Whole Cell Protein Profiling of Human Pathogenic Bacteria Isolated from Clinical Samples

M. Mahendrakumar and M. Asrar Sheriff

1P.G and Research Department of Plant Biology and Plant Biotechnology, Government Arts College for Men (Autonomous), Nandanam, Chennai, Tamil Nadu, India
2P.G and Research Department of Zoology, The New College, Chennai, Tamil Nadu, India

Corresponding Author: M. Asrar Sheriff, P.G and Research Department of Zoology, The New College, Chennai, Tamil Nadu, India Tel: +91-9840433658

ABSTRACT

The present investigation deals with the protein profiling of clinical pathogens isolated from clinical samples. For the purpose, four different bacteria were isolated from the clinical samples and biochemically characterized. The 16S rRNA sequence analysis revealed the isolated bacteria were Escherichia coli, Staphylococcus epidermidis, Bacillus subtilis, and Klebsiella pneumonia. The bacteria were cultured in liquid medium and the protein profile of the bacteria was investigated through ultra-sonication followed by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis. The results revealed that the unique protein bands observed in the bacteria at defined size can act as biomarker for the identification and differentiation of the bacteria from the other species.

Key words: Biomarkers, whole cell protein profiling, 16S rRNA analysis, SDS-PAGE

INTRODUCTION

Many bacterial pathogens including opportunistic pathogens also act as commensals in our body. Proper diagnosis is required to distinguish these pathogens from indigenous bacteria. There are many specialized procedures employed to distinguish these organisms effectively. Identification of pathogens among the beneficial commensal bacteria can be quite challenging. Various bacterial membrane proteins are involved in adhesion, invasion and intracellular survival of pathogens in the human host and are hence, of potential interest in the search for drug targets or biomarkers (Carlsnohn et al., 2006). The protein profile of the bacteria seems to be the reflection of the genome of the strain; hence, the determination of the whole protein content plays an important role in their identification, classification and comparative studies (Kustos et al., 1998). SDS-PAGE is also an important molecular technique used for identification of bacteria at the species level (Durrani et al., 2008).

In microbiological analysis involving epidemiological outbreaks, it is necessary to obtain a more detailed characterization of the organisms by methods such as whole cell protein and plasmid analysis. Whole cell protein studies in bacteria using SDS-PAGE have been supported by many researchers to evaluate the whole cell lysate (Ardanuy et al., 1998; Aly et al., 2003). Electrophoretic analysis of whole cell provides a rough measure of the number and physio-chemical properties of gene products. SDS-PAGE of proteins has been used extensively for the identification and classification at the strain and species level (Katircioglu et al., 2003). In this study, four pathogenic...
bacteria of medical importance were isolated from the clinical samples and employed for protein profiling with a view to enable their easy identification and differentiation based on the presence of unique proteins. The present investigation deals with the protein profiling of clinical pathogens isolated from clinical samples.

**MATERIALS AND METHODS**

**Chemicals and reagents:** With the insight of getting accurate results, all the chemicals used in the present study were of analytical grade and were procured from Sigma Alrich, USA and SRL, India.

**Collection of clinical samples:** For the isolation of bacteria, the clinical samples such as blood, pus, sputum, etc. were collected from the patients of Voluntary Health Services (VHS) Chennai. The samples were collected in sterile containers, brought to the laboratory and stored in refrigerator till further processing.

**Isolation of bacteria:** The bacteria present in the samples were enumerated by swabbing the collected clinical samples using sterile nutrient agar plates. The plates were incubated for 3 days and observed for bacterial growth. The enumerated bacterial colonies were separated based on colony morphology and sub-cultured till the purity of bacterial cultures was ensured.

**Morphological and biochemical characterization of bacteria:** The morphological characteristics of the bacteria i.e., shape, motility, endospore producing ability, Gram’s properties and biochemical characteristics of the bacteria like oxidase, catalase, indole, methyl red, Voges Proskeaur and citrate utilization test were determined using standard methods.

**Molecular characterization of bacteria:** The isolated bacteria were ascertained to their respective systematic position by molecular characterization using 16S rRNA sequence analysis. For the purpose, the genomic DNA of the isolated bacteria was isolated using Cetyl Trimethyl Amino Bromide (CTAB) extraction procedure. The isolated bacterial DNA was confirmed and their size was determined using Agarose Gel Electrophoresis (AGE). The required 16S rRNA was further amplified using PCR (Thermocycler) in all the samples using universal primer set (B27F (5'-AGAGTTTTGATCCTGGCTCAG-3') and U1492R (5'-GGTTACCTTGTTACGACTT-3')). The purity and size of the amplified product was tested using AGE. The sequencing of the amplified 16S rRNAs was performed using ABI3100 automated sequencer. Using the obtained sequence, the isolated bacteria were identified using Basic Local Alignment Search Tool (BLAST) hosted by National Centre for Biotechnological Information (NCBI) server. The unknown organisms were identified with the help of sequences showing maximum homology and identity with the query sequence.

**Protein profiling**

**Culture and harvesting of bacteria:** About 50 mL of nutrient broth was prepared aseptically and inoculated with isolated bacteria separately. The inoculated broths were kept in shaker (250 rpm) and left for 24 h at room temperature. The growth of bacteria was monitored by turbidity developed. All the bacterial cells were harvested by centrifugation in eppendorf tubes (3000 rpm for 15 min). The harvested cells were washed twice with Phosphate Buffer Saline (PBS) and suspended in 1 mL distilled water.
**Ultrasonication of cells:** The whole cell protein/intracellular proteins were obtained by sonicating the bacterial cells using sonicator. The cells suspended in distilled water were sonicated in sterile environment. The operational conditions including sonication for 30 sec and 10 cycles with an amplitude of 100. The inner proteins of the cells were released by disturbing the cell membrane using 3 mm width×90 mm length probe.

**SDS-PAGE analysis:** The protein profile of the sonicated cells was studied using SDS-PAGE analysis (Laemmli, 1970). In the present study, 5% stacking gel and 12% separating gel were employed to determine the protein profile of isolated four bacteria. The size of the dissociated proteins was determined using known molecular weight protein ladder mixture. After separation of dissociated proteins using SDS-PAGE, the gel was stained using Coomassie Brilliant Blue (CBR) R-250 dye and left overnight under shaken condition. The gel was destained using methanol, acetic acid and water mixture until bands are visible. The size of the dissociated proteins was determined by comparing the distance traveled by the dissociated proteins and the ladder protein.

**RESULTS AND DISCUSSION**

Among 12 bacteria isolated, 4 isolates (MKAS01, MKAS02, MKAS03 and MKAS04) were considered for the present investigation. The 4 isolates were isolated, purified and cultivated in broth medium and agar medium. Initially all the four strains were biochemically characterized and their results were presented in the Table 1. The molecular identification of the bacteria was performed using 16S rRNA sequence analysis. The 16S rRNA of all the four bacteria was sequenced and compared with the available database using BLAST search. The BLAST results revealed that the 16S rRNA sequence of the isolated strains, MKAS01, MKAS02, MKAS03 and MKAS04 shows maximum homology with *Escherichia coli*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Klebsiella pneumoniae*, respectively. The phylogeny of the isolated bacterial strains was also obtained through BLAST (Fig. 1-4). The sequences were published in the NCBI-GENBANK databases under the accession numbers, KP726932, KP726933, KP726934 and KP726935 respectively. The sizes of the 16S rRNA, Genbank accession number were shown in Table 2.

The whole cell protein profiles of the isolated bacterial strains were determined using SDS-PAGE. SDS-PAGE method alleviates the need for culturing and the sample is analyzed in a more direct manner. It is also more economical compared to AFLP fingerprinting, relatively...
easy and aids in the analysis of many samples at the same time (Aly et al., 2003). Moreover, the results obtained by SDS-PAGE of whole cell proteins can discriminate at much the same level as DNA fingerprinting (Priest and Austin, 1993). More than 15 protein bands could be resolved ranging in size from 94 kDa to below 20 kDa as determined by visual assessment of their approximate molecular masses (Fig. 5).

Multiple protein bands were detected in all the bacteria. Protein bands of 38 and 53 kDa appeared as major bands in *K. pneumoniae* and *E. coli*. Whereas, protein band of 38 kDa was found as major band in *B. subtilis*. An unique protein band correspond to molecular weight of 42 kDa was expressed in *S. epidermidis* which corresponds to Ornithine carbamoyl transferase in agreement with the work of Hussain et al. (1999). Another unique band of 25 kDa was expressed in *B. subtilis*,
Fig. 4: Phylogeny of *Klebsiella pneumoniae* MKAS04

but is not expressed in the other three bacteria investigated. Hence, the whole cell protein analysis revealed the differences and uniqueness in the protein expression which could serve as a tool in the identification and classification of the bacteria.

Biochemical and molecular markers are versatile and highly informative tools for the identification and diagnosis of bacterial and fungal pathogens (Mare *et al.*, 2001). They can be used to evaluate levels of genetic diversity and phenotypic relationships within and between species and to identify particular races and pathotypes (Brown, 1996). Protein profile analysis of clinical isolates of *K. pneumonia* involved in nosocomial outbreaks revealed the existence of high level of antibiotic resistance due to deficiency in porin protein expression (Ardanuy *et al.*, 1998). This protein is of medical importance as it is implicated as an “adhesion molecule” involved in the attachment to biological surfaces which is a prerequisite for causing invasive disease (Foster and Hook, 1998).
Cacoub and Thiolières (1993) outlined the importance and significance of protein profiling in the diagnosis and follow up of patients in an Internal medicine department. Fegatella et al. (1999) assessed the protein profiles from the marine bacterium *Sphingomonas* sp. for investigating the molecular basis of the unique physiology of this organism. De Lacroix-Szmania et al. (2002) and Skopkova-Zarnayova et al. (2005) have highlighted the utility and value of protein profiles analysis in the diagnosis of human bacterial infections and antibiotic resistant strains of bacteria.

**CONCLUSION**

Whole cell protein profiling using SDS-PAGE served as a useful tool in the identification and differentiation of clinical pathogens. Our result suggests that the same with the help of unique protein bands obtained from the four pathogenic bacteria isolated from clinical samples. However, for the definitive characterization of bacteria, alternative molecular techniques such as PFGE or plasmid analysis should be employed.

**REFERENCES**

Aly, I.N., M.A. Abdel-Sattar, K.A. Abd-Elsalam, M.S. Khalil and J.A. Verreet, 2003. Comparison of multi-locus enzyme and protein gel electrophoresis in the discrimination of five *Fusarium* species isolated from Egyptian cottons. Afri. J. Biotechn., 2: 206-210.

Ardanuy, C., J. Linares, M.A. Dominguez, S. Hernandez-Alles, V.J. Benedi and L. Martinez-Martinez, 1998. Outer membrane profiles of clonally related *Klebsiella pneumoniae* isolates from clinical samples and activities of cephalosporins and carbapenems. Antimicrobial Agents Chemotherapy, 42: 1636-1640.

Brown, J.K.M., 1996. The choice of molecular marker methods for population genetic studies of plant pathogens. New Phytol., 133: 183-195.

Cacoub, P. and J.M. Thiolières, 1993. [Importance of the protein profile in internal medicine]. Allergie et Immunologie, 25: 416-424.

Carlsohn, E., J. Nystrom, H. Karlsson, A.M. Svennerholm and C.L. Nilsson, 2006. Characterization of the outer membrane protein profile from disease-related *Helicobacter pylori* Isolates by Subcellular fractionation and Nano-LC FT-ICR MS analysis. J. Proteome Res., 5: 3197-3204.

De Lacroix-Szmania, I., P. Hausfater, R. Dorent, J.M. Thiolières and M.J. Foglietti et al., 2002. [Value of the protein profile in the diagnosis of infectious endocarditis when hemolysis is present]. La Revue de Medecine Interne, 23: 432-435.

Durrani, R., M. Abubakar, M.J. Arshed, S. Saleha, I. Ullah and Q. Ali, 2008. Biological characterization and protein profiles of two model bacteria by SDS-PAGE and FT-IR. ARPN J. Agric. Biol. Sci., 3: 6-16.

Fegatella, F., M. Ostrowski and R. Cavicchioli, 1999. An assessment of protein profiles from the marine oligotrophic ultramicrobacterium, *Sphingomonas* sp. strain RB2256. Electrophoresis, 20: 2094-2098.

Foster, T.J. and M. Hook, 1998. Surface protein adhesins of *Staphylococcus aureus*. Trends Microbiol., 6: 484-488.

Hussain, M., G. Peters, G.S. Chhatwal and M. Herrmann, 1999. A lithium chloride-extracted, broad-spectrum-adhesive 42-kilodalton protein of *Staphylococcus epidermidis* is ornithine carbamoyltransferase. Infect. Immun., 67: 6688-6690.

Katircioglu, H., B. Aslım, Z.N. Yükseldao, N. Mercan and Y. Beyati, 2003. Production of poly-β-hydroxybutyrate (PHB) and differentiation of putative *Bacillus* mutant strains by SDS-PAGE of total cell protein. Afr. J. Biotechnol., 2: 147-149.
Kustos, I., B. Kocsis, I. Kerepesi and F. Kilar, 1998. Protein profile characterization of bacterial lysates by capillary electrophoresis. Electrophoresis, 19: 2317-2323.
Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.
Mare, L., L.M.T. Dicks and M.L. Van der Walt, 2001. Characterization of South African isolates of Salmonella enteritidis by phage typing, numerical analysis of RAPD-PCR banding patterns and plasmid profiles. Int. J. Food Microbiol., 64: 237-245.
Priest, F.G. and B. Austin, 1993. Modern Bacterial Taxonomy. Springer Science and Business Media, UK., ISBN: 9780412461200, Pages: 228.
Skopkova-Zarnayova, M., E. Siebor, D. Rovna, H. Bujdakova and C. Neuwirth, 2005. Outer membrane protein profiles of clonally related Klebsiella pneumoniae isolates that differ in cefoxitin resistance. FEMS Microbiol. Lett., 243: 197-203.