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Heat Resisting Bacteria from Soil: A Simple Method for Isolation and Extraction of DNA

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During the period January/ 01/ 2012 to February /02 /2013, hundred fifty soil samples were collected from Draia, Nasiria and Umalhamaam, Riyadh Saudi Arabia. Soil suspension was obtained by using pyrophosphate to separate soil particles. Small amount of soil (0.3g) were centrifuged at 5000 g/min for 10 minutes. Serial dilution and plating on nutrient agar solid medium suitable to the bacteria was adopted. In order to obtain DNA from bacterial soil, we used a method based on direct lysis of cells. Many methods have been described for isolating DNA from prokaryotic cells. The choice of method depends on the degree of the purity of the DNA required for the analysis to be performed. We have adopted simple and rapid method allowing DNA to be released from bacterial cells. In general, techniques with extraction kits were easily and rapidly performed but were more expensive than the manually prepared in our laboratory. DNA was released from bacterial cells by an organic method and alcohol precipitation was followed and was electrophoresed. Gram, single staining and sensitivity test of bacterial colonies from soil against some antibiotics were also performed.

Key words: Heat resistant, Bacteria, DNA isolation, Soil, Antibiotics.

Specific endospore formers have become important contaminants in industrial food processing. The direct or indirect soil route of contamination or dispersal is the start of events or processes in the agro food chain that eventually leads to important problems or concerns for food safety and/or quality (Schliemann *et al*; 2006). Antibiotic resistance genes are typically isolated by cloning from cultured bacteria or by polymerase chain reaction (PCR) amplification from environmental samples. These methods do not access the potential reservoir of undiscovered antibiotic resistance genes harbored by soil bacteria because most soil bacteria are not cultured readily, and PCR detection of antibiotic resistance genes depends on primers that are based on known genes (Christian *et al*; 2004). The genetic relatedness among *Bacillus cereus* group strains was assessed by multilocus sequence typing (MLST) using an optimized scheme based on seven chromosomal housekeeping genes. The *B. cereus* group constitutes a coherent population unified by the presence of ubiquitous and specific genetic elements which do not show any pattern, either in their sequences or genomic locations, which allows differentiating between the member species of the group. Nevertheless, the population is very dynamic, as particular lineages of clinical origin can evolve to form clonal complexes. (Guinebretière *et al*; 2008). Isolating and characterizing DNA sequences for use in molecular methods are integral to evaluating microbial community diversity in soil. Any isolation protocol should maximize nucleic acid isolation while minimizing copurification of enzymatic inhibitors. Although several methods that focus on extraction of total community DNA from environmental soil and water samples have been published the lack of a standard nucleic acid isolation protocol reflects the difficulty in

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accomplishing these goals, most likely due to the complex nature of the soil environment (Kweku *et al*;2009). A rabid isolation method of campylobacter DNA using mechanical disruption combined with the guanidine-based reagent DNAzol has been developed by (Englen *et al*; 2000). Osmo-tolerant bacteria from the Thar soils were isolated and characterized. Osmotic tolerance capacity of isolates was examined on glycerol, NaCI and alcohol; and sequencing of 16S rRNA gene was also performed for bacterial identification (Sharma *et al*; 2013). Several spore formers either need or tolerate specific conditions for germination and growth, which all can occur in food even in combination, such as low or high temperatures and anaerobic or acidophilic conditions (Marc Heyndrickx 2011). Bacteria that are hidden in the soil need more attention and investigation. Advice and awareness of the pathogenic microorganism hidden in the soil is necessary for children playing in the gardens around the residences with soil. For the past few years, bacteria were changing and will keep on changing and resistances to antibiotics are increasing and are distributed widely in different habitats and may or may not possess unique physiological properties.

MATERIALS AND METHODS

During the period January 01/ 2012 to February 02 /2013, hundred fifty soil samples were collected from Direa, Umalhamaam and Nasiria areas (50 samples each), Riyadh Saudi Arabia. From each of the three areas, triplicates of 0.3 gram specimen were taken. Soil suspension was obtained by using 10ml pyrophosphate and triplicates of 0.3 gram specimen in 15mL Screw Cap Conical Bottom Centrifuge Tubes mixed for 5 minutes and were centrifuged at 5000 g/min for 10 minutes. One ml of each supernatant was taken and was serially diluted in 9ml of sterile distilled water and 100µl from each diluted tube was smeared on nutrient agar plates and were incubated at 70^0C over nigh. As bacterial shape is indicative of the bacterial prototype, Colonies of overnight growth were single stained by using crystal violet dye and were also gram stained with crystal violet and safranin dyes. Sensitivity test of bacterial colonies from soil against some antibiotics were also performed. In order to obtain a rapid isolation of DNA from bacterial soil, we used a method based on direct cell lysis of non-ionic detergents (Triton X-100 treatment) (Robert *et al*; 2008). The concentration and purity of DNA was determined spectrophotometric ally (BIO-RAD Smart Spec 3000; USA); for this purpose, DNA absorbance was measured at 260 nm (¼g DNA/g sample; 1 A 260 = 50 ¼ g / mL DNA) and protein impurities were checked at 280 nm. The quality of the extracted DNA was evaluated by the $A_{260/280}$ ratio, and values obtained was close to 1.8 indicating a good DNA extract with little protein contamination.

RESULTS

Soil suspension was obtained by using pyrophosphate to separate soil particles. Small amount of soil (0.3g) were centrifuged at 500g/min

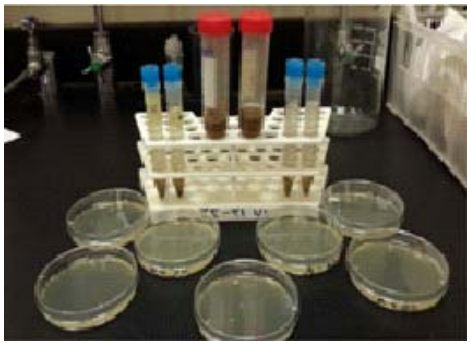

Fig. 1. The soil samples were centrifuged. The superminants were inoculated in the petridishes and then after drying were incubated at 70°C overnight

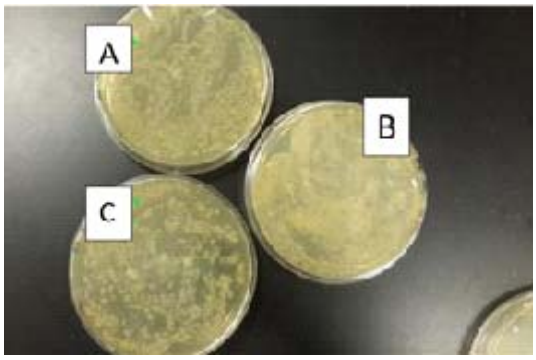

Fig. 2. Plages A, B and C were inoculated 100 µl each of different serial diluation 0,001, 0.0001 and 0.00001 respectively

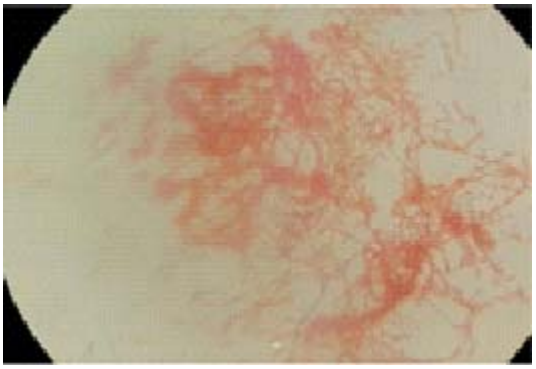

Fig. 3. Heat resistant bacteria from soil stained with safranin only show rode shaped streptobacilli

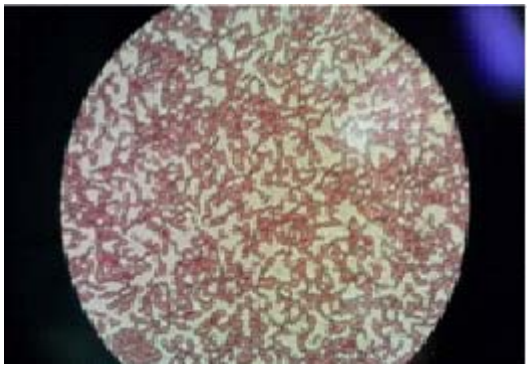

Fig. 4. Heat resistant bacteria isolated from soil stained with crystal violet and safranin showed gram negative

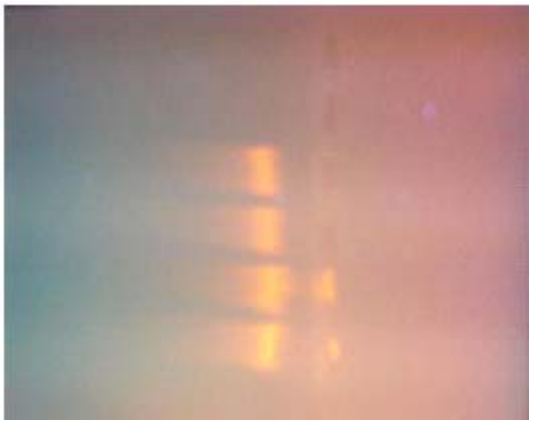

Fig. 5. Results of Deoxyribonucleic acid (DNA) electrophoresis from colonies of heat resistant bacterial soil

Fig. 6. Isolates from soils showing sensitive to Neomycin (NE) 30 micro gram and Gentamicin (GM) 10 microgram

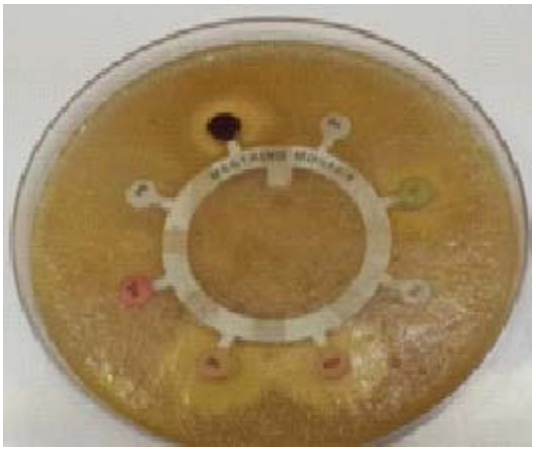

Fig. 7. Isolates from soil showing sensitive to : Neomycin (NE) 30 micro gram. Gentamicin (GM) 10 micro gram, Chloramphenicol (C) 30 micro gram and Tetracycline (T) 30 microgram

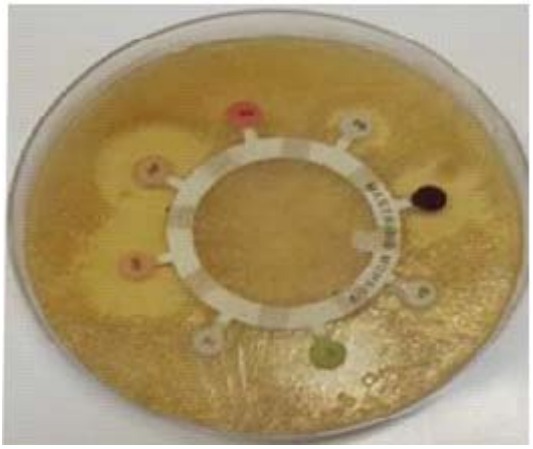

Fig. 8. Isolates from soil showing sensitivity to Chloramphenicol (C) micro gram, Gentamicin (GM) 10 microgram, Neomycin (NE) 30 micro gram, Penicillin g (PG) 10 units and tetracycline (T) 30 microgram

for 10 minutes see (Fig1). Serial dilutions and plating on nutrient agar solid medium suitable to the bacteria were adopted. Inoculated different serial dilution of bacterial suspension on nutrient agar plates and incubated at 70^0C. Bacterial growths of overnight incubation see Fg2. Single staining results see Fig3. And Gram staining results see Fig4. Based on direct cell lysis bacterial DNA was isolated form soil by treatment of non-ionic detergents" Triton X-100" see Fig5.Some colonies of the bacterial isolates were sensitive to Neomycin(NE) and Gentamycin (GM) only see Fig6. Other colonies were sensitive to Neomycin (NE), Gentamycin (GM) and Chloramphenicol(C) and Tetracycline (T) see Fig7. Others showed resistance to Chloramphenicol (C) 30 microgram, Gentamicin (GM) 10micro gram, Neomycin (NE) 30 micro gram, Penicillin g (PG) 10 units and tetracycline (T) 30 microgram see Fig8.

DISCUSSION

Isolation of bacteria from soil and the extraction of bacterial DNA should be done very carefully and precisely. (Englen *et al*; 2000). Working on bacterial soil is very important as there is a clear association between soil-borne endospore forming bacteria and food contamination (Marc Heyndrickx 2011). Most antibiotics target a single gene or pathway, to get resistance bacteria need to alter pathway or gene or make a pump that takes the antibiotic out of the cell (Remco *et al*; 2005). Soil typically contain 109 to 1010 microorganisms per gram (dry weight), which may represent more than a million bacterial species. Under nutrient-limited conditions, vegetative cells of *Bacillus* species undergo the cell differentiation process of sporulation. The resulting spores are metabolically dormant and show besides resistance to heat and resistance to other potentially lethal treatments that include radiation, high pressure, chemicals, and desiccation. Although spore dormancy and associated resistance are very stable, spores may survive over hundreds and even millions of years. Bacteria in the phylum *Acidobacteria* are widely distributed and abundant in soils, but their ecological roles are poorly understood, owing in part to a paucity of cultured representatives (Stephanie *et al*; 2007). In order to obtain a rapid and specific detection test for bacteria in soil, we used pyrophosphate to separate soil particles and small amount of soil (0.3g) was centrifuged at 5000 g/min for 10 minutes. For bacterial genomic DNA isolation we used a method based on direct lysis of cells, treatments of non-ionic detergents (Triton X-100 (Robert *et al*; 2008). The number of methicillin resistant *Staphylococcus aureus* or MRSA infections has been increasing and become a serious problem in public heath worldwide. The use of bacteriocin as an alternative agent to overcome this problem is promising (Ratchaneewan *et al*; 2013). Accurate and early detection of methicillin-resistant *S. aureus* (MRSA) is great important for the management of infected patients and select the appropriate infection control measures. Accordingly, evaluation of the accuracy of the phenotypic and genotypic methods commonly used to determine the profile of antimicrobial resistance is essential to ensure that the most appropriate therapy is chosen (Mounir *et al;* 2013). In a study curried by Jewie Tian and his team obtained 97 halophilic or halotolerant eubacteria from saline-alkali soil in Shache County, Xinjiang Province, China, by selective media. (Jiewei Tian *et al ;* 2013). Antibiotic sensitivity test of nine Gram negative organisms isolated from septicemia patients were resistant to ampicillin, gentamicin, followed by nitrofurantoin, streptomycin, tetracycline, colistin, cotrimazole and nalixidic acid (Ella *et al*; 2007).Our findings of bacterial soil resistant to antibiotics such as Chloramphenicol (C) 30 microgram, Gentamicin (GM) 10micro gram, Neomycin (NE) 30 micro gram, Penicillin g (PG) 10 units and tetracycline (T) 30 microgram is a signal of the need for percussions of the bacterial pathogens in the soil and of the Specific endospore formers that have become important contaminants in industrial food processing.

CONCLUSION

In many types of food and feed, soil can be considered as the initial contamination source for spore formers. Usually, when direct transfer from soil is involved, levels of these spore formers in foods, ingredients, or feeds are too low to cause problems. However, because of the complexity of the food chain, particular spore-forming species or types may encounter niches where proliferation

occurs. This can happen on the primary production level like silage, bedding material, in the processing line like storage tanks, during distribution such as temperature abuse during refrigerated storage, or in the final product, complex foods, fruit juice. These proliferation steps enable the endospore former such as *B. cereus*, *A. acidoterrestris*, *C. tyrobutyricum*, non-proteolytic *C. botulinum* or *C. estertheticum*, either to enter as a contaminant from the dust into a next step of the production chain or to provoke food quality or safety problems in the final product. Families for the safety of their children playing in the recreation gardens with dust contaminated pathogens resistant to(Chloramphenicol 30 microgram), (Gentamicin) 10micro gram, (Neomycin) 30 micro gram, (Penicillin g) 10 units and (Tetracycline) 30 microgram as our research findings show, can be ingested with food or injected into cuts on the skin. It is a challenge to gain insight into the whole contamination flow of endospore formers originating from soil as well as in the conditions permitting their proliferation.

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