotometer (Peqlab, Erlangen, Germany). The quantification of RNA was done in triplicates. After RNA concentrations in the samples were analyzed, RNA integrity was determined in 1 μl of total RNA using the RNA Nano (succinate and phenol) and Pico chips (2,4-TDA) assays and Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, United States) in accordance with the manufacturer’s protocols. Samples with an integrity number (RIN) above 7.0 were selected for further downstream applications.

Results and discussion

Yield and quality of total RNA isolated from *P. capeferrum* TDA1

Routine molecular applications such as RT-PCR, NGS, RNA-seq require RNA with high purity and integrity [23,26]. At present, several methods and kits are available for extracting RNA from samples rich in polysaccharides, phenols and other secondary metabolites but they are mostly applied to plants tissues, leaves and woody species [20,21,23,26,33,37,38]. For this particular reason, five RNA isolation methods were compared in order to obtain high-quality RNA from *Pseudomonas capeferrum* TDA1 growing on aromatic compounds (phenol and 2,4-TDA) and succinate.

First, two commercial kits (RNeasy and RNeasy power plant kit, Qiagen) based on spin columns following the manufacturer’s recommendations yielded low RNA quantity for phenol (ranged from 5.3 to 18.9 ng/μL) and 2,4-TDA samples (ranged from 2.0 to 2.1 ng/μL), compared to RNA isolated from cells grown on succinate that showed higher concentrations for both protocols.

In addition, one method based on phenol/chloroform [35] and another containing guanidium-thiocyanate-phenol and chloroform (TriFast, VWR) were tested and the RNA yields were the lowest for cell samples grown on the aromatic compounds among all assays conducted. Finally, the modified RNAzol RT method achieved high RNA concentrations for *P. capeferrum* TDA1 grown on succinate, phenol and 2,4-TDA (Table 1).
Table 1. Total RNA quantity (ng/μL) and purity (A_{260}/A_{280} and A_{260}/A_{230}) for different RNA isolation methods applied on cells from *Pseudomonas capeferrum* TDA1 growing on different carbon sources (succinate, phenol and 2,4-TDA). Values represent mean ± SD.

| Method                  | Concentration (ng/μL) | A_{260}/A_{280} | A_{260}/A_{230} |
|-------------------------|-----------------------|-----------------|-----------------|
|                         | Succinate | Phenol | TDA | Succinate | Phenol | TDA | Succinate | Phenol | TDA |
| RNeasy                  | 67.5±14.17 | 5.3±1.77 | 2.0±0.74 | 1.98±0.19 | 1.60±0.12 | 2.71±1.44 | 2.02±0.13 | 0.33±0.18 | 0.2±0.13 |
| RNeasy P.Plant          | 48.7±12.35 | 18.9±3.02 | 2.1±0.25 | 2.25±0.06 | 2.06±0.09 | 1.30±0.46 | 2.12±0.05 | 2.02±0.18 | 2.48±1.58 |
| Phenol/Chloroform       | 54.0±25.07 | 37.3±25.66 | 0.3±0.14 | 1.67±0.15 | 1.20±0.57 | LOD | 1.63±0.52 | 0.81±0.62 | LOD |
| Trifast                 | 102.7±9.38 | 88.2±1.17 | 1.1±0.71 | 1.66±0.37 | 1.85±0.07 | LOD | 2.17±0.08 | 1.65±0.28 | LOD |
| Modified RNAzol RT      | 130±32.07   | 73.6±8.80  | 5.3±0.16 | 2.11±0.02 | 2.10±0.11 | 2.02±0.16 | 2.32±0.07 | 2.10±0.27 | 1.95±0.11 |

1Column purification;
2Guanidium thiocyanate, phenol and chloroform;
3Guanidium thiocyanate and phenol. LOD: below limit of detection.

https://doi.org/10.1371/journal.pone.0260002.t001

A_{260}/A_{280} and A_{260}/A_{230} ratios are often used for providing a rough indication of purity. A ratio of 2.0–2.2 is generally accepted as pure RNA [26,39,40]. Table 1 shows the A_{260}/A_{280} ratio for the five protocols demonstrating that RNeasy kit (1.98 ± 0.19; succinate), RNeasy power plant kit (2.25 ± 0.06; succinate, 2.06 ± 0.09; phenol) and modified RNAzol RT (2.11 ± 0.02; succinate, 2.10 ± 0.11; phenol, 2.02 ± 0.16; 2,4-TDA) method were effective inhibiting protein and phenol contamination [39,40].

The A_{260}/A_{230} ratio is a sensitive indicator of contaminants such as: guanidine thiocyanate (GTC), guanidine hydrochloride (GuHCl), EDTA, polysaccharides and other secondary metabolites. The RNeasy kit (2.02 ± 0.13; succinate), RNeasy power plant kit (2.12 ± 0.05; succinate, 2.02 ± 0.18; phenol) and modified RNAzol RT (2.32 ± 0.07; succinate, 2.10 ± 0.27; phenol, 1.95 ± 0.11; 2,4-TDA) showed values that correspond to high RNA purity (Table 1). On the other hand, phenol/chloroform and TriFast methods revealed lower A_{260}/A_{230} (≤ 1.85) and A_{260}/A_{230} (≤ 1.65) ratios for succinate and phenol samples, which indicates organic contamination that compromises the RNA quality [23,26,38,41,42]. For these two methods, the ratios could not be measured for 2,4-TDA because the RNA concentration was below the detection limit [43].

RNA isolation of cells grown on 2,4-TDA using spin columns presented A_{260}/A_{280} and A_{260}/A_{230} ratios out of the acceptable range, suggesting possible problems in the extraction due to the presence of polyphenolics, polysaccharides and secondary metabolites that precipitated with the nucleic acids [26,42].

These results are consistent with previous reports which demonstrated that commercial kits using spin columns are not suitable for RNA extraction from plants rich in polysaccharides and polyphenols. Phenolic substances reduce the efficiency of the column and can bind irreversibly to proteins and nucleic acids, leading to degradation and subsequent low-quality RNA [23,26,44]. Therefore, the low RNA concentration yielded by commercial kits has been proven previously in several plant tissues, seeds, roots and woody perennials with high content of polysaccharides and polyphenols [23,25–27,42,45].

However, not all RNA yields from cells grown on aromatic compounds had the same result. In the case of 2,4-TDA, the poor RNA yield and quality demonstrated protein and organic contamination. During incubation, a browning effect in the media was observed, which suggests the presence of PPO enzymes catalysing the oxidation of diphenols to quinones [28,46] that can irreversibly bind to the RNA and interfere with the extraction process and downstream applications [18,23,31]. Studying the annotated genome of *Pseudomonas capeferrum* TDA1 reveals the presence of the gene *yfiH* encoding for a polyphenol oxidoreductase laccase.
(EC 1.10.3.2) (data not showed). This enzyme oxidizes a broad range of phenolic and non-phenolic compounds and has been isolated from several Pseudomonas species [47]. In this study, the browning effect was not visible in phenol samples probably due to the hydroxylation of monophenols to diphenols that produces colourless intermediates [46,48]. Previous work also demonstrated that high-quality total RNA could be isolated from bacterial strains grown on phenol and benzoate using commercial kits [49–51], however not all the metabolic pathways related to bio-degradation of aromatic compounds have been identified and many intermediates as well as secondary metabolites are still unknown.

Methods (phenol/chloroform and TriFast) based on guanidinium thiocyanate–phenol–chloroform were less efficient for the isolation of RNA from P. capeferrum TDA1. RNA extracted from cells grown on phenol presented low $A_{260}/A_{280}$ and $A_{260}/A_{230}$ ratios (Table 1) for both protocols. These results are consistent with previous reports that suggested that chloroform can affect the isolation and quantification of the RNA [23,55]. Thus, polysaccharides can co-precipitate with RNA during the phenol/chloroform extraction steps [25]. Despite of these drawbacks, some modified protocols have been tested in plant leaves [52], seeds [37] and seedlings [53] obtaining high-quality RNA.

In the case of 2,4-TDA samples, the phenolic compounds and secondary metabolites in the oxidized form could interfere with the RNA yield. Negligible quantities of RNA extracted from plant tissues was reported earlier using a reagent based on guanidinium thiocyanate [54]. Also, it has been demonstrated that this substance participates in the precipitation of considerable amount proteins with the nucleic acids, reducing the RNA isolation efficiency [23,55].

Further analysis of RNA integrity using an Agilent 2100 Bioanalyzer showed RIN values $\geq 8.50$ (Fig 2A–2C) for RNA from cells grown on phenol (TriFast and modified RNAzol RT) and for 2,4-TDA (modified RNAzol RT) indicating no degradation of RNA. Generally, RNA with a RIN value above 7.0 is suitable to ensure sequencing quality [42]. The three remaining methods revealed RIN values below 5.50.

On the other hand, RNA isolation from succinate samples (RNeasy and RNeasy power plant kit, TriFast and modified RNAzol RT) revealed RIN values $\geq 7.90$, which confirms the complexity of high-quality RNA extraction from cells grown on aromatic compounds. In contrast, the phenol/chloroform protocol obtained low RIN values ($\leq 5.20$) for all the samples.

RNA samples (succinate and phenol) extracted with the TriFast method exhibited $A_{260}/A_{280}$ and $A_{260}/A_{230}$ ratios out of the acceptable range for high-quality RNA (lower than 1.8 or over 2.0) and a RIN over 8. This finding is compatible with other studies which propose that RNA purity and RNA integrity are unrelated and there is no significant correlation between them [56,57]. However, RNA quality control (integrity and purity) is critical and must be assessed independently in order to assure reliable and reproducible results. Different reports suggest that low-quality RNA has a severe effect in pPCR quantification [58], transcript estimation [59], differential expression [60] and cDNA synthesis [61] that interferes with gene expression studies.

As it has been discussed above, to obtain high-quality RNA represents a fundamental step for high technology platforms including NGS that have provided many valuable insights into biological systems.

The modified protocol presented in this study demonstrated an increase in RNA yield and quality from all the carbon sources (Fig 2c and Table 1) compared to commercially available kits, which have been reported as the first option for Gram-negative bacteria, because they are rapid, capable of high-throughput analysis and cost-effective [35,62,63]. The use of RNAzol® RT as a single step procedure removed DNA contamination without DNase treatment and reduced RNA time handling and helped diminished sample degradation, as has been tested in similar methods [63].
Fig 2. Bioanalyzer results. Electropherograms of total RNA extracted from *Pseudomonas capeferrum* TDA1 grown on: A) phenol (with TriFast method), B) phenol (with modified RNAzol RT method) and C) 2,4-TDA (with modified RNAzol RT method). The main peaks correspond to ribosomal RNA (16S and 23S).

https://doi.org/10.1371/journal.pone.0260002.g002
Regarding the cells grown on 2,4-TDA, pooling was an important key in the modified method. Pooling samples may provide a solution when RNA input is insufficient in a single sample for subsequent analyses [64]. A previous report showed that RNA isolated from pooled Gyrodactylus salaris samples was useful for increasing total RNA quality and yield [65]. Moreover, the use of pooling biological samples has been tested for the detection of gene expression changes via microarray [66]. Considering the low RNA or DNA inputs (ng/μL) which cannot be efficiently precipitated, the use of a carrier material has been studied as an effective alternative in some protocols [67]. Glycogen is regularly used in several molecular biology applications precipitating nucleic acids in solution to improve the formation of a visible pellet that simplifies downstream sample processing [67,68]. Finally, the use of a purification and concentration step is highly recommended for removing any phenol trace in RNA extraction involving guanidine-phenol based reagents as RNAzol RT [69]. This final step has been reported previously in other RNA extraction protocols due to it ensures the recovery of highly concentrated and pure RNA that be used for downstream applications afterwards [70–72].

Conclusions
In the present study, five different methods for RNA isolation from Pseudomonas capeferrum TDA1 grown on succinate, phenol and 2,4-TDA were compared. Conventional methods failed to yield high quality RNA from cells grown on 2,4-TDA (Table 1). Therefore, a modified RNAzol RT protocol was developed and demonstrated to be the most efficient to obtain high-quality total RNA from 2,4-TDA grown cells. The modified RNAzol RT method tackles the problem of RNA degradation, its interaction with phenolic compounds and the removal of organic contaminants effectively. Furthermore, the protocol showed to yield high quality RNA for cells grown on phenol, another aromatic carbon source as well as cells grown on succinate. In fact, all bacteria known to aerobically degrade complex aromatic compounds use the same machinery of oxygenation enzymes that release metabolic degradation by-products known to interfere with RNA. Therefore, we are convinced that the present protocol can be used as a guideline as a guideline to improve total RNA extraction from all bacterial on samples from all bacterial cultures growing on complex aromatic carbon sources.

Supporting information
S1 Fig. Bioanalyzer results of total RNA isolated from P. capeferrum TDA1 grown on 2–4 TDA using the RNeasy method.
(TIF)

S2 Fig. Bioanalyzer results of total RNA isolated from P. capeferrum TDA1 grown on 2–4 TDA using the RNeasy power plant method.
(TIF)

S3 Fig. Bioanalyzer results of total RNA isolated from P. capeferrum TDA1 grown on 2–4 TDA using the phenol/chloroform method.
(TIF)

S4 Fig. Bioanalyzer results of total RNA isolated from P. capeferrum TDA1 grown on 2–4 TDA using the TriFast method.
(TIF)

Acknowledgments
We thank Kerstin Ethner and Ines Màusezahl for their technical support.
Author Contributions

Conceptualization: Hermann J. Heipieper, Christian Eberlein.

Data curation: María José Cárdenas Espinosa, Tabea Schmidgall, Georg Wagner, Uwe Kappelmeyer, Stephan Schreiber.

Formal analysis: María José Cárdenas Espinosa.

Funding acquisition: Hermann J. Heipieper.

Investigation: María José Cárdenas Espinosa, Uwe Kappelmeyer, Hermann J. Heipieper.

Methodology: Stephan Schreiber, Christian Eberlein.

Supervision: Uwe Kappelmeyer, Stephan Schreiber, Hermann J. Heipieper, Christian Eberlein.

Validation: María José Cárdenas Espinosa, Uwe Kappelmeyer, Hermann J. Heipieper.

Visualization: María José Cárdenas Espinosa.

Writing – original draft: María José Cárdenas Espinosa.

Writing – review & editing: Uwe Kappelmeyer, Hermann J. Heipieper, Christian Eberlein.

References

1. Pimviriyakul P, Wongnate T, Tinkul R, Chaiyen P. Microbial degradation of halogenated aromatics: molecular mechanisms and enzymatic reactions. Microb Biotechnol. 2020; 13(1):67–86. Epub 2019/10/01. https://doi.org/10.1111/1751-7915.13488 PMID: 31566582

2. George KW, Hay AG. Bacterial strategies for growth on aromatic compounds. Adv Appl Microbiol. 74: Elsevier; 2011. p. 1–33. https://doi.org/10.1016/B978-0-12-387022-3.00005-7 PMID: 21459192

3. Singh P, Kumar R. Critical review of microbial degradation of aromatic compounds and exploring potential aspects of Furfuryl alcohol degradation. J Environ Polym Degrad. 2019; 27(5):901–16.

4. Díaz E, Prieto MaA. Bacterial promoters triggering biodegradation of aromatic pollutants. Curr Opin in Biotechnol. 2000; 11(5):467–75. https://doi.org/10.1016/s0958-1669(00)00126-9 PMID: 11024365

5. Semple KT, Cain RB, Schmidt S. Biodegradation of aromatic compounds by microalgae. FEMS Microbiol Lett. 1999; 170(2):291–300.

6. Atashgahi S, Sánchez-Andrea I, Heipieper HJ, van der Meer JR, Stams AJ, Smidt H. Prospects for harnessing biocide resistance for bioremediation and detoxification. Science. 2018; 360(6390):743–6. https://doi.org/10.1126/science.aar3778 PMID: 29773745

7. Caño B, Nagarajan K, Loh K-C. Biodegradation of aromatic compounds: current status and opportunities for biomolecular approaches. Appl Microbiol Biotechnol. 2009; 85(2):207–28. https://doi.org/10.1007/s00253-009-2192-4 PMID: 19730850

8. Fuchs G, Boll M, Heider J. Microbial degradation of aromatic compounds—from one strategy to four. Nat Rev Microbiol. 2011; 9(11):803–16. https://doi.org/10.1038/nrmicro2652 PMID: 21963803

9. Teufel R, Mascaraque V, Ismail W, Voss M, Perera J, Eisenreich W, et al. Bacterial phenylalanine and phenylacetate catabolic pathway revealed. PNAS. 2010; 107(32):14390–5. https://doi.org/10.1073/pnas.1005399107 PMID: 20660314

10. Pérez-Pantoja D, González B, Pieper DH. Aerobic degradation of aromatic hydrocarbons. In: Rojo F, editor. Aerobic Utilization of Hydrocarbons, Oils and Lipids. Cham: Springer International Publishing; 2016. p. 1–44.

11. Çınar Ö. Biodegradation of central intermediate compounds produced from biodegradation of aromatic compounds. Bioprocess Biosyst Eng. 2004; 26(5):341–5. https://doi.org/10.1007/s00449-004-0364-2 PMID: 15300479

12. Shrivastava R, Phale PS. Biodegradation of mono-aromatic compounds by bacteria. Microorganisms in Environmental Management: Springer; 2012. p. 451–76.

13. Arias DG, Doria CMM, Ramos LR, Morocho HCN. Molecular characterization of the polyphenol oxidase gene in lulo (Solanum quitensis Lam.) var. Castilla. Braz J Plant Physiol. 2012; 24(4):261–72.
14. El-Naijar N, Gali-Muhtasib H, Ketola RA, Vuorela P, Urtti A, Vuorela H. The chemical and biological activities of quinones: overview and implications in analytical detection. Phytochem Rev. 2011; 10 (3):353–70.

15. Loomis W. Overcoming problems of phenolics and quinones in the isolation of plant enzymes and organelles. Methods in enzymology. 31: Elsevier; 1974. p. 528–44. https://doi.org/10.1016/0076-6879(74)31057-9 PMID: 4418408

16. Shu C, Sun S, Chen J, Chen J, Zhou E. Comparison of different methods for total RNA extraction from sclerotia of *Rhizoctonia solani*. Elec J Biotechnol. 2014; 17(1):9.

17. Güray MZ. Partial purification and characterization of polyphenol oxidase from thermophilic Bacillus sp. İzmir Institute of Technology; 2009.

18. Choudhary S, Kumar M, Chowdhury I, Singh R, Pandey S, Sharma H, et al. An efficient and cost-effective method of RNA extraction from mucilage, phenol and secondary metabolite rich tissue of tosa jute (C. *olitorius* L.) actively developing phloem fiber. 3 Biotech. 2016; 6(1):100. https://doi.org/10.1007/s13205-016-0415-9 PMID: 28330170

19. Kalinowska E, Chodoraska M, Paduch-Cichal E, Mroczkowska K. An improved method for RNA isolation from plants using commercial extraction kits. Acta Biochim Pol. 2012; 59(3). PMID: 22826824

20. Ramadoss N, Basu C. Extraction of rna from recalcitrant tree species *Paulownia elongata*. BIO-PROTOCOL. 2018; 8(14). https://doi.org/10.21769/BioProtoc.2925 PMID: 34395749

21. Sánchez C, Villacreses J, Blanc N, Espinoza L, Martínez C, Pastor G, et al. High quality RNA extraction from maqui berry for its application in next-generation sequencing. SpringerPlus. 2016; 5(1):1243. https://doi.org/10.1186/s40064-016-2906-x PMID: 27536526

22. Badejo AC, Badejo AO, Shin KH, Chai YG. A gene expression study of the activities of aromatic ring-cleavage dioxygenases in *Mycobacterium gilvum PYR-GCK* to changes in salinity and pH during pyrene degradation. PloS One. 2013; 8(2):e58066. https://doi.org/10.1371/journal.pone.0058066 PMID: 23469141

23. Carpinteti PdA, Fioretti VS, Igniez da Cruz T, de Almeida FAN, Canal D, Ferreira A, et al. Efficient method for isolation of high-quality RNA from *Psidium guajava* L. tissues. PloS One. 2021; 16(7): e0255245. https://doi.org/10.1371/journal.pone.0255245 PMID: 34310664

24. Breitler J-C, Campa C, George F, Bertrand B, Etienne H. A single-step method for RNA isolation from tropical crops in the field. Sci Rep. 2016; 6(1):1–6. https://doi.org/10.1038/s41598-016-0001-8 PMID: 28442746

25. Lan T, Yao B, Shen Y, Wang Xa. Isolation of high-quality total RNA from lipid-rich seeds. Anal Biochem. 2013; 438(1):1–3. https://doi.org/10.1016/j.ab.2013.03.012 PMID: 23524018

26. Liu L, Han R, Yu N, Zhang W, Xing L, Xie D, et al. A method for extracting high-quality total RNA from plant rich in polysaccharides and polyphenols using *Dendrobium huoshanense*. PloS One. 2018; 13(5): e0196592. https://doi.org/10.1371/journal.pone.0196592 PMID: 29715304

27. Xiao H, Kim W-S, Meng B. A highly effective and versatile technology for the isolation of RNAs from grapevines and other woody perennials for use in virus diagnostics. Virol J. 2015; 12(1):1–15. https://doi.org/10.1186/s12035-015-0503-7 PMID: 26482551

28. Carbón-Manso MJ, Blanco AC, Schmidgall T, Atanasoff-Kardjaliyev AK, Kappelmeyer U, Tischler D, et al. Toward biorecyclin g: isolation of a soil bacterium that grows on a polyurethane oligomer and monomer. Front Microbiol. 2020; 11:404. https://doi.org/10.3389/fmicb.2020.00404 PMID: 32292389

29. Utomo RNC, Li W-J, Tiso T, Eberlein C, Doeker M, Heipieper HJ, et al. Defined microbial mixed culture protocols for utilization of polyurethane monomers. ACS Sustain Chem Eng. 2020; 8(47):17466–74.

30. Dash PK. High-quality RNA isolation from ployphenol-, polysaccharide- and protein-rich tissues of lentil (*Lens culinaris*). 3 Biotech. 2013; 3(2):109–14. https://doi.org/10.1007/s13205-012-0075-3 PMID: 28324564

31. Japelaghi RH, Haddad R, Garroso G-A. Rapid and efficient isolation of high quality nucleic acids from plant tissues rich in polyphenols and polysaccharides. Mol Biotechnol. 2011; 49(2):129–37. https://doi.org/10.1007/s12033-011-9384-8 PMID: 21302150

32. Rubio-Piña JA, Zapata-Pérez O. Isolation of total RNA from tissues rich in polyphenols and polysaccharides of mangrove plants. Electron J Biotechnol. 2011; 14(5):11-.

33. Sangha JS, Gu K, Kaur J, Yin Z. An improved method for RNA isolation and cDNA library construction from immature seeds of *Jatropha curcas*. BMC Res Notes. 2010; 3(1):1–6. https://doi.org/10.1186/1756-0500-3-126 PMID: 20444276

34. Yang G, Zhou R, Tang T, Shi S. Simple and efficient isolation of high-quality total RNA from *Hibiscus tiliaceus*, a mangrove associate and its relatives. Prep Biochem Biotechnol. 2008; 38(3):257–64. https://doi.org/10.1080/10826060802164991 PMID: 18569872
35. 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. https://doi.org/10.1016/j.mex.2018.05.011 PMID: 29984193
36. RNAzol RT (R4533)—Technical Bulletin—Sigma-Aldrich. https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/1/r4533bullet.pdf.
37. Kanani P, Shukla YM, Modi AR, Subhash N, Kumar S. Standardization of an efficient protocol for isolation of RNA from Cuminum cyminum. J King Saud Univ Sci. 2019; 31(4):1202–7.
38. Liao X, Li H, Khan A, Zhao Y, Hou W, Tang X, et al. A simple and rapid method for isolating high-quality RNA from kenaf containing high polysaccharide and polyphenol contents. bioRxiv. 2020:2020.07.06.189506. https://doi.org/10.1101/2020.07.06.189506
39. Desjardins P, Conklin D. NanoDrop microvolume quantitation of nucleic acids. JoVE. 2010;(45):e2565. https://doi.org/10.3791/2565 PMID: 21189466
40. Koetsier G, Cantor E. A practical guide to analyzing nucleic acid concentration and purity with microvolume spectrophotometers. NEB Inc. 2019.
41. Acosta-Maspons A, González-Lemos I, Covarrubias AA. Improved protocol for isolation of high-quality total RNA from different organs of Phaseolus vulgaris. Biotechniques. 2019; 66(1):161–2. https://doi.org/10.2144/btn-2018-0129 PMID: 30744411
42. Ma Z, Huang B, Xu S, Chen Y, Li S, Lin S. Isolation of high-quality total RNA from chinese fir (Cunninghamia lanceolata) (Lamb.) Hook. PLoS One. 2015; 10(6):e0130234. https://doi.org/10.1371/journal.pone.0130234 PMID: 26083257
43. ND-1000 Spectrophotometer V3.5 User’s Manual In: Technologies N, editor. Wilmington, DE 19810 USA 2007.
44. Wang L, Stegemann JP. Extraction of high quality RNA from polysaccharide matrices using cetyltrimethylammonium bromide. Biomaterials. 2010; 31(7):1612–8. https://doi.org/10.1016/j.biomaterials.2009.11.024 PMID: 19962190
45. Footitt S, Awan S, Finch-Savage WE. An improved method for the rapid isolation of RNA from Arabidopsis and seeds of other species high in polyphenols and polysaccharides. Seed Sci Res. 2018; 28(4):360–4.
46. Toivonen PM, Brummell DA. Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. Postharvest Biol Technol. 2008; 48(1):1–14.
47. Mandic M, Djokic L, Nikolaivits E, Prodanovic R, O’Connor K, Jeremic S, et al. Identification and characterization of new laccase bio catalysts from Pseudomonas species suitable for degradation of synthetic textile dyes. Catalysts. 2019; 9(7):629.
48. Snidle V, Lakshmi M, Manasa M, Sravan M. Metabolic pathways for the biodegradation of phenol. Int J Eng Adv Technol. 2012; 2(3):695–705.
49. Bhardwaj P, Sharma A, Sagarkar S, Kapley A. Mapping atrazine and phenol degradation genes in Pseudomonas sp. EGD-AKNS. Biochim Eng J. 2015; 102:125–34.
50. Gu Q, Wu Q, Zhang J, Guo W, Ding Y, Wang J, et al. Isolation and transcriptome analysis of phenol-degrading bacterium from carbon–sand filters in a full-scale drinking water treatment plant. Front Microbiol. 2018; 9:2162. https://doi.org/10.3389/fmicb.2018.02162 PMID: 30298058
51. Haußmann U, Qi SW, Wolters D, Rögnar M, Liu SJ, Poetsch A. Physiological adaptation of Corynebacterium glutamicum to benzoate as alternative carbon source—a membrane proteome-centric view. Proteomics. 2009; 9(14):3635–51. https://doi.org/10.1002/pmic.200900025 PMID: 19639586
52. Sah SK, Kaur G, Kaur A. Rapid and reliable method of high-quality RNA extraction from diverse plants. Am J Plant Sci. 2014; 5(21):3129.
53. Box MS, Coustham V, Dean C, Myline JS. Protocol: A simple phenol-based method for 96-well extraction of high quality RNA from Arabidopsis. Plant Methods. 2011; 7(1):1–10. https://doi.org/10.1186/1746-4811-7-1 PMID: 21266074
54. Ghawana S, Paul A, Kumar H, Kumar A, Singh H, Bhardwaj PK, et al. An RNA isolation system for plant tissues rich in secondary metabolites. BMC Res Notes. 2011; 4(1):1–5.
55. Mornkham T, Wangsomnuk PP, Fu Y-B, Wangsomnuk P, Jitjai K, Poetsch A. Physiological adaptation of Corynebacterium glutamicum to benzoate as alternative carbon source—a membrane proteome-centric view. Proteomics. 2009; 9(14):3635–51. https://doi.org/10.1002/pmic.200900025 PMID: 19639586
56. Die JV, Román B. RNA quality assessment: a view from plant qPCR studies. J Exp Bot. 2012; 63(17):6069–77. https://doi.org/10.1093/jxb/era276 PMID: 23045609
57. Ruocco N, Costantini S, Zupo V, Romano G, Ianora A, Fontana A, et al. High-quality RNA extraction from the sea urchin Paracentrotus lividus embryos. PLoS One. 2017; 12(2):e0172171. https://doi.org/10.1371/journal.pone.0172171 PMID: 28199408
58. Carvalhais V, Delgado-Rastrollo M, Melo LD, Cerca N. Controlled RNA contamination and degradation and its impact on qPCR gene expression in *S. epidermidis* biofilms. J Microbiol Methods. 2013; 95(2):195–200. https://doi.org/10.1016/j.mimet.2013.08.010 PMID: 23999274

59. Wan L, Yan X, Chen T, Sun F. Modeling RNA degradation for RNA-Seq with applications. Biostatistics. 2012; 13(4):734–47. https://doi.org/10.1093/biostatistics/kxs001 PMID: 22353193

60. Sigurgeirsson B, Emanuelsen O, Lundeberg J. Sequencing degraded RNA addressed by 3’tag counting. PLoS One. 2014; 9(3):e91851. https://doi.org/10.1371/journal.pone.0091851 PMID: 24632678

61. Riesgo A, PÉREZ-PORRO AR, Carmona S, Leys SP, Giribet G. Optimization of preservation and storage time of sponge tissues to obtain quality mRNA for next-generation sequencing. Molecul Ecol Resour. 2012; 12(2):312–22. https://doi.org/10.1111/j.1755-0998.2011.03097.x PMID: 22136287

62. Chomczynski P, Willfinger W, Kennedy A, Rymaszewski M, Mackey K. RNAzol® RT: a new single-step method for isolation of RNA. Nat Methods. 2010; 7(12):4–5.

63. Jahn CE, Charkowski AO, Willis DK. Evaluation of isolation methods and RNA integrity for bacterial RNA quantitation. J Microbiol Methods. 2008; 75(2):318–24. https://doi.org/10.1016/j.mimet.2008.07.004 PMID: 18674572

64. Assefa AT, Vandesompele J, Thas O. On the utility of RNA sample pooling to optimize cost and statistical power in RNA sequencing experiments. BMC Genomics. 2020; 21(1):1–14.

65. Fromm B, Harris PD, Bachmann L. MicroRNA preparations from individual monogenean *Gyrodactylus salaris*-a comparison of six commercially available totalRNA extraction kits. BMC Res Notes. 2011; 4(1):1–6. https://doi.org/10.1186/1755-0646-4-217 PMID: 21714869

66. Kendzierski C, Irizarry R, Chen K-S, Haag J, Gould M. On the utility of pooling biological samples in microarray experiments. PNAS. 2005; 102(12):4252–7. https://doi.org/10.1073/pnas.0500671102 PMID: 15755808

67. Gautam A, Kumar R, Dimitrov G, Hoke A, Hammaineh R, Jett M. Identification of extracellular miRNA in archived serum samples by next-generation sequencing from RNA extracted using multiple methods. Mol Biol Rep. 2016; 43(10):1165–78. https://doi.org/10.1007/s11033-016-4043-6 PMID: 27510798

68. Duy J, Koehler JW, Honko AN, Minogue TD. Optimized microRNA purification from TRizol-treated plasma. BMC Genomics. 2015; 16(1):1–9. https://doi.org/10.1186/s12864-015-1299-5 PMID: 25765146

69. Center UDG. [16.08.2021]. https://dnatech.genomecenter.ucdavis.edu/faqs/how-should-i-purify-my-samples-how-should-i-remove-dna-or-rna-contamination

70. Goh YJ, Barrango R, Klaenhammer TR. In vivo transcriptome of *Lactobacillus acidophilus* and colonization impact on murine host intestinal gene expression. MBio. 2021; 12(1):e03399–20. https://doi.org/10.1128/mBio.03399-20 PMID: 33500337

71. Kraushar ML, Krupp F, Harnett D, Turko P, Ambrozkiewicz MC, Sprink T, et al. Protein synthesis in the developing neocortex at near-atomic resolution reveals Ebp1-mediated neuronal proteostasis at the 60S tunnel exit. Mol Cell. 2021; 81(2):304–22. e16. https://doi.org/10.1016/j.molcel.2020.11.037 PMID: 33357414

72. Manfredonia I, Nithin C, Ponce-Salvatierra A, Ghosh P, Wirecki TK, Marinus T, et al. Genome-wide mapping of SARS-CoV-2 RNA structures identifies therapeutically-relevant elements. Nucleic Acids Res. 2020; 48(22):12436–52. https://doi.org/10.1093/nar/gkaa1053 PMID: 33166999