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RESEARCH ARTICLE :

Comparison of rapid methods for the extraction of bacterial DNA using scar marker for commercial liquid biofertilizer *Azospirillum lipoferum* (Az204) from TNAU

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SUMMARY : The increasing uses of DNA methodologies like SCAR marker to authenticate the commercial biofertiliser as a quality control requires an rapid, simple and efficient recovery of bacterial DNA from the sample. Hence, this study was made to determine which DNA extraction methods are most effective for liquid biofertilizer samples. Five routinely used nucleic acid extraction procedures were compared based upon quantity and purity of extracted DNA.The quantity of total DNA recovered by each extraction method was determined andcompared. Among the five methods followed for rapid DNA extraction and strain authentication, simple boiling of cells in water gave high quality and quantity DNA and positive for Sequence characterized amplified regions (SCAR) PCR followed by TE buffer extraction. The SDS and lysozyme based methods yielded less quality DNA and are not suitable for SCAR PCR.

KEY WORDS :
DNA extraction, Polymerase chain reaction, Biofertilizer

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BACKGROUND AND OBJECTIVES

In the context of both the cost and environmental impact of chemical fertilizers, excessive reliance on the chemical fertilizers is not a viable strategy in the long run because of the cost, both in domestic resources and foreign exchange, involved in setting up of fertilizer plants and sustaining the cooperatives or private sector production. Therefore, biofertilizers would be the viable option for farmers to increase productivity per unit area.Among the bacterial bioinoculants, *Azospirillum* and phosphate solubilizing bacteria shared nearly half of the total annual production. For example, in India, it was reported an increased *Azospirillum* bioinoculant production from 1174 t in 2005 (11.5 % of total bioinoculant) to 9985 t in 2014, which occupies nearly 20 per cent of annual bioinoculant production (NCOF, 2014).

Most of the microbial bioinoculants and organic fertilizers available in the market have

to address a common problem: quality control and its regulation. Quality is the key factor for the success of any product and is applicable for microbial inoculants too. The development of PCR-based molecular markers for bioinoculant strains, allowing the detection of a specific DNA sequence of the target offers the possibility to differentiate it even from closely related strains of same species (Olive and Bean, 1999). Sequence characterized amplified regions (SCAR) markers are based on the inherent genomic variability of a strain from others allow for rapid identification of it from a complex sample. Hence, SCAR markers could be a potential tool, as a new molecular quality standard, for strain authentication of commercial inoculants. However, the quality of results from such molecular techniques depends upon anefficient recovery of bacterial DNA from the sample. Efficiency of DNA extraction typically is affected by various factors, such as incomplete cell lysis, DNA sorptionto particulate material, and degradation or damage of DNA (Miller *et al.*, 1999).

Protocols for DNA extraction vary from in situ lysis of cells to less direct methods that extract the cells from samples prior to lysis. These approaches often involve combinations of detergents, physical disruption, solventextraction, and enzymatic lysis to obtain crude extracts of nucleic acid. Usually, two factors have to be particularly considered during the extraction procedure. The first is to maximize the DNA yield. The second is to ensure that the extracted DNA is amenable to several enzymatic treatments like PCR amplification (Spaniolas *et al.*,2008). In other words, the greatest challenge is the extraction of high-quality PCR-compatible DNA from the samples. Several methodshave been evaluated for bacterial cell wall lysis and DNA extraction using detergents, proteolytic enzymes, lysozyme, mechanical disruption, temperature changes alone or invarious combinations, DNA extraction kit, etc. Although the DNA extraction kit method is convenient, rapid and highly efficient, it is not widely applied on account of its high cost (Aldous *et al.*,2005). While various methods for DNA extraction from different samples have been published, there is little comparative information of these methods that would indicate which are superior in regards to extraction efficiency and DNA purity.Thus, this study compared five routinely used nucleic acid extraction procedures. These different extraction methods have contrasting physical, chemical, and enzymatic protocols, and were evaluated with regards to the quantity of total DNA extracted from commercial liquid biofertilizer sample. In this study, 5 methods of extracting bacterial DNA were compared and the main purpose of this study was to establish an economical, simple, and convenient method for extracting genomic DNA from the commercial liquid biofertilizer for its detection as a quality control using SCAR PCR.

RESOURCES AND METHODS

Sample preparation :

For this, liquid formulation of biofertilizer *Azospirillum lipoferum* bioinoculants (Az204) obtained from Tamil Nadu Agricultural College (TNAU) were prepared as described byVendan and Thangaraju (2007) under lab-scale. The standard quality parameters of these commercial preparations were assessed by following BIS standards (BIS, 2000) which includes pH, viable cell count and contaminants.

DNA preparation :

In order to develop a protocol for quick DNA extraction using SCAR marker based authentication of mother cultures of bioinoculant, five simple methods were compared for their efficiency to yield PCR-ready-DNA. The methods adopted are as follows:

Method 1 : Boiling method: The boiling method mainly uses a boiling step. The bacterial pellet was re-suspended in 100 µL of sterile distilled water and the tubes were incubated at 95°C for 20 min and centrifuged at 14000 × *g* at 4°C for 3 min. The supernatant was transferred to a clean tube and used for spectrophotometric and PCR analysis.

Method 2 : TE Boiling and extraction method: The pellet was suspended in 200 µL TE buffer [10 mMTris-HCl (pH 8.0), 1 mM EDTA], and the mixture was briefly mixed on a vortex mixer. The suspension was placed in a boiling water bath for 1 min, subjected to 3 freeze-thaw cycles alternating between -70°C for 3 min and 100°C for 2 min and then centrifuged at 10000 x g for 5 min. A 100 µL aliquot of the supernatant was transferred to a sterile tube and used for spectrophotometric and PCR analysis.

Method 3 : SDS Method: The pellets were treated as described above for the TE buffer, except that 200 µL of the non-ionic detergent mix *i.e.* 2% SDS was substituted for the TE buffer.

Method 4 : Water boiling and extraction method: A 200 µL aliquot of ultrapure water was added to the pellet and the suspension was treated as described above for the TE buffer.

Method 5 : Lysozyme method:The pellet was suspended in 100 µL of 200 mM $CaCl_2$ and 1 % lysozyme and incubated at 42°C for 2–5 min. After incubation, 300 µLof 96 % ethanol was added; the samples were mixed briefly by vortexing; and DNA was collected by centrifugation at 13,000 rpm for 5 min. Precipitated DNA was air dried at room temperature for 10 min and dissolved in 50 µL TE; cell debris was spun down by brief centrifugation at 13,000 rpm for 2 min and supernatant containing purified DNA was directly used for the subsequent experiments.

Quantification of DNA :

DNA concentration of each extract was determined spectrophotometrically according to manufacturer specifications (Nanodrop, 2000, Thermo Scientific, USA), where DNA was quantified by measuring the A260 : A280 ratiominus the background as measured by the A230 value. All extracts were diluted until the measured DNA concentration was linear between at least two different dilutions. This minimized any error resulting from a loss of linear correlation between the measured absorbance and the actual DNA concentration of the extract.

Genomic DNA detection by different extraction methods :

For each method tested, the presence and quality of the extracted genomic DNA from one of the triplicate samples was analyzed using a 0.5% agarose gel containing ethidium bromide. Ten microliters of the DNA extracted by each method was added into the gel and electrophoresed for 30 min at 150 V. Gel images were acquired as tagged image file format (TIFF) files with a Gel Imaging System (Alpha imager TM1200).

Statistical analysis:

The mean was calculated for the DNA concentrations of the five samples obtained by each extraction method. A 95% confidence interval (CI) was then obtained for each mean. This CI was used to compare mean DNA concentrations from each extraction procedure.

OBSERVATIONS AND ANALYSIS

The mean concentration of DNA obtained from each extraction method is summarized in Table 1. Among the five methods followed for rapid DNA extraction and strain authentication, simple boiling of cells in water gave high quality and quantity DNA and positive for SCAR PCR followed by TE buffer extraction. The SDS and lysozyme based methods yielded less quality DNA and are not suitable for SCAR PCR.

Some important factors that should be considered when choosing a DNA extraction method are the time required to complete the extraction, the cost of extraction and the safety of the chemical reagents employed. Moreover, DNA fragmentation should be avoided duringthe extraction. For purposes of this study, the primary use of the extracted DNA was PCR amplification. Thus, fragmentation of the DNA during extraction was not measured. A variety of previously published DNA extraction methods were evaluated in this study. These methods employed different approaches to cell lysis and DNA extraction, including freeze-thawing and chemical lysis.

From the extraction methods already published forvarious bacteria, here compared five methods for extracting DNA from the sample. The aim of an extraction procedure is to obtain ahigh quality and high yield of DNA from the samples.The extracted DNA should contain the least amount of proteins, RNA, or any other PCR inhibitors (Deuter *et al.*,1995).Removing those inhibitors is one of the key factors for a successful PCR.SCAR-PCR results was found successful in

Table 1 : Performance of DNA extraction methods for rapid authentication of standard strains by SCAR marker PCR

Method	DNA extraction method	DNA yield (ng/ µL)	A260/280	SCAR marker amplification
1.	Boiling water	1150.97(± 141.16)a	1.94 (± 0.01)a	+++
2.	TE boiling and extraction	920.10 (± 191.67)a	1.92 (± 0.04)a	+++
3.	SDS boiling and extraction	586.00(± 132.52)ab	1.75 (± 0.02)ab	-
4.	Water boiling and extraction	792.67 (± 57.65)ab	1.88 (±0.02)ab	+++
5.	Lysozyme method	185.60 (± 13.16)b	1.79 (±0.02)b	-

Values are mean (± SE) of three replicates and values followed by the same letter in each column are not significantly different from each other as determined by DMRT ($p \leq 0.05$).

removal of these inhibitors. DNA absorbance was measured at 260 nm (*A*260) toevaluate the quantity of the extracted DNA, and the ratio of the absorbance at 260 nm to that at 280 nm (*A*260/280) was used to evaluate the DNA quality. This method wasemployed previously by other researchers to compare different DNA extraction methods (Ki *et al.*, 2007).

In this study, the results of the concentration andthe purity for each method were correlated. The SDS and lysozyme methods seemed to yield a lower quantity of DNA and also their *A*260/280 ratio indicated a high protein contamination. On the other hand, the concentrations of the DNA obtained using the TE boiling and extraction and water boiling and extraction methods given considerable good amount of DNA and also showed *A*260/280 ratio was a high purity of theDNA obtained. When applied to engorged individuals, the boiling water method resulted in 100% successful DNA amplification having high concentration and purity of DNA.

Bacterial lysis is the key to obtain bacterial DNA. Although the SDS and lysozyme methods can provide the DNA yield, the SDS residue inhibits the PCR process. This result is consistent with that reported by Khan and Yadav (2004). The excessive SDS above 0.01%has been shown to inhibit PCR by denaturing the Taqpolymerase. Theoretically, column-purified DNA should be the cleanest, containing the least PCR-inhibitory substances. One purpose of this study was to identify a method forrapid DNA extraction that did not compromise PCR sensitivity. Results showedthat for PCR, column purification was unnecessary for DNA extracted from the sample. Compared to normal traditional DNA extraction that takes several hours and other method by DNA extraction kit which also atleast requires two hours, DNA extraction with boiling method can be completed within less than 1 h, and it does notinvolve 3-4 transfers of samples to new tubes.

Conclusion :

From the above study, it was standardized that commercial biofertilizer of *Azospirillum lipoferum* strain Az204 of TNAU can be extracted by boiling water extraction or TE based freezing and thawing method could be used for SCAR-marker based detection. In conclusion, of the five extraction methods evaluated,the boiling method is technically simpler, extremely easy,less expensive, and more rapid than the DNA extraction kit method; it is the best method for extracting genomic DNA from the samples. Although the extraction with TE buffer and ultrapure water is and inexpensive, the DNA yield is the lowest. The SDS and lysozyme method consistently inhibit PCR, therefore, they cannot berecommended for DNA extraction from the sample.

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REFERENCES

Aldous, W.K., Pounder, J.I. Cloud, J.L. and Woods, G.L. (2005). Comparison of six methods of extracting *Mycobacterium tuberculosis* DNA from processed sputum for testing by quantitative real-time PCR. *J. Clin. Microbiol.*,**43**: 2471-2473.

Deuter, R., Pietsch, S., Hertel, S. and Muller, O. (1995). A method for preparationof fecal DNA suitable for PCR. *Nucleic Acids Res.*, **23** : 3800-3801.

Khan, I.U. and Yadav, J.S. (2004). Development of a single-tube, cell lysisbased, genus-specific PCR method for rapid identification of mycobacteria: optimization of cell lysis, PCR primers and conditions, and restriction pattern analysis. *J. Clin. Microbiol.*, **42** : 453-457.

Ki, J.S., Chang, K.B., Roh, H.J., Lee, B.Y., Yoon, J.Y. and Jang, G.Y. (2007). Direct DNA isolation from solid biological sources without pretreatments with proteinase-K and/or homogenization through automated DNA extraction. *J. Biosci. Bioeng.*, **103** : 242-246.

Miller, D.N., Bryant, J.E., Madsen, E.L. and Ghiorse, W.C. (1999). Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. *Appl. Environ. Microbiol.*, **65** : 4715-4724.

NCOF (2014). Annual Report 2013-14. National centre for Organic farming, Ghaziabad, India.

Olive, D.M. and Bean, P. (1999). Principles and applications of methods for DNA-based typing of microbial organisms. *J. Clin. Microbiol.*, **37** : 1661-1669.

Spaniolas, S., Tsachaki, M., Bennett, M.J. and Tucker, G.A. (2008). Evaluation of DNA extraction methods from green androasted coffee beans. *Food Control.*, **3** : 257-262.

Vendan, R. and Thangaraju, M. (2007). Development and standardization of cyst based liquid formulation of *Azospirillum* bioinoculant. *Acta Microbiol. Immunol. Hung.*, **54** :167-177.