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Narrative Review of Bacterial DNA Extraction: Key Points and Challenges

*Samar Mohamed Mansour Solyman [1, *], Manar El Samak [2] .*

[1]Faculty of Pharmacy, Sinai University, Kantara, Egypt
[2] Department of Microbiology & Immunology, Faculty of Pharmacy , Suez Canal University , Egypt
**Corresponding author*

Correspondence:

Samar Mohamed Mansour Solyman
samar.mansour@su.edu.eg

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ABSTRACT

Extraction of bacterial DNA is a critical step in obtaining high-quality DNA. The overall quality, yield, and integrity of the genomic DNA isolated affects the success of any downstream applications. Many factors can affect the quality and yield of genomic DNA obtained. By understanding some of the key steps underlying DNA chemical and physical properties, the methodology of DNA extraction can be modified to deal with specific sample types for particular research purposes. This narrative review aimed to present a comprehensive overview of bacterial DNA extraction protocols by summarizing research articles from various databases such as PubMed and Science Direct. Keywords were used for the research, such as 'DNA extraction, bacterial DNA isolation, and optimization of DNA extraction protocol. Research articles included in this review were English-language articles published between 1999 and 2023 that fulfilled the study objectives. After thoroughly assessing the included articles, 36 relevant articles were selected for this review. This review sheds light on the key steps of DNA extraction and several challenges facing researchers in the field of molecular biology. This narrative review serves as a simple guide for molecular biology researchers.

KEYWORDS: DNA Extraction, Bacterial DNA, Lysis protocol, DNA purity.

1. INTRODUCTION

Deoxyribonucleic acid (DNA) extracted from bacteria has become a significant practice in many areas of biology. It is widely used for molecular biology studies, clinical disease detection, and the study of epidemiology. In the field of molecular biology, the need to obtain a large amount of pure DNA is an important aspect in order to gain a better understanding of the genomics and proteomics of an organism. Many molecular methods (such as PCR, gene cloning, genomics) require pure DNA as a substrate. The knowledge gained from studying DNA can often point the way to new discoveries, which may have important implications for human welfare. However, the quality of the DNA that has been isolated might be an issue. If the DNA is of poor quality, it will be challenging to work with and may even give misleading information. This is particularly problematic when working with the DNA of bacteria, with a wide range of families and cell wall structure differences. Sometimes, DNA from two different organisms could be mixed up, or important DNA fragments could be lost.

Therefore, it is essential to have pure DNA which has been isolated from a single bacterial strain. In this review, the challenges of bacterial DNA extraction methods will be demonstrated, and some key points to overcome them will be discussed.

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2. METHODS / RESEARCH SELECTION

Across various electronic databases, including PubMed, SCOPUS, and Science Direct. The search utilized keywords such as "DNA extraction, bacterial DNA extraction, DNA Isolation, Nucleic acid extraction" In addition to manual electronic searches for relevant articles. Articles published in English between the year 1999 and 2023 that fulfilled the study's objectives were included. The article selection process resulted in 88 articles, 65 articles were selected based on their titles and abstracts. An additional Fourteen articles were obtained through a manual search (including the words gram-positive bacteria, Actinomycetes, Mycobacterium tuberculosis, marine bacteria, and environmental bacteria), resulting in a total of 79 articles. After evaluating the full texts and applying the journal regulation to the authors, 36 articles were chosen for the review that met the study's objectives.

3. RESULTS & DISCUSSION

Although slightly different methods for DNA extraction exist, the chemical and physical processes of breaking the cells open to release the DNA, purifying the DNA away from the cellular components and then precipitating the DNA from an aqueous solution are all the same. Some key steps are recommended in DNA extraction in some classes of bacteria. Performing further techniques to get a high-quality DNA during the DNA extraction stage can be costly in both time and materials. This is especially true for those who work with environmental samples where inhibitors are common. Therefore, it is better to extract DNA to be of satisfactory purity for the intended use.

3.1. Selection of Appropriate Method for DNA Extraction

The selection of an appropriate bacterial DNA extraction method is crucial for obtaining reliable results in various molecular biology applications. Different studies have highlighted the importance of choosing the proper extraction method based on the specific requirements of the study. Previous studies emphasize the importance of selecting appropriate DNA extraction methods for efficient bacterial detection and quantification. Furthermore, the influence of DNA extraction methods on microbial profiles has been investigated by [1] in the context of oral microbiome analysis. They found that different extraction procedures introduced bias in DNA yield and bacterial species representation. Another study [2] compared the performance of various DNA extraction methods for analyzing human oral microbes. They suggested that the selection of the DNA extraction method should be based on the specific aims and specimens of the study. Overall, these studies highlight the importance of considering factors such as sample type, downstream applications, and desired outcomes when selecting a bacterial DNA extraction method.

3.1.1. Physical Methods of DNA Extraction

Physical methods for bacterial DNA extraction have been widely studied and developed over the years. Zhang, and Coworkers [3] described a new method for isolating bacterial genomic DNA using sonication, which resulted in highly concentrated and fragmented DNA suitable for microarray analysis. Wu, et al 2009 [4] developed an effective cell lysis method for extracting bacterial genomic DNA from compost, comparing enzymatic disruption, physical-chemical combination, and commercial kit methods for DNA yield and cell lysis efficiency. While Govindarajan, et al. 2011 [5] introduced a microfluidic origami device for

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point-of-care extraction of bacterial DNA from viscous samples, showcasing innovative approaches to DNA extraction technology. Bruner and Collegueas study compared physical cell lysis methods for DNA extraction from soil, emphasizing the importance of diversity and quantity measures in assessing extraction bias [6]. The effectiveness of boiling, freezing-thawing, and basic chemical extraction techniques for bacterial DNA extraction was assessed in a study by Hershan, et al. 2005, which found that these physical techniques were comparable to expensive kit-based techniques for PCR investigations [7]. To identify the best DNA extraction techniques for studying rumen microbial communities, a recent study [8] modified and improved manual techniques for community DNA extraction from goat rumen digesta. These studies showed the variety of physical techniques used to extract bacterial DNA as well as the continuous attempts to increase extraction accuracy and efficiency across a range of study domains.

3.1.2. Optimization of DNA Extraction Protocol

Several studies have focused on optimizing DNA extraction protocol for various bacterial samples. Zoetendal,and Collegueas [9] presented a DNA isolation protocol suitable for gastrointestinal tract samples based on mechanical disruption and phenol: chloroform: isoamyl alcohol extraction. . Biesbroek, et al. 2012 [10] highlighted the difficulties caused by bacterial density and sought to develop precise microbiota analysis procedures for low-density microbial communities. Similarly, Mathay, et al. 2015[11] suggested an ideal strategy for 16S rRNA gene-based analysis and validated the effect of PCR primers and DNA extraction procedures on the accuracy of fecal microbiota profiling. In a different investigation [12], the MSM I extraction method demonstrated higher DNA quantity and quality after optimizing a stool processing protocol for DNA extraction. For plant-associated bacterial communities, Cecelia, et al. 2021 [13] contrasted 16S rRNA gene amplification and DNA extraction techniques, pointing out biases in both processes and suggesting particular kits and procedures for researchers working in this area. These results highlight how crucial it is to optimize DNA extraction procedures for various bacterial samples to guarantee precise and effective downstream analyses.

3.1.3. Optimization of Lysis Conditions

The optimization of DNA lysis conditions is crucial in various research fields, including clinical applications, environmental studies, and industrial processes. Different studies have focused on developing efficient methods for DNA extraction and purification to ensure high yields and quality of DNA samples. Bead mill homogenization in a lysis combination comprising chloroform, SDS, NaCl, and phosphate-Tris buffer was found to be successful as reported previously by Miller and collegueas [14] that assessed and improved DNA extraction and purification protocols for soil and sediment samples. Qamar, et al 2016 [15] used the SDS-proteinase K approach to improve conditions for the extraction of high-quality DNA from whole blood for PCR analysis. To sum up, these studies show how crucial it is to optimize DNA lysis conditions to guarantee effective DNA extraction, purification, and analysis across a range of research domains. Every study emphasizes distinct approaches and strategies catered to their own research goals, highlighting the importance of optimization in producing trustworthy and superior DNA samples.

3.2. Common Challenges in Bacterial DNA Extraction

Extractions of bacterial DNA possess several challenges that can impact the quality and yield of the extracted genetic material. To overcome the difficulties caused by slow growth and

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different cell wall composition, a prior study created a DNA extraction technique tailored for mycobacteria that improved the quantity and purity of DNA preparations [16]. Different strategies have been developed to prevent bacterial DNA contamination and ensure the safety of products and samples. Efficient strategies for the broad-range detection of low-abundance bacteria without DNA decontamination of PCR reagents have been proposed [17]. Abusleme and collegueas assessed different DNA extraction techniques for bacterial species linked to humans, highlighting the significance of DNA yield, shearing, repeatability, and microbial diversity representation in choosing a suitable technique [18]. Mirsepasi, et al. 2014 showed how the extraction technique affects the investigation of microbial diversity by contrasting two DNA extraction techniques for intestinal bacterial diversity [19]. However, work by Gobbi, et al. 2019 [20] addressed the difficulties in extracting DNA from soil, focusing on the impact on DNA yield and purity, especially in samples with lower biomass, as well as the biases brought about by indirect extraction techniques. A study by Nishitani and coworkers [21] examined DNA methylation analysis from saliva samples made clear how important it is to distinguish between bacterial and human DNA in mixed samples in order to perform an appropriate analysis. To characterize Shiga toxin-producing Escherichia coli isolates, a recent study [22] highlighted the influence of DNA extraction on whole genome sequencing analysis, underscoring the necessity of consistent workflows to get robust data quality. Bauer, et al. 2022 [23] described an optimized method for bacterial nucleic acid extraction from positive blood culture broths, focusing on efficient inhibitor removal and DNA preservation to obtain high-quality genetic material suitable for metagenomic sequencing and antimicrobial susceptibility prediction. These studies collectively highlight the significance of selecting appropriate DNA extraction methods to overcome common challenges in bacterial DNA extraction and ensure the quality and integrity of the extracted genetic material.

3.3. Challenges in Extracting DNA from Gram-Positive Bacteria

Challenges in extracting DNA from gram-positive bacteria can be attributed to the unique characteristics of various species within this group. For instance, Desulfosporosinus youngiae, a spore-forming sulfate-reducing bacterium isolated from a constructed wetland treating acid mine drainage, despite being phylogenetically a member of the Gram-type-positive phylum Firmicutes, cells stained Gram-negative at all growth phases [24]. This discrepancy in staining behaviour can complicate DNA extraction procedures, as traditional methods may not yield accurate results. Similarly, Anoxybacillus Kamchatkensis, a novel thermophilic facultative aerobic bacterium with a broad pH optimum from the Geyser Valley, Kamchatka, presents challenges due to its unique environmental adaptations [25]. The extremophilic nature of Anoxybacillus Kamchatkensis may require specialized techniques for DNA extraction to ensure the preservation of genetic material for downstream analysis. Furthermore, the presence of specific metabolic pathways in gram-positive bacteria, such as those involved in sulfate reduction or carboxydotrophy, can also impact DNA extraction processes. Similarly, Thermincola Carboxydiphila, a novel anaerobic, carboxydotrophic, hydrogenogenic bacterium from a hot spring in the Lake Baikal area, may have unique cellular structures requiring specialized DNA isolation methods [26]. Overall, the challenges in extracting DNA from gram-positive bacteria stem from a combination of factors, including staining behaviour, metabolic pathways, environmental adaptations, and genetic sequences. Addressing these challenges may require the development of specialized DNA extraction protocols tailored to the unique characteristics of each bacterial species. Further research in this area is essential to improve the efficiency and accuracy of DNA extraction from gram-positive bacteria for various applications in microbiology and biotechnology.

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3.4. Challenges in DNA Extraction from Actinomycetes

Actinomycetes are a group of bacteria known for producing bioactive compounds with potential pharmaceutical applications. The cell wall of Actinomycetes is more complex than that of other species of bacteria with high Guanine-Cytosine (GC) content. However, extracting DNA from these organisms poses several challenges. One major issue is the poor quality of genomic DNA obtained using conventional techniques. To address these challenges, researchers have developed specialized DNA extraction methods tailored to actinomycetes. For example, a study focused on environmental DNA extraction specific for vegetative cells of actinomycetes demonstrated the importance of using methods optimized for the unique characteristics of these bacteria [27] This can be achieved by culturing the bacteria in an especial media, Reasoner's 2A agar (R2A media) is a low nutrient containing agar. The decreased nutrient levels result in slower growth for many bacteria, but Actinomycetes can still be cultured over time periods of around two weeks. This longer growth period resulted in larger, more robust cells. A Comparative DNA extraction methods was performed using five methods with a novel improved method (28), which includes several steps for cell lysis, briefly: precipitated cell mass was suspended in a buffer containing salts, EDTA, Tris HCL and glass beads. The samples were homogenized with crushing machine, incubated at 100°C for 5 minutes, and frozen in liquid N2 for 3 minutes. An additional lysis step includes mixing with SDS, proteinase K in Tris HCL, CaCl2 and Lysozyme. Samples were incubated at 55°C for 2 hours, the sample tubes were inverted and 50 ml Phenol: chloroform: iso-amyl alcohol (25:24:1) were added and the mixture was centrifuged at 8000 rpm for 5 minutes at room temperature. Optimal lysis techniques are likely species-specific and seem to be a critical step [28].

3.5. DNA Extraction from Marine Samples

The high extraction efficiency and quantitative result based on the length of lysozyme and proteinase K enzyme treatment, which greatly affected the DNA extraction efficiency, were highlighted in a study conducetd by Kjärstin and collegueas [29] that improved a technique for DNA extraction from saltwater samples. Additionally, the precipitation of low-concentration DNA was enhanced by the inclusion of yeast tRNA as a co-precipitant. In contrast to other methods, a simpler phenol-chloroform extraction approach devised in an study conducted by Urakawa, et al. 2010 [30] demonstrated higher DNA recovery and purity. Nucleic acid extraction techniques for examining bacterial populations linked to corroded carbon steel in maritime environments were assessed and improved previously [31]. Portas, et al. 2021,compared extraction techniques for describing marine eukaryotic biofilms at offshore wind farm locations [32]. These investigations emphasize the difficulties and significance of effective DNA extraction.

3.6. Bacterial DNA Extraction from Environmental Samples

The difficulties in extracting bacterial DNA from environmental samples have been investigated by several researchers. Urakawa, et al. 2010 [30] created a modified DNA extraction technique that targets ammonia-oxidizing archaea in particular to effectively recover DNA from coastal waters. Marotz and collegueas [33] found a pipeline that shortens sample processing times without sacrificing taxonomic or abundance information by comparing the extraction efficiencies of various platforms for metagenomics of diverse environmental samples. To guarantee accurate and consistent biodiversity data across investigations, Gavin, et al. 2018 [34] suggested standardized protocols for DNA extraction from diverse environmental samples, suggesting particular kits for various sample types.

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3.7. Challenges in DNA Extraction of Mycobacterium Tuberculosis

The difficulties in extracting DNA from Mycobacterium tuberculosis have been a topic of interest in tuberculosis research, with several studies focusing on different aspects of DNA extraction and its implications. Conventional extraction methods use highly hazardous chemicals, such benzene or chloroform, and methods using chloroform alone have also been described; these agents are unsuitable for diagnostics in the hospital or research laboratory and may be harmful to health and the environment. High-speed centrifugation is an effective lysis method, but it can lose nucleic acid from the mycobacterial complex from the pellet formed, and resuspension of the cell lysate is ineffective. Izhar, et al. 2004 [35] developed a single-tube, cell lysis-based, genus-specific PCR method for quick identification of mycobacteria, confirming the importance of cell lysis optimization. The study conducted by Pan, et al 2013 [36] compared four DNA extraction methods for detecting mycobacterium tuberculosis by real-time PCR, emphasizing the clinical application in diagnosing pulmonary tuberculosis. Presence of inhibitors in mycobacterial cells: Inhibitors can either degrade the extracted DNA or interfere with the amplification of the extracted DNA. Agents used for the disruption of mycobacterial cells are likely to inhibit PCR by binding to DNA or interfering with polymerase activity or both. Although the specific agents that account for inhibitor activity have not been clearly defined, the mycobacterial cell wall contains complex lipids that interfere with the recovery of the DNA and RNA, which can reduce PCR sensitivity. Often, lipid components released from disrupted mycobacterial cells interfere with the extraction of protein-free nucleic acid that is required for downstream processing.

4. CONCLUSION

In conclusion, the literature reviewed emphasizes the critical role of proper bacterial DNA extraction techniques in various microbiological studies ranging from environmental microbiology to public health and bacterial diversity research. Ensuring complete and consistent DNA extraction methods is essential for obtaining reliable results and advancing our understanding of bacterial communities and pathogens.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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