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BIOLOGICAL CHARACTERIZATION AND PROTEIN PROFILES OF TWO MODEL BACTERIA BY SDS-PAGE AND FT-IR

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ABSTRACT

Bacteria are simple, single celled microorganisms that can exist in a variety of different shapes. They are identified and classified largely in a series of biochemical tests or through molecular characterization. Two bacterial species i.e., Pseudomonas aeroginosa, and E. coli were characterized by biochemical tests, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and FT-IR (Fourier Transform Infrared Spectroscopy). Complex protein bands in SDS-PAGE could not differentiate all isolates completely. E. coli samples (stool and urine) when compared with the marker, were similar in their molecular weights. P. aeroginosa samples showed slight similarity in their protein bands having major proteins as 85kDa, 133kDa, 148kDa and 180 kDa, respectively. FT-IR provides a better method of characterization but it requires high precision and care during work because a slight humidity can overlap all the spectral analysis. When all the spectral images were compared with the control i.e., the distilled water, almost all the bacterial samples showed similarity in their spectra except the P. aeroginosa from pus sample, which showed multiple peaks in its spectra at a wave number 3500-1000cm^{-1}.

Keywords: bacteria, protein, characterization, peptide analysis, E. coli, P. aeroginosa, SDS-PAGE, FTIR.

INTRODUCTION

The human body is host to an active ecosystem. Microbes such as bacteria, viruses, fungi and protozoa exist in our mouth, eyes, ears, nose, intestines and skin. Although microbes are disease-causing microorganisms but they perform many important functions for us, producing vitamins, help in digestion of food, and work as an essential part of our immune system. Among microorganism bacteria are relatively simple, single celled organisms. Because their genetic material is not enclosed in a special nuclear membrane, therefore these bacterial cells are called prokaryotes (Tortora *et al.* 1995).

Molecular techniques are major tools for the characterization of bacteria from food and other biological substances. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), DNA Amplification Finger Printing (DAF) and Restriction Fragment Length Polymorphism (RFLP) are the molecular techniques used for the characterization of bacterial macromolecules and are of significance importance (Vos *et al.*, 1995). Bacterial typing is carried out by DNA Amplification Finger Printing (DAF) e.g, Salmonella enterica obtained from human, animal (clinical), and food sources (Daly *et al.*, 2000).

Kabadjova *et al*. (2002) used a rapid PCR-RFLP-based identification scheme for four closely related Carnobacterium species (C. divergens, C. piscicola, C. gallinarum, and C. mobile). Amplified fragment length polymorphism (AFLP) analysis was used for the characterization of bacterial macromolecules. AFLP analysis is one of the robust multiple-locus fingerprinting techniques among genetic marker techniques that have been evaluated for genotypic characterization (Koeleman *et al.*, 1997).

SDS-PAGE is an important molecular technique used for the identification at species level of whole cell proteins and it has the advantage of being fairly simple and rapid to perform. But for the identification this technique requires extensive data to cover all known target species (Leisner *et al.* 1994).

FT-IR (Fourier Transform Infrared Spectroscopy) is an important analytical technique that provides information related to the fundamental molecular property of the bacteria. It is used to study whole organism biochemical fingerprints (spectra), which are reproducible and distinct for different bacteria (Burgula *et al*. 2007).

E. coli and Pseudomonas aeroginosa are the simplest bacteria that can be grown easily on their respective media. E. coli and Pseudomonas aeroginosa are the normal flora of humans, but they can cause diseases in many cases. Their whole cell protein profiles can be easily studied on SDS-PAGE and to study whole organism biochemical fingerprints (spectra) FT-IR is an important analytical technique to be used. Therefore present study was conducted to meet the main objectives, to isolate and bio-chemically characterize the bacterial pathogens involved in diseases in clinically suspected samples, to characterize the bacterial proteins of E. coli and Pseudomonas aeroginosa, by using SDS-PAGE and to study whole cell microorganism biochemical fingerprints (spectra) by using FT-IR

MATERIALS AND METHODS

This study was conducted in National Veterinary Laboratory, Islamabad where samples of E. coli (Extracted form stool, urine, pus, human vaginal swabs i.e., HVS) and samples of Pseudomonas aeroginosa (Extracted form stool, urine, pus, HVS) were analyzed using Biochemical

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tests, SDS-PAGE and Fourier Transform Infrared Spectroscopy (FT-IR).
For Gram staining the protocol was followed as described by Cappucino and Sherman, (1998) for all samples of E. coli and Pseudomonas aeroginosa.

Sub-culturing and purification

E. coli

After staining, a known pure colony was inoculated on two MacConkey agar plates with the help of sterilized wire loop. They were incubated at 37°C for 24 hours. Stained slides from pure culture plates were examined under microscope for their purity.

Pseudomonas aeroginosa

Same procedure mentioned above was carried out for the purification of the Pseudomonas aeroginosa.

Biochemical characterization

The tests (Catalase test, oxidase test, growth in the absence of CO_2, Urease production test, H_2S production test, Nitrate reduction test, Motility test) were employed for the biochemical characterization of E. coli and Pseudomonas aeroginosa following Brough (1984).

Harvesting of culture

All bacteria i.e., E. coli and Pseudomonas aeroginosa were harvested by centrifugation in eppendorf tubes (Speed 13,000rpm (Ultra centrifuge, Germany). Supernatant was discarded and the pellet was suspended in 1ml-distilled water. The pellet was first suspended in 0.5ml PBS (Phosphate Buffer Saline) and then they were centrifuged. The supernatant was discarded and the pellet was suspended in 1ml of distilled water.

Sonication (disruption) of cells for whole cell protein

The pellet was dissolved in distilled water and then it was sonicated (dr. hielscher, type UP 400S) type sonicater in sterile conditions, for 30 seconds and 10 cycles, keeping the amplitude on 100 and the cycle as 1. Sonication is carried out to disrupt the cells and release the inner protein. The cells were disrupted with the conical probe having 3mm width and 90mm length.

Sodium Dodecylsulphate-Polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoresis was carried out in the discontinuous sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) system of Laemmli (1970) using 12% (w/v) separating gel and 5 %(w/v) stacking gel. Comparative protein profile of E. coli and Pseudomonas aeroginosa were observed by SDS-PAGE.

The electrophoresis was carried out using slab type SDS-PAGE Model (V20-CDC, UK) with 12% polyacrylamide gel. The molecular weight of the dissociated proteins was estimated by using high molecular weight standard mixture (Sigma SDS-7H) with molecular weight ranging 30,000-200,000. A 12% running gel (1.5M Tris-HCl (Scharlau) pH 8.9, 10% SDS (Scharlau), 10% acryl amide + bisacrylamide (Merk), 5% stacking gel (0.5 M Tris-HCl pH 6.9, 10% SDS, 30% acryl bis) were prepared and polymerized, chemically running gel solution contain 20 µl N, N', N', N' tertamethylenediamine (Sigma), and 15 µl to the stacking gel solution. A 0.3 g in 3ml ammonium persulphate (Sigma) was added to both solutions.

A marker of known molecular weight (SDS marker, Sigma-7H and Fermentas SM 0661 protein ladder) was also loaded (40µl) along with the samples. The apparatus was connected with constant electric current (30mA) till the bromophenol blue (BPB) reached the bottom of the plate.

Staining and destaining of gel

The gels were put into a container with staining solution-containing coomassie brilliant blue (CBR) R-250 dissolved in methanol (Scharlau) with acetic acid (Merck, Germany) and water i.e. the double distilled water. Gels were left in the staining solution for overnight and destained in methanol, acetic acid and water with shaking (IKA- VIBRAX type VXR, Germany) until the bands became visible above the background. Both staining and destaining steps were carried out while shaking.

Determination of molecular weight of proteins

Molecular weights of whole cell proteins are analyzed by SDS-PAGE by using a high molecular weight standard marker DALTON MARK SIGMA -7H and Fermentas protein ladder SM0661 for SDS Gel Electrophoresis. The molecular weight of Sigma -7H ranged between 205kDa to 29kDa and the molecular weight of the fermentas SM0661 ranged between 200kDa to 10kDa.

The sigma-7H has seven proteins and fermentas SM0661 has 14 proteins. The distance traveled by bands of the marker was measured. By taking the distance traveled by the bands of marker (in mm) along x-axis and molecular weight (in kDa) of the proteins present in the marker along y-axis, a standard graph was obtained. The distance covered by each band of the sample was calculated and with the help of standard graph, molecular weights of proteins present in the sample were determined.

Characterization of bacteria by FT-IR

The samples were prepared by dilutions in the test tubes by taking 0.1ml of the sample in 0.9ml of the distilled water and then its Optical density was adjusted at 0.1nm. The wavelength of the spectrophotometer was 520nm when it was on 15 minutes before for the calibration. After OD was adjusted then the samples were taken to the FT-IR (Nicolet Nexus 670 FT-IR) room for generation of the spectra. The Germanium crystal was first cleaned with the help of alcohol and then the sample was put onto that Germanium crystal and then the spectra of the sample was taken by making the contact of the probe with that of the Germanium crystal. At each and every step before addition of the sample, the background spectra was taken that was the most important step in FT-IR. The already feeded software OMNIC-FT-IR read the spectra of

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the sample. During generation of the spectra, the FT-IR starts auto-gaining of the sample and the spectra was generated by the four sources power which provides the current, scan which scans the image, laser generates the spectra and the source generates the light created in that spectra.

RESULTS AND DISCUSSIONS

Characterization of bacteria

On BHI (Brain Heart Infusion) agar and nutrient agar, convex colonies with entire edges, mucoid and sticky nature with approximate size of 2-3 mm diameter were observed. All the pure culture showed luxuriant growth on blood agar having translucent grayish or yellowish green colonies. No haemolysis on blood agar was observed. Pseudomonas aeroginosa when grown on nutrient agar produced smooth mucoid colonies. E. coli when grown on MacConkey produced pink smooth colonies, which showed that it is lactose fermenter.

Staining

The Gram staining of E. coli showed that it was gram negative and it had rod shape with a size of 2µm while Pseudomonas aeroginosa showed that it was also gram negative and rod in shape 0.5 to 0.8 µm by 1.5 to 3.0 µm.

Biochemical characterization

The biochemical results of organisms showed that E. coli was citrate positive, oxidase negative, TSI positive while the biochemical results of Pseudomonas aeroginosa showed that it was citrate positive, oxidase positive, catalase positive, Urease positive, H_2S negative, nitrate positive and Motility test positive (Table-1).

Major and minor protein profiles of the organisms studied in SDS-PAGE

When SDS-PAGE was used to observe protein pattern, complex protein profiles of these bacteria (E. coli and P. aeroginosa) were obtained. Presence of a large number of bands in whole- cell profile could not differentiate these three bacteria. The E. coli bands of SDS-PAGE showed that stool and urine samples were similar in their molecular weight. They had a major protein of 97.4 kDa. While HVS and Pus sample had a major protein of 106 kDa and 133 kDa (Figures 1-3). Hafeez and Sultana (1992) investigated the whole cell protein profile of 20 clinical isolates that were taken from the NIH Islamabad using SDS-PAGE to determine the variability in the gene pool of E. coli.

The SDS-PAGE results of P. aeroginosa of stool and urine sample showed similarity in their protein pattern. They had a common protein of 60 kDa while major protein of stool sample was 85 kDa. The urine sample had a major protein of 133 kDa. The HVS sample had a major protein of 148 kDa while pus sample had 180 kDa. Khan in 1996 studied 42 strains of P. aeroginosa by SDS-PAGE and indicated the presence of 45 protein bands of different molecular weights (Tables 2 and 3).

Results of Fourier Transform Infrared Spectroscopy (FT-IR)

The samples were then analyzed by FT-IR to find similarities and dissimilarities in their pattern. The results of FT-IR showed that E. coli samples (HVS, stool, urine and Pus) show similarity and they were comparable but E. coli HVS sample was highly similar to stool. Urine sample show dissimilarity at a wave number below 500 cm^{-1}. So, the results showed that E. coli samples were comparable. The spectra of E. coli were similar because they were isolated from the same patient.

The spectral analysis of Pseudomonas aeroginosa samples when compared showed no similarity in their pattern. But stool and HVS samples show slight similarity at a wave number 500cm^{-1}.Pseudomonas aeroginosa pus sample showed multiple peaks because they were isolated from different patients (Figures 4-10).

Kim *et al.* (2006) in a similar type of study investigated that outer membrane spectral analysis of Salmonella enterica serotypes by FT-IR showed that most spectra was in 1800-1500cm-1 regions separated the serotypes and provided better classification. Similarly Burgula *et al.* (2007) found that FT-IR methods enable chemical discrimination of intact bacterial cells and produce complex whole-organism biochemical finger prints (spectra), which are reproducible and distinct for different bacteria.

When all the spectral analysis was compared with the control i.e., the distilled water, both the bacteria showed similarity in their pattern.

CONCLUSIONS AND FUTURE PROSPECT OF STUDY

Successful approach for the whole cell peptide analysis of three bacterial species has been attempted through Biochemical tests, SDS-PAGE and FT-IR (Fourier Transform Infrared Spectroscopy). This study concluded that SDS-PAGE is a better technique for the identification at species level as compared to FT-IR. Further studies can devise methods of better control for diseases in human caused by these bacterial species. If the peptides of these bacterial species are isolated then we can construct a vaccine by antigenic peptides and we can design a vaccine by computational biology.

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Table-1. Biochemical properties of E. coli and Pseudomonas aeroginosa.

	E. coli		**Pseudomonas aeroginosa**	
Biochemical test	**Reaction positive /negative**	**Inference**	**Reaction positive /negative**	**Inference**
Citrate test	Positive	Blue and turbid medium	Positive	Blue and turbid medium
Oxidase test	Negative	No purple colour on reagent paper	Positive	Deep purple colour on reagent paper
TSI test	Positive	Yellowing of the medium, strong lactose fermenter	Negative	No yellow colour
Catalase test	Positive	Release of oxygen bubbles	Positive	Release of oxygen bubbles
Nitrate test	Positive	Reddening of the medium	Positive	Reddening of the medium
Motility Indole Urease	Positive	Motility positive layers are produced in medium, Indole positive red colour is seen and Urease negative	Positive	Motilility positive, Indole negative and Urease vary

Table-2. E. coli whole cell profile results of SDS-PAGE.

Stool		**Urine**		**Human viginal sample**		**Pus**	
Mol. wt (kDa)	**Distance** (mm)	**Mol. wt** (kDa)	**Distance** (mm)	**Mol. wt** (kDa)	**Distance** (mm)	**Mol. wt** (kDa)	**Distance** (mm)
97.4	28	97.4	28	106	25	133	20
27	76	27	76	66	40	80	35
21	90	21	90	57	50	57	50
20	96	20	96	25	79	48	57
18	103	18	103	-	-	-	-

Table-3. Pseudomonas aeroginosa whole cell SDS-PAGE profile.

Stool		**Urine**		**Human viginal sample**		**Pus**	
Mol. wt (kDa)	**Distance** (mm)	**Mol. wt** (kDa)	**Distance** (mm)	**Mol. wt** (kDa)	**Distance** (mm)	**Mol. wt** (kDa)	**Distance** (mm)
85	32	133	19	148	17	180	15
73	37	93	29	93	29	108	25
60	44	60	44	85	32	65	40
28	68	47	57	-	-	50	52
24	80	-	-	-	-	-	-
22	87	-	-	-	-	-	-
19	97	-	-	-	-	-	-

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Table-4. Fermentas molecular weight marker SM0661.

Molecular weight (kDa)	**Distance traveled by marker** (mm)
200	7
150	10
120	15
100	22
85	28
70	35
60	48
50	60
40	73
30	92
25	126
20	142
15	152
10	160

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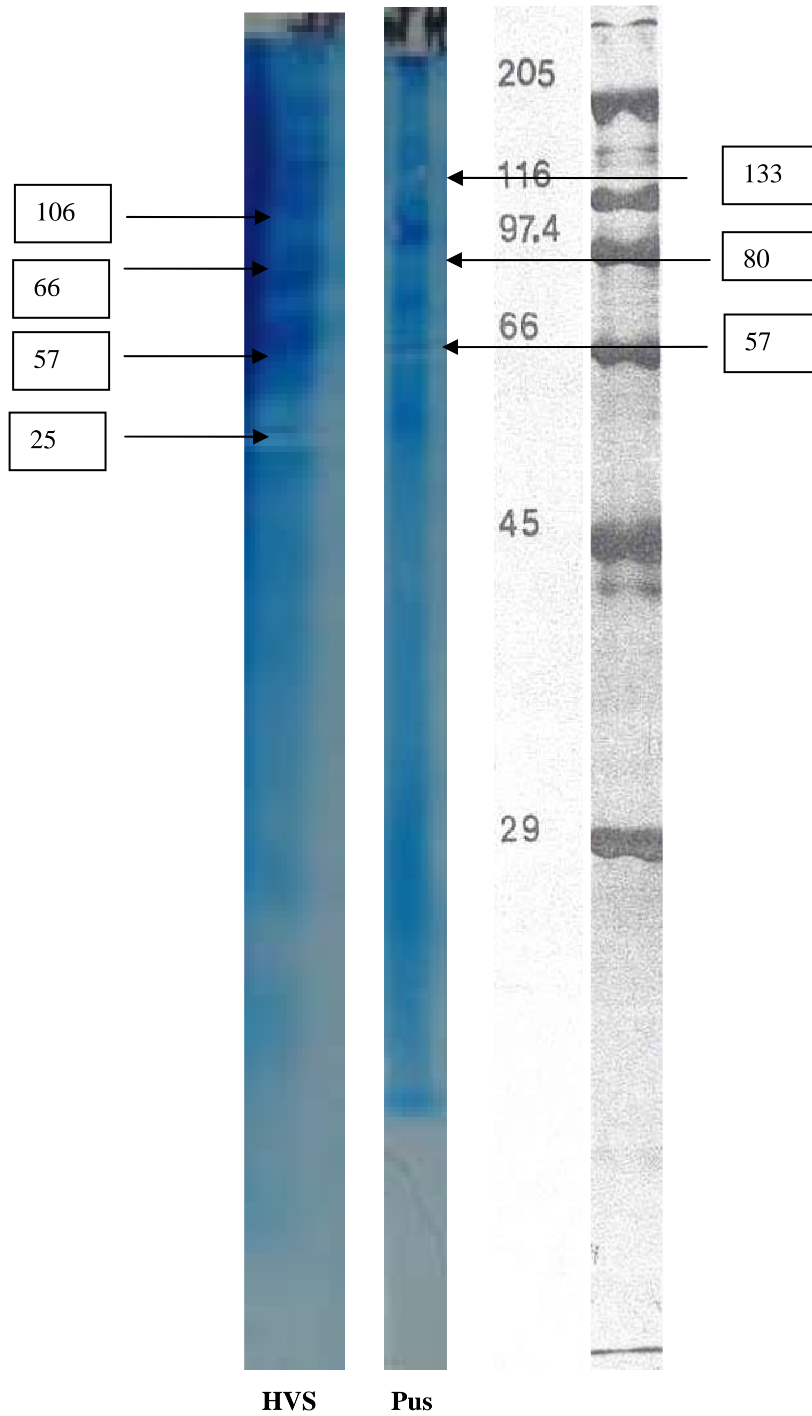

Figure-1. E. coli HVS and Pus sample with molecular weights in kDa.

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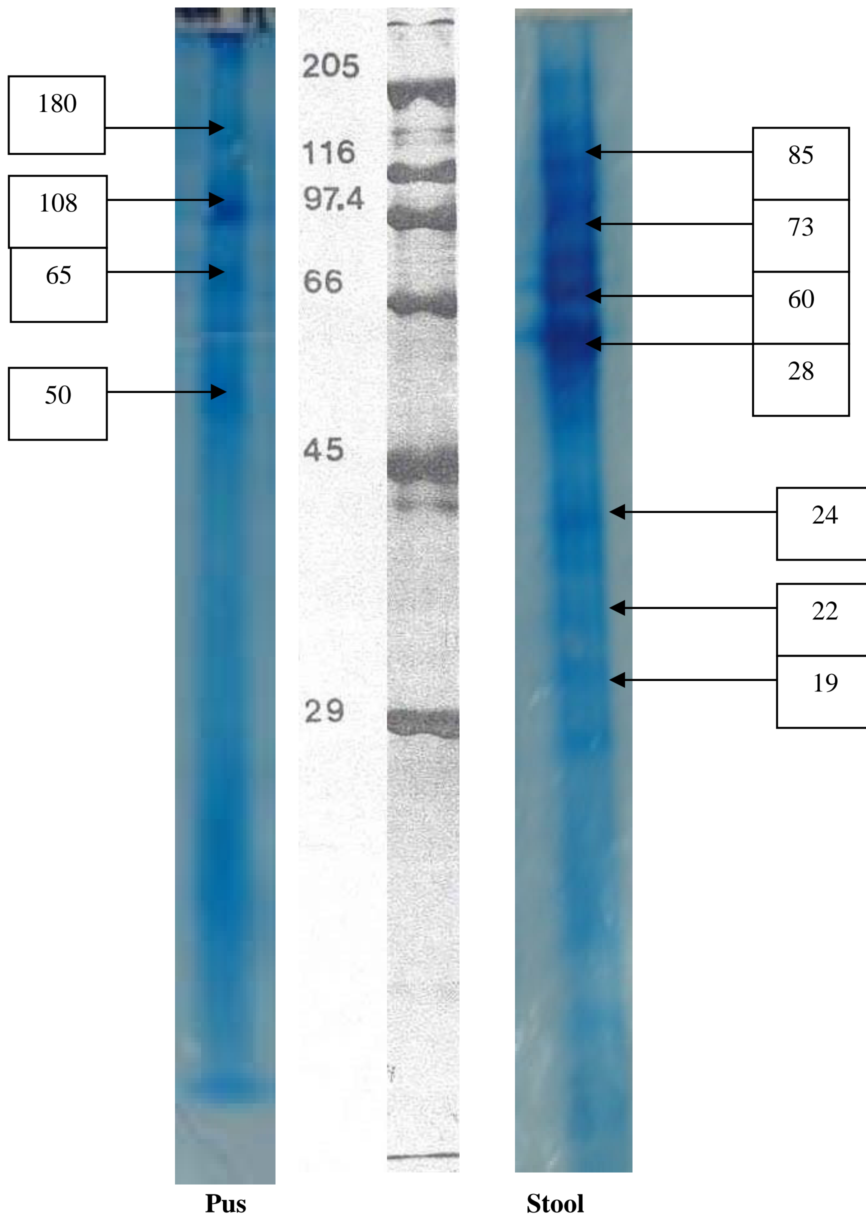

Figure-2. Pseudomonas aeroginosa stool and pus samples molecular weight (kDa) with marker.

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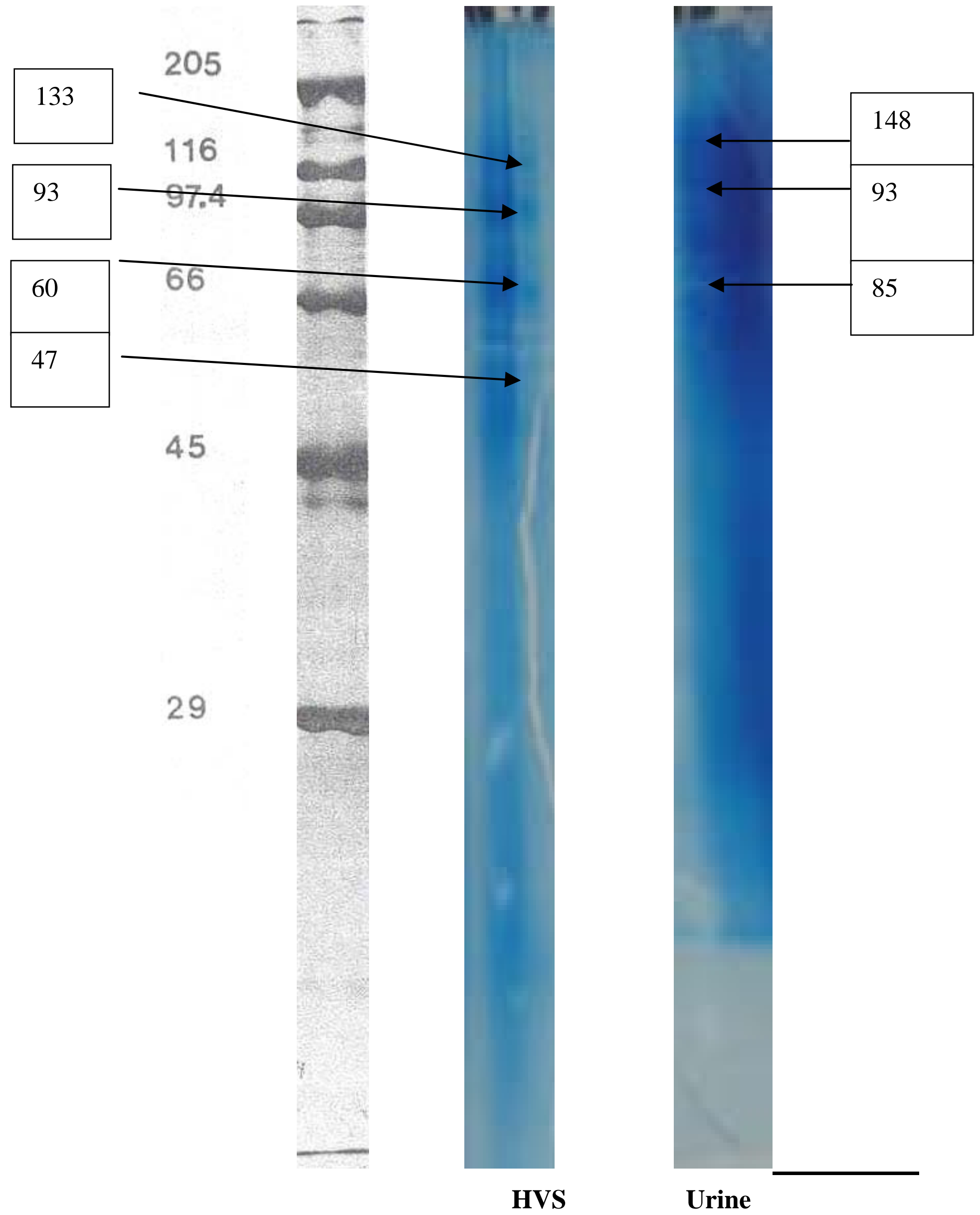

Figure-3. Molecular weight (kDa) Pseudomonas aeroginosa Urine and HVS.

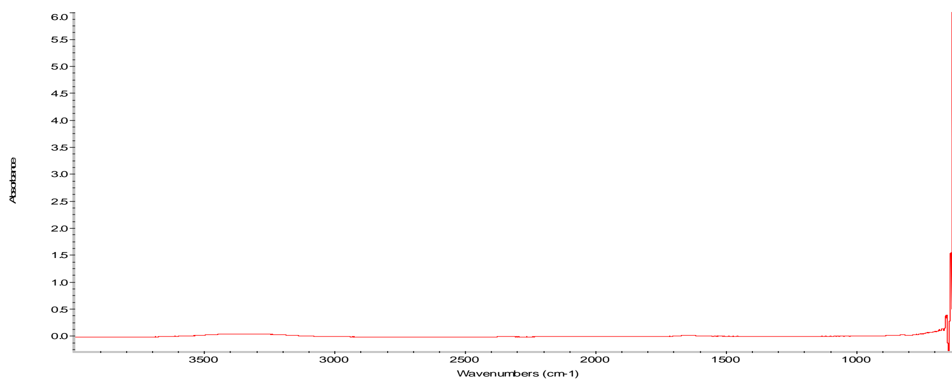

Figure-4. E. coli whole cell stool sample.

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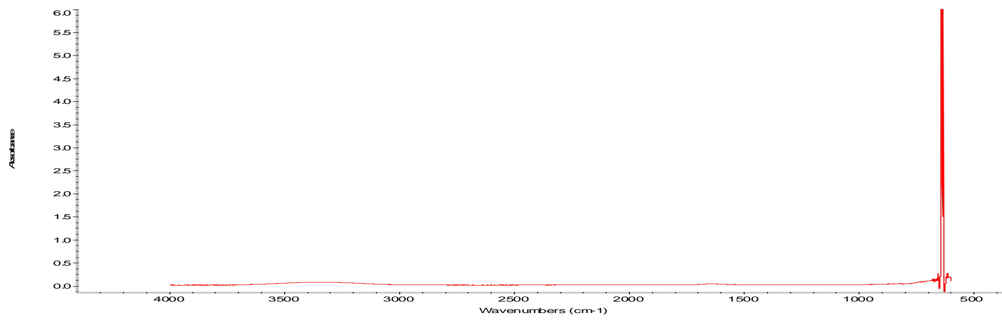

Figure-5. E. coli whole cell urine sample.

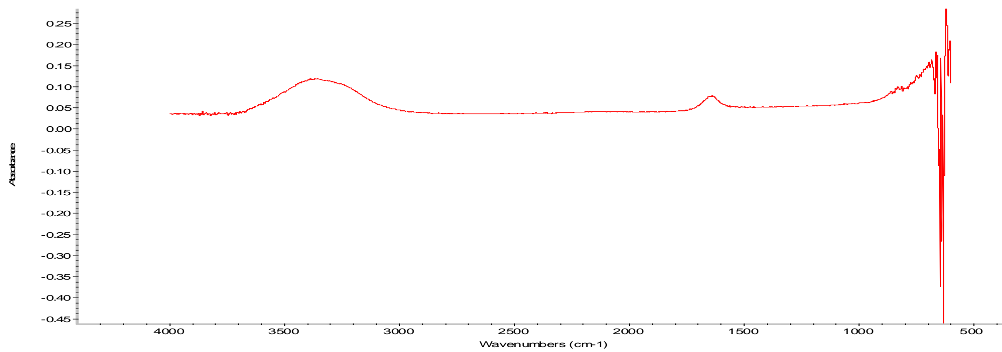

Figure-6. Pseudomonas aeroginosa whole cell urine sample.

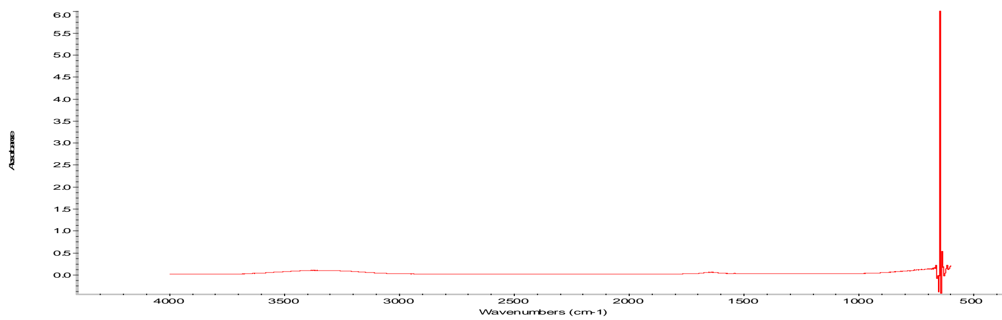

Figure-7. Pseudomonas aeroginosa whole cell stool sample.

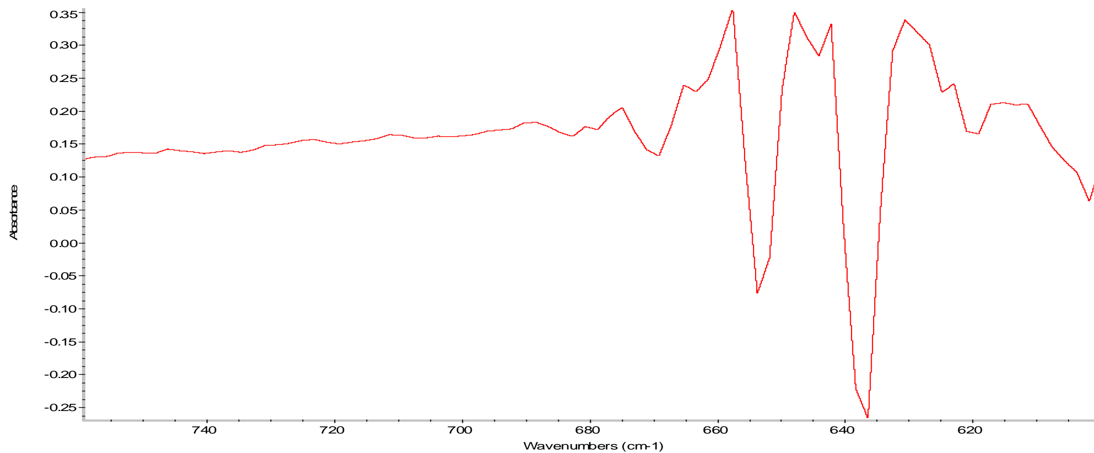

Figure-8. Control (Distilled water).

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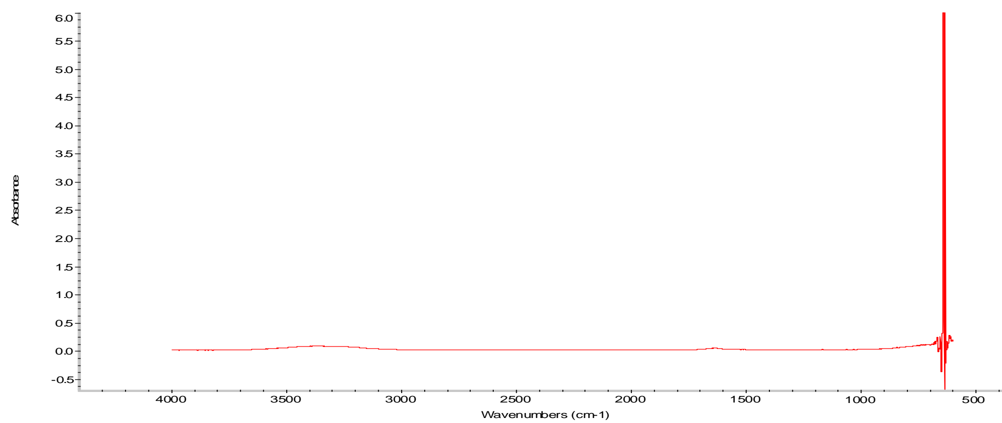

Figure-9. Pseudomonas aeroginosa whole cell human viginal sample.

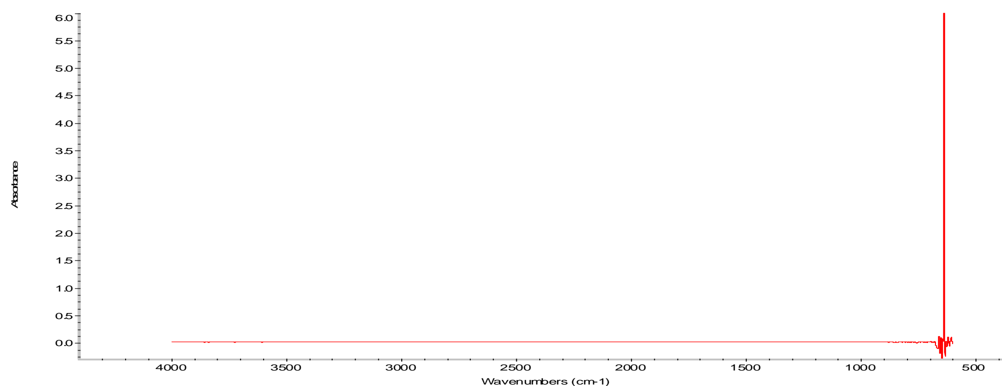

Figure-10. E. coli whole cell human viginal sample.

REFERENCES

Brough C. 1984. District laboratory Practice in Tropical countries. (PART 2) Monica Cambridge. ISBN No 0-521-66546-9.

Burgula Y., D. Khali, S. Kim, S. S. Krishnan, M. A. Cousin, J. P. Gore, B. L. Reuhs and L. J. Mauer. 2007. Review of Mid-Infrared Fourier Transform-Infrared Spectroscopy Applications for Bacterial detection. Journal of Rapid methods and Automation in Microbiology. 15: 146-175.

Cappucino J. G. and N. Sherman. 1998. Microbiology: A laboratory Manual. 5th Ed., Benjamin/Cummings imprint. ISBN 0-8053-7646-1.

Daly M., J. Buckley, E. Power, C. O'Hare, M. Cormican, B. Cryan, P. G. Wall and S. Fanning. 2000. Molecular characterization of Irish Salmonella enterica Serotype Typhimurium: detection of class I integrons and assessment of genetic relationships by DNA amplification fingerprinting. Appl. Environ. Microbiol. 66: 614-619.

Hafeez A. and A. Sultana. 1992. Study of the whole cell and outer membrane protein profile of E. coli by SDS-PAGE. Pakistan Journal of Zoology.1:66-68.

Kabadjova. P., X. Dousset, V. Le Cam, and H. Prevost. 2002. Differentiation of closely related carnobacterium food isolates based on 16S-23S ribosomal DNA intergenic spacer region polymorphism. Appl. Environ. Microbiol. 68: 5358-5366.

Khan F.G. 1996. A preliminary study of finger printing of Pseudomonas aeroginosa of whole cell protein analysis by SDS-PAGE. Indian Journal of medical research.104: 342-348.

Kim S., Reuhs B.L. and Mauer L.J. 2005. Use of Fourier transform Infrared spectra of crude bacterial lipopolysaccharides and chemometrics for differentiation of Salmonella enterica serotypes. J. Appl. Microbiol. 99: 411-417.

Koeleman J. G. M., J. Stoof, D. J. Biesmans, P. H. Savelkoul and C. M. Vandenbroucke-Grauls. 1997. AFLP finger printing of Acinetobacter. J Clin Microbiol. 36: 2522-2529.

Laemmli U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London). 227: 680-685.

Leisner J. J., J. C. Millan, H. H. Huss and L. M. Larsen. 1994. Production of Histamine and tyramine by Lactic

ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science
©2006-2008 Asian Research Publishing Network (ARPN). All rights reserved.

Acid bacteria isolated from Vacuum packed sugar-salted fish. J Appl. Bacteriol. 76: 417-423.

Tortora G. J., B. R. Funke and C. L. Case. 1995. Microbiology. An Introduction. 5th Edition. The Benjamin/Cummings Publishing Co., Inc., Redwood City, CA. pp. 273-294.

Vos P, R. Hogers, M. Bleeker, M. Reijans, T. van der Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.