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Fast Protocol for DNA Isolation of DNA from Bacterial Isolated from a Hyper-Arid Environment

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The extraction of high quality genomic DNA for PCR amplification from phosphate solubilising microbes (gram positive) is complicated due to the presence of a high percentage of secondary metabolites or PCR inhibitors which bind to or co-precipitate with nucleic acids during DNA extraction. In the present study we report a modified sodium dodecyl sulfate/phenol protocol that includes elimination of lysis solution to the bacteria cell wall and the washing of pellet with ethanol. The present study the relation A_{260}/A_{280} and A_{260}/A_{230} were of 1.84 ± 0.17 and 1.92 ± 0.62, respectively. These results showed that the DNA fraction is pure and may be used for PCR analysis future. To confirm this, the DNA's purity was evaluated across a PCR amplification of fragment of the *16S* gene using cell´s biomass from native phosphate solubilising bacteria (gram positive). Finally, the advantages of this procedure is that in the DNA extraction is not necessary the use of Proteinase K, and sonication to lyse samples prior to or in conjunction with lysis solutions.

Key words: DNA extraction, Native phosphate solubilising bacteria, Gram positive, PCR.

Phosphorus (P) is one of the major essential macronutrients for plants (making up about 0.2% of a plant's dry weight), which is applied to the soil in the form of phosphatic manure (Zhao and Lin, 2001). This element is a component of key molecules such as nucleic acids, phospholipids, and ATP, and, consequently, plants cannot grow without a reliable supply of this nutrient. Pi is also involved in controlling key enzyme reactions and in the regulation of metabolic pathways (Vance 2003). In the Mexicali Valley, which is contiguous with the Imperial Valley in southern California, is a desert environment, characterized by high production of crops (Vargas-Bejarano *et al*., 2012). However, during the applications of phosphorus (P) fertilizer at crops, one great amounts of this element is converted to insoluble forms in the calcareous soils, limiting long term ecosystem productivity (Walpola and Yoon, 2012). In recent year, the used of Phosphate solubilising microbes (PSM) have been reported. In this sense, Seshachala and Tallapragada, (2012) report that phosphate solubilising microbes (PSM) are known to bring about mobilisation of insoluble phosphates and this can stimulate plant growth even under the conditions of Phosphorus deûciency. In this sense, our laboratory has isolated native phosphate solubilising microbes from rhizospheres of native plants from Mexicali Valley

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that may contribute to their stimulatory effect on plant growth and demonstrate the capability to adaptability to the agroclimatic conditions (eg., temperature and salinity). In this context, the isolation of pure DNA from these native phosphate solubilising microbes is crucial for the study of gene expression because it is a prerequisite for several molecular biology techniques, including gene isolation with polymerase chain reaction (PCR), Southern blot, and the construction of DNA libraries (Rubio-Piña and Zapata-Perez, 2011).

A variety of methods are currently available for the isolation of genomic DNA from microorganism (Kumar *et al*., 2010), though the quantity and quality of DNA obtained by these methods is generally satisfactory, these protocols are not universally applicable at all microorganins (*eg*., native phosphate solubilising microbes). Some methods have been reported to minimize the DNA extraction steps but they need a large amount of biomass or time, and lysis steps such as enzymatic or sonication (Kumar *et al*., 2010). Therefore, the objective of this study was to develop a rapid protocol for the isolation of good quality total DNA from native phosphate solubilising microbes for the implementation of molecular biology techniques such as gene isolation by PCR (polymerase chain reaction).

MATERIALS AND METHODS

The rhizospheric soil adhered to roots (100 g) were collected in the native forest of *Pluchea sericea*, in the Valley Mexicali, located in Baja California, Mexico (32°26' 54.399" N 114° 57' 39.299"W). Sampling was performed around five trees and three subsamples of roots with adhering rhizospheric soil were collected and homogenized, resulting three composite samples. The serial dilution and pour plate method were used for the isolation of native phosphate solubilising microbes on Pikovskaya's (PKV) agar medium. Bacterial colonies showing phosphate solubilizing zone around them were considered as PSB. Pure culture of the isolates were then grown overnight in tripticase soy broth at 30°C without agitation and mixed with sterile glycerol (40% or 80%) for preservation at -81°C.

DNA extraction buffer and solutions

Total DNA from native phosphate solubilising microbes was extracted, based on the method of Gonzalez-Mendoza *et al*. (2008) with modifications. The glassware (tubes and bottles) were treated with 0.1% DEPC solution at 30 °C overnight, autoclaved twice at 121 °C for 20 min, and then dried at 100 °C before use.

Tips used for DNA extraction were DNase and RNase-free (Axygen®, USA). The extraction buffer was prepared used 2.5 % SDS (w/v), 0.6 mM EDTA and 0.2 mM hydroxymethyl-hydrochloride (Tris-HCl, pH 8.0). The extraction of nucleic acids was realised using a combination of chloroform/ phenol (1:1 v/v) previously prepared.

DNA extraction procedure

Extraction buffer (0.25 mL) was added to 200 mg of biomass of each native phosphate solubilising microbes, (previously obtained by centrifuge) and shaken vigorously for 10 s. Then equal volume of chloroform-phenol mix was slowly added and incubated at 65°C for 5 min. Finalized the period of incubation the mixture was centrifuged at 11000 x g, 4 °C for 5 min. The supernatant (80 µl) was transferred to a new microtube, and 0.6 fold volume of cold absolute isopropanol was added and mixed thoroughly for precipitating total DNA at -20 °C for 30 min; the mixture was then centrifuged at 11000 x g for 10 min at 4 °C. The supernatant was discarded and the pellet was re-suspended in 0.03 ml DEPC-treated MiniQuantum (deionised) water, and stored at 020 °C for further use. Concentration, yield, and quality control indices such as $A_{260/280}$ ratios and $A_{260/230}$ ratios were carried out with 3 µl of re-suspended total DNA using UV-Vis *Spectrophotometer*. Finally, 5 µl of total DNA solution were loaded onto 1.2 % agarose gel, which was stained with EtBr and electrophoresed to visualized DNA under UV light.

PCR Reaction

Specific DNA was amplified by PCR with Taq DNA polymerase according to the manufacturer's instructions (Invitrogen). The PCR analysis was performed according to the method described by Gonzalez-Mendoza *et al.* (2008), where 2 µl of DNA (20 ng) were used as PCR template.

The PCR reactions were performed using 16S rRNA gene with universal primers 27 forwad (AGA GTT TGA TCC TGG CTC AG) and 27 reverse (AAG GAG GTG ATC CAG CCG CA), PCR reactions

were carried out using the following protocol: 95 °C for 5 min (1 cycle), 55 °C for 40 seconds and 72 °C for 1 min (35 cycles). The quality of the PCR reactions was run in 1.5 % Tris acetate EDTA agarose gel, and bands were visualized by staining with ethidium bromide. Images were captured and stored with the Multidoc-It Digital Imaging system (UVP).

RESULTS AND DISCUSSION

The first step in the genetic characterization of any isolate of Phosphorus solubilizing microorganisms from soils is the obtaining of moderately pure, PCR amplifiable DNA from the cells. However, the presence of a high peptidoglycan content in the cell walls of microorganism (*eg., Bacillus* spp.,), and associated secondary metabolites or PCR inhibitors could be responsible for the absence of nucleic acids during DNA extraction (Sachinandan *et al*., 2010). In the actuality, many protocols are laborious, time-consuming, and, moreover, restricted to certain types of bacteria (Kleines *et al*., 2003). In the present study we adapted a rapid RNA isolation method from plants (Gonzalez-Mendoza *et al*., 2008) combining SDS extraction buffer without lysis solution of the bacteria cell wall. Additionally, the washing of pellet with freshly prepared 75% ethanol is not necessary because produced poor results. The present study showed good yields of high-quality genomic DNA (Figure 1). Where, the relation A_{260}/A_{280} and A_{260}/A_{230} were of 1.84 ± 0.17 and 1.92 ± 0.62, respectively. These results showed that the DNA fraction is pure and may be used for PCR analysis future. To confirm this, the DNA's purity was evaluated across a PCR amplification of fragment of the *16S* gene using cell´s biomass from native phosphate solubilising bacteria.

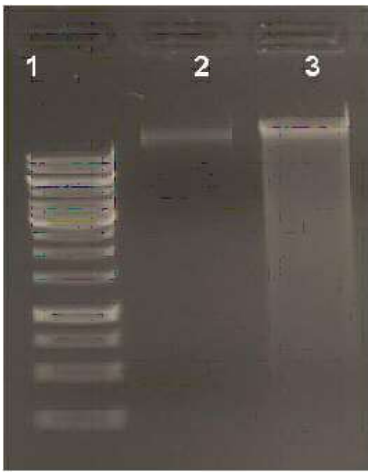

Fig. 1. Gel electrophoresis of the total DNA extracted from *Bacillus subtilis* strain Bs-Cach Lane 1: DNA marker; lane 2 to 3: DNA isolated from *Bacillus subtilis* strain Bs-Cach

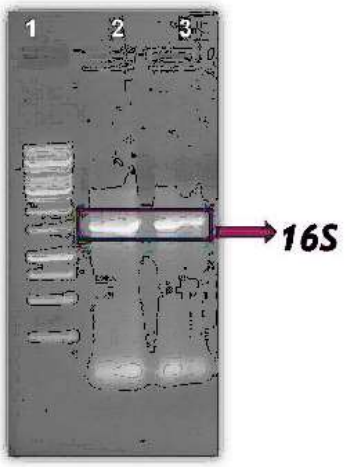

Fig. 2. Agarose electrophoresis of the RT-PCR products Lane 1: DNA marker; lane 2 to 3: *16S* gen, 1500 pb

In this sense, the results showed that PCR amplification produced a single band of approximately 1500 bp specific to *16S* gen (Figure 2) corresponding to the presence *Bacillus subtilis* strain Bs-Cach, (*Genbank,* number accession: KC256786). Finally, the advantages of this procedure is that in the DNA extraction is not necessary the use of Proteinase K, sonication, and grinding in liquid nitrogen to lyse difficult samples prior to or in conjunction with lysis solutions.

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