Therefore, an effort in this direction is likely to bring a significant breakthrough for diagnosis of listeriosis which is a neglected disease in various tropical countries, including India.

Conflict of interest: Authors declare no conflict of interest.

1. http://www.searo.who.int/topics/listeria_infections/en/ (accessed on 1 June 2016).
2. https://www.cdc.gov/listeria/ (accessed on 1 June 2016).
3. Yang, L., Banada, P. P., Liu, Y. S., Bhunia, A. K. and Bashir, R., *Biotechnol. Bioeng.*, 2005, **92**, 685–694.
4. Ueda, S. and Kuwabara, Y., *Biocontrol Sci.*, 2010, **15**, 91–95.
5. Liu, D., *J. Med. Microbiol.*, 2006, **55**, 645–659.
6. Soni, D. K. and Dubey, S. K., *Mol. Biol. Rep.*, 2014, **41**, 8219–8229.
7. Soni, D. K., Singh, R. K., Singh, D. V. and Dubey, S. K., *Infect. Genet. Evol.*, 2013, **14**, 83–91.
8. Soni, D. K., Singh, M., Singh, D. V. and Dubey, S. K, *BMC Microbiol.*, 2014, **14**, 241.
9. Soni, D. K., Singh, D. V. and Dubey, S. K., *J. Microbiol.*, 2015, **53**, 653–660.
10. Koo, K. and Jaykus, L. A., *Lett. Appl. Microbiol.*, 2000, **31**, 187–192.
11. Burtscher, C. and Wuertz, S., *Appl. Environ. Microbiol.*, 2003, **69**, 4618–4627.
12. Rodriguez-Lazaro, D., Pla, M., Scortti, M., Monzo, H. J. and Vazquez-Boland, J. A., *Appl. Environ. Microbiol.*, 2005, **71**, 9008–9012.
13. O'Grady, J., Sedano-Balbas, S., Maher, M., Smith, T. and Barry, T., *Food Microbiol.*, 2008, **25**, 75–84.
14. Ligaj, M., Oczkowski, T., Jasnowska, J., Musiał, W. G. and Filipiak, M., *Pol. J. Food Nutr. Sci.*, 2003, **12**, 61–63.
15. Farabullini, F., Lucarelli, F., Palchetti, I., Marrazza, G. and Mascini, M., *Biosens. Bioelectron.*, 2007, **22**, 1544–1549.
16. Gao, H. W., Qin, P., Lin, C., Shang, Z. M. and Sun, W., *J. Iran. Chem. Soc.*, 2010, **7**, 119–127.
17. Wu, L. W., Liu, Q. J., Wu, Z. W. and Lu, Z. H., *Yi Chuan*, 2010, **32**, 512–516.
18. Sun, W. *et al.*, *Electrochim. Acta*, 2012, **85**, 145–151.
19. Kashish, Soni, D. K., Mishra, S. K., Prakash, R. and Dubey, S. K., *J. Biotechnol.*, 2015, **200**, 70–76.

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A rapid and effective method for extraction of bacterial DNA from crude oil-contaminated soil

Crude oil contamination causes a major environmental problem. The crude oil changes the microbial population present in the soil, thereby affecting its properties[1]. The microbial community present in crude oil-contaminated soil has significant ecological importance. These microbes can be used as an effective tool for bioremediation. It has been well documented that soil contains the highest level of prokaryotic diversity than any other environment. One gram of soil can have more than 10 billion microorganisms comprising different species[2]. Two approaches can be used for isolating bacterial DNA from the soil. These are culturable and unculturable techniques, the latter being more effective as less than 1% of bacteria can be cultured in artificial media[3]. The basic steps in any unculturable technique comprises (a) processing of soil samples, (b) extraction of bacterial DNA directly from the soil, (c) downstream analysis of DNA which includes sequencing, phylogenetic analysis and other multivariate data analysis. The extraction of DNA is the most important step, as the purity of DNA determines its use for further downstream analysis. Sometimes contaminants such as humic acid precipitate with the DNA and hinder its amplification[4]. Various kits are available commercially for extraction of soil bacterial DNA, but only a few give good results with crude oil-contaminated soil[5]. Moreover, commercial DNA extraction kits are more expensive. Few reports have been documented on extraction of DNA from the soil using manual method[6]. However, no reports are available for the extraction of DNA from crude oil-contaminated soil. Therefore, we have developed a rapid, cost-effective method for extraction of pure DNA from crude oil-contaminated soil.

Soil samples were collected from the surrounding areas of Digboi oil refinery, i.e. Dubbs Para and Digboi Cenetary Park, Assam, India. They were processed by drying in a hot-air oven under 100°C for 2 h and sieved in a 2 mm mesh to separate the debris. Then 10 g of soil sample was weighed and 27 ml of extraction buffer (Tris-HCl, 100 mM EDTA, 1.5 M NaCl and 1% CTAB, pH 8) was added. Next, 1 ml of lysozyme (10 mg/ml) was added to this mixture (soil and extraction buffer) and incubated at 37°C for 2 h with continuous shaking at 150 rpm. Then 100 µl of proteinase K (10 mg/ml) and 3 ml of 20% SDS were added and the mixture was incubated at 65°C for 2 h in a water bath. After final incubation, the solution was centrifuged at 5981 rpm for 10 min. Subsequently, 8 ml of the supernatant was transferred to 50 ml centrifuge tube and equal volume of phenol : chloroform : isoamyl alcohol was added. The mixture was vortexed at maximum speed for 5 min. The aqueous phase was collected and 0.6 volumes of chilled ethanol was added and allowed to precipitate for 30 min. After precipitation, the solution was centrifuged at 8459 rpm for 10 min. The supernatant was discarded and pellet was dissolved in TE buffer. DNA was visualized by loading 4 µl in agarose gel (0.8%) for 50 min. The experiment was conducted in three replicates and repeated five times.

Table 1. DNA concentration and purity using manual method and DNA extraction kit

Method	Site	DNA concentration (ng/µl)	$A_{260/280}$
Manual extraction	Dubbs Para	15.77 ± 0.01	1.76
Manual extraction	Digboi Cenetary Park	17.57 ± 0.45	1.74
DNA isolation kit	Dubbs Para	8.05 ± 0.77	1.80
DNA isolation kit	Digboi Cenetary Park	8.50 ± 0.31	1.80

Critical difference (CD) = 1.689 for DNA concentration; CD computed at $P = 0.05$; ± Standard deviation (SD).

Table 2. Concentration of 16S rDNA using the manual method and DNA extraction kit

Method	Concentration (ng/µl)	$A_{260/280}$
Manual extraction	57.8 ± 1.57	1.73
DNA isolation kit	45.2 ± 1.23	1.8

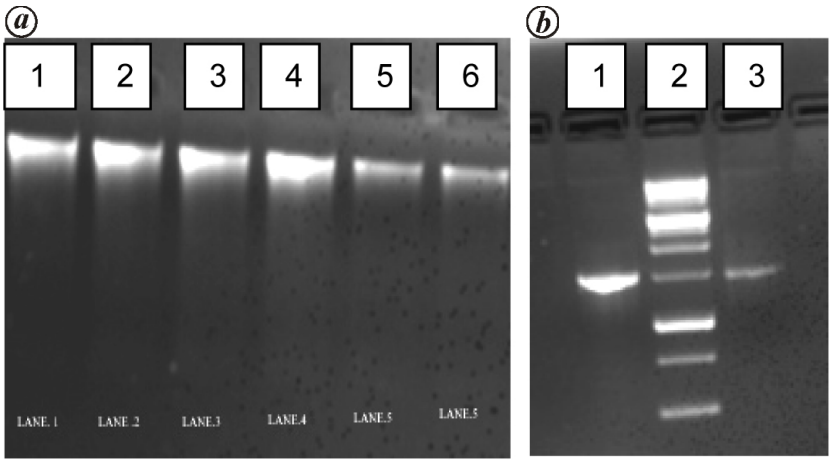

Figure 1. ***a***, Agarose gel (0.8%) showing bands of DNA extracted by the manual method and commercial kit. Lanes 1–4, DNA extracted by the manual method for site Dubbs Para (lanes 1 and 2), Digboi Cenetary Park (lanes 3 and 4). Lanes 5 and 6, DNA extracted using the commercial kit; 4 µl of DNA was loaded for each sample. ***b***, Lane 1, 16S rDNA amplification of the manually extracted soil bacterial DNA. Lane 2, 1 kb DNA ladder. Lane 3, 16S rDNA amplification of DNA isolated using the commercial kit was loaded in 1.5% agarose gel.

We observed clear bands on the gel for all sites. The intensity of the bands was more than that using the commercial kit (Cat 1288-50; Figure 1 *a*). The DNA extracted by both methods was quantified using BioSpectrometer (Table 1). The yield of DNA extracted by manual method was significantly higher ($P = 0.05$) than that using the commercial kit. The concentration of DNA extracted by manual method was 16.67 ng/µl in comparison with that using the commercial kit (8.05 ng/µl). Also, 16S rRNA gene amplification of DNA extracted manually was done to determine whether the DNA can be further used for downstream analysis. Fifty microlitres of reaction mixture was prepared which consisted of 5 µl 10× buffer, 3 µl dNTP solution, 3 µl FD1 and RP2 primer (10 pmol each), 4 µl DNA template, 2.5 U *Taq* polymerase and 30 µl nuclease free water. The PCR cycle was adjusted as initial denaturation of 95°C for 5 min and 94°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 min. The reaction was set for 30 cycles. The product was visualized in 1.5% agarose gel for 2 h. A clear ~1.5 kb band of DNA was observed for both the methods (Figure 1 *b*). The concentration of DNA extracted by the manual method was more in comparison with that using the commercial kit (Table 2). Therefore, there was no interference by contaminants present in the soil during amplification.

In conclusion, this is a rapid and cost-effective method for the extraction of bacterial DNA directly from crude oil-contaminated soil to study bacterial community structure. The efficiency of this protocol will largely depend on processing of the soil, which in turn will depend upon the type of soil samples.

1. Abbasian, F., Lockington, R., Megharaj, M. and Naidu, R., *Curr. Microbiol*., 2016, **72**, 663–670.
2. Delmont, T. O. *et al*., *Appl. Environ. Microbiol.*, 2011, **77**, 1315–1324.
3. Scholss, P. D. and Handelsman, J., *Curr. Opin. Biotechhnol.*, 2003, **14**, 303–310.
4. Tebbe, C. C. and Vahjen, W., *Appl. Environ. Microbiol*., 1993, **59**, 2657–2665.
5. Jurelevicius, J. D. *et al*., *Appl. Environ. Microbiol*., 2013, **79**, 5927–5935.
6. Zhou, J., Bruns, M. A. and Tiedje, J., *Appl. Environ. Microbiol.*, 1995, **62**, 316–322.

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