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Protein and DNA Typing of Some Disease Agents Isolated from Both Solid Wastes and Associated House Flies

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Abstract: Five bacterial genera were isolated from both solid wastes collected from different localities within Cairo governorate and flies associated with these wastes. Protein profile of *Staphylococcus xylosus* showed identical bands of 184.14 to 101.39 KD. While its plasmid DNA arrays ranged from 40978 to 3354 bp. Proteins and DNA analysis of *Klebsiella pneumonia* and *Enterobacter sakazaki* isolated from garbage and breeding flies did not show identical configuration of each bacterial strain. *Pseudomonas aeruginosa* protein showed one fraction of Mwt. 39.66 KD and 3 plasmid arrays (165, 133 and 19 Kbp). *E.coli* isolated from flies and garbage showed 4 sharing protein bands ranged from 43.03 to 14.4 KD. And Plasmid arrays were identical and of MWt 195, 165, 98, 15, 8 and 7 Kbp.

Key words: Pathogenic bacteria · protein profile · plasmid isolation · DNA characterization

INTRODUCTION

The high fly density associated to wastes of solid type is a major problem faced developing countries [1] House fly is a dangerous vector for many disease agents [2, 3]. All previous studies used routine morphological and biochemical methods to identify bacterial isolates carried by flies and contaminate its waste breeding places [4, 5]. Our study used new molecular techniques to assure the similarity or dissimilarity between bacterial isolates carried by flies and its breeding places to incriminate vector for pathogens transmitted to man and animals total protein characterization and be used to characterize isolated bacteria.These techniques are recently used by many scientists to assure bacterial identification [6-9].

MATERIALS AND METHODS

Sampling sites: six different Egyptian localities resembling rural and urbanized areas were chosen as sites for different waste and fly collecting samples. The sites are El-Demerdash hospital, El mokatem hill, fifteen of May city, Manshaat El Sadr, Manshaat El naser and El Obour market different sites in each locality were visited as sources of pathogenic bacteria. Isolated bacteria from flies and Breeding soiled wastes were previously identified by [5].

Protien profile and DNA fingerprint

Bacterial protein characterization: Bio-Rad protein assay kit was used to estimate the total bacterial proteins, while SDS polyacrylamide gel electrophoresis was used to characterize the isolated protein samples according to the method described by [10].

Typing of bacterial plasmid DNA: Plasmid DNA that encoding the toxin production property of the bacteria was isolated. The High pure plasmid isolation kit from (Boehringer Mannhein) was used for isolation and purification of DNA according to the method of Gonzalez and Carlton [11]. Agarose gel electrophoresis and the computer program (Progel analysis) were used to detected and calculate molecular weight of plasmid arrays according to the description in Rady [12].

Plasmid DNA digest: Endonucleases *Eco* R1 and *Sma* I were used to digest isolated DNA for characterization according to the method mentioned in Maniatis *et al.* [13].

RESULTS

Isolation of common bacterial species from different solid waste locations at Cairo governorate (EL-Demerdash hospital garbage, Fifteenth of May, Manshaat EL-Sadr, Manshaat Nasser and EL-Obour Market) and from associated flies which may breed in different

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Table 1: Molecular weight estimates of (KD) protein of different bacterial strain isolated from garbage and housefuly samples

Lanes	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8	Lane 9	Lane 10	Lane 11	Lane 12
Rows												
r1												212
r2	184.57											
r3		181.14										
r4			138.29	136.57								
r5												116
r6			103.16	102.71								
r7	101.39	101.83										
r8					97.843	97.4						97.4
r9					86.48	86.48						
r10												66.2
r11			64.336	64.129								
r12					63.3	63.3						
r13			62.471									
r14			60.193	59.986								
r15					59.364							
r16			67.914									
r17				57.707								57.5
r18					56.154	56.154						
r19			55.817	55.481								
r20					52.788							
r21						52.115						
r22			50.769	50.433								
r23			48.077									
r24					46.058	46.058						
r25									43.029	43.029		
r26			42.692	42.356								
r27					41.01	41.01						
r28			40.337	40								40
r29					38.594		39.063	39.063				
r30			26.408	25.94								
r31				-								
r32									22.659	22.956		
r33					21.253	21.253						
r34			18.159	18.006								19.378
r35									18.159	18.172		
r36					17.397	17.397						
r37					16.33	16.33						
r38									14.748		14.748	
r39										14.384		
r40				13.294								
r41					12.931	12.931						

sites of these locations was previously described in (Fallatah *et al*. 5), five isolates were found to be common between each waste site and flies collected from the same site (*Enterobacter*,*Klebsiella*, *Pseudomonas*, *Staphylococcus* and *Escherichia* spp.)

Protein profile of the shared isolated bacteria: Gel electrophoretic run of bacterial proteins in Fig. 1 and Table 1 proved that *Staphylococcus xylosus* isolated from garbage (El Demerdash hospital main garbage) and its associated flies are sharing protein bands of MWt of range (184.57-184.14), (101.39-101.83) KD.

Protein profile of *Enterobacter sakazaki* (Table 1 and Fig. 1 lane 3, 4) isolated from both garbage and flies of the same site did not show identical configuration. The same result is observed when comparing protein profile of

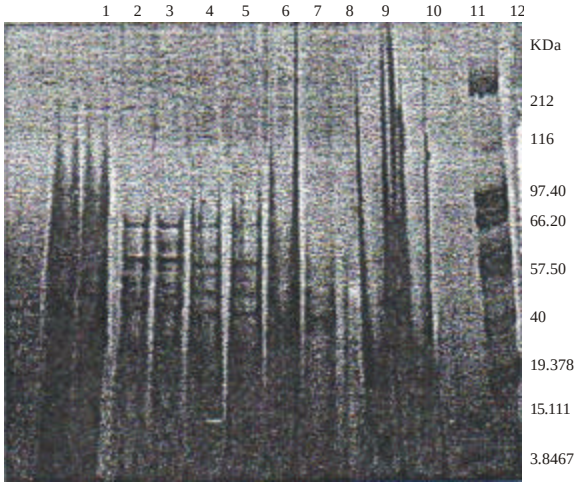

Fig. 1: Protein profile of different bacterial strains isolated from garbage and housefly samples

1. Protein profile of *Staphylococcus xylosus* isolated from garbage sample
2. Protein profile of *Staphylococcus xylosus* isolated from fly sample
3. Protein profile of *Enterobacter sakazakii* isolated from garbage sample
4. Protein profile of *Enterobacter sakazakii* isolated from fly sample
5. Protein profile of *Klebsiella pneumonia* isolated from garbage sample
6. Protein profile of *Klebsiella pneumonia* isolated from fly sample
7. Protein profile of *Pseudomonas aeruginosa* isolated from garbage sample
8. Protein profile of *Pseudomonas aeruginosa* isolated from fly sample
9. Protein profile of *Escherichia coli* isolated from garbage sample
10. Protein profile of *Escherichia coli* isolated from fly sample
11. Low molecular weight protein marker
12. High molecular weight protein marker

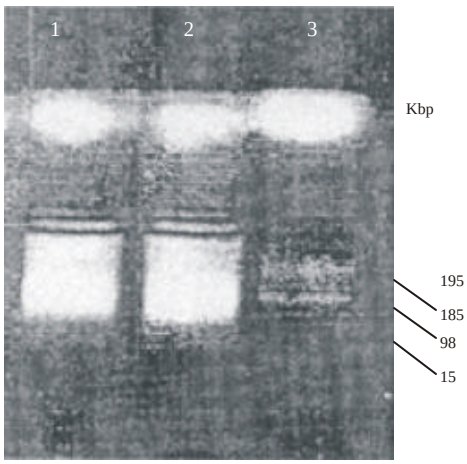

Fig. 2: DNA plasmid profile of two undigested isolates of *Pseudomonas aeruginosa*

1. Total plasmid DNA (Garbage sample)
2. Total plasmid DNA (Fly sample)
3. Standard plasmid DNA

Table 2: Bacterial plasmid sizes and digestion with endonucleases

	Total plasmid DNA (kbp)		*Sma* 1 (bp)		*Eco* RI (bp)	
Bacterial isolate	Waste	Fly	Waste	Fly	Waste	Fly
Psuedomonas aeruginosa	165	165	4600	4600	9800	9800
	133	133			8600	8600
	19	19			5000	5000
Escherichia coli	231	231	44467	44467	40978	40986
	195	195	30787	30787	17377	17377
	165	165	17377	17377	6557	6557
	44	44	16014	16014	3354	3354
			6557	6557		
			3535	3535		
			2322	2322		
Staphylococcus xylosus	165	165	11788	11788	23130	23130
	160	160	5169	5169	16512	16512
	98	98			7578	7578
					6126	6126
					4162	4162
Klebsiella pneumonia	195	195	11818	11636	13091	12364
	165	165	10727	10545	10909	11455
		148	9273	9091	9455	9636
Enterobacter sakazaki	195	210	9897	9590	9949	9744
			8821	8513	8821	

the bacteria *Klebsiella pneumonia* (Table 1 and Fig. 1 Lane 5, 6) the isolated bands from flies contain 13 protein fractions while eleven protein bands isolated from bacteria collected from wastes. Protein bands of MWt (59.36, 38, 59) KD. Are not found in fly bacterial isolates.

Fractionation of waste and fly isolates of *Pseudomonas aeruginosa* reveal one protein fraction of 39.063 KD. While *E. coli* isolates from wastes and collected flies show 4 identical bands of MWt. 43.029, 22.66 (18.159-18.172) (14.748-14.384) KD.

Plasmid DNA characterization: Comparing plasmid profile of identical bacterial isolates (*Pseudomonas*, *Escherichia* and *Staphylococcus* spp.) from both wastes and associated flies is important to incriminate house flies as vectors capable in transmitting disease causing agents. As shown in Fig. 2 and Table 2, three plasmid arrays of MWt. 165, 133, 19 kbp are separated from *Pseudomonas aeruginosa* of both flies and waste samples. *Sma* I digested DNA of *Pseudomonas aeruginosa* shows one cut for both isolates at 4600 bp (Fig. 3 and Table 2), while digestion using *Eco* RI cut both plasmid isolates three times at 9800, 8600 and 5000bp (Fig. 3, Table 2). DNA characterization of isolated *E.coli* from wastes and associated flies show 4 equal plasmid arrays of MWt. 231, 195, 165 and 44 kbp (Fig. 4, Table 2) while digestion using *Eco* RI cut plasmid DNA at sites revealing fragments of M.Wt. 40978, 17377, 6557, 3354, bp. *Sma* I digests both DNA samples to 7 fragments of the same MWt. 44467, 30787, 17377, 16014, 6557, 3535, 2322 (Fig. 5, Table 2).

Staphylococcus xylosus isolated from both garbage and flies show three identical plasmid arrays of MWt. 165, 160 and 98 kbp. *Eco* RI (Fig. 6, Table 2). Digested plasmids showed 5 cuts (Fig. 7, Table 2) of equal molecular sizes 23130, 16512, 7578, 6126, 4162 bp. *Sma* I endonuclease splits both DNA samples at 11788 and 5169 bp. while *Klebsiella pneumonia* of both wastes and associated flies did not show similarties in plasmid arrays as shown in (Fig. 8, Table 2). Endonuclease digestion also proved that the two isolates are not identical. *Sma* I cut plasmid DNA of *Klebsiella* (from flies) at 11636, 10545, 9091 bp while cuts of *Klebsiella* from garbage reveal fragments of M.Wt. 11818, 10727 and 9273. *Eco* RI cuts the bacterial plasmid (from fly samples) at MWt. 12364, 11455 and 9636 bp. while that of *Klebsiella* from garbage have molecular weights 13091, 10909 and 9455 bp. Which proved that the two isolates are not identical. (Fig. 9, Table 2). Characterization of plasmid DNA from *Enterobacter sakazaki* of fly and garbage samples showed that the

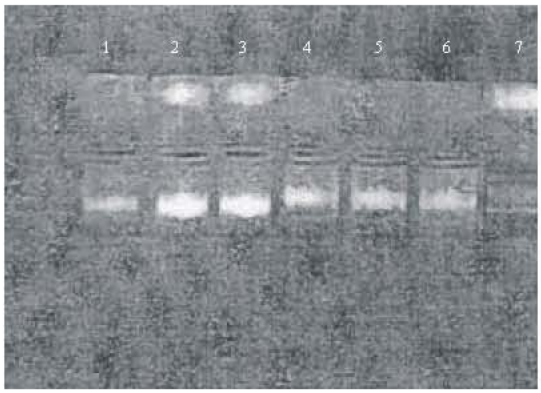

Fig 3: Endonucleases digestion of *Pseudomonas aeruginosa* plasmid DNA
1. *Sma* I cutted DNA (Garbage sample)
2. *Sma* I digested DNA (Fly sample)
3. *Sma* I digested DNA (Fly sample)
4. *Eco* RI digested DNA (Fly sample)
5. *Eco* RI digested DNA (Fly sample)
6. *Eco* RI digested DNA (Garbage sample)
7. DNA marker

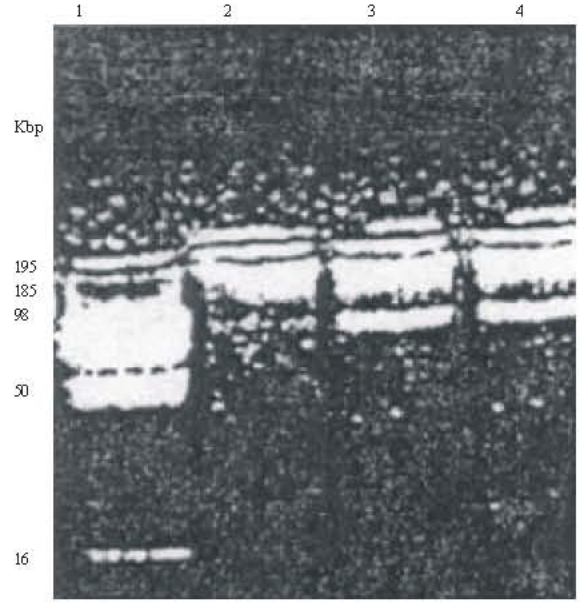

Fig. 4: DNA plasmid profile of two undigested isolates of *Escherichia coli*
1. Standard plasmid DNA (Fly sample)
2. Undigested plasmid DNA (Garbage sample)
3. Undigested DNA (Garbage sample)
4. Undigested DNA (Garbage sample)

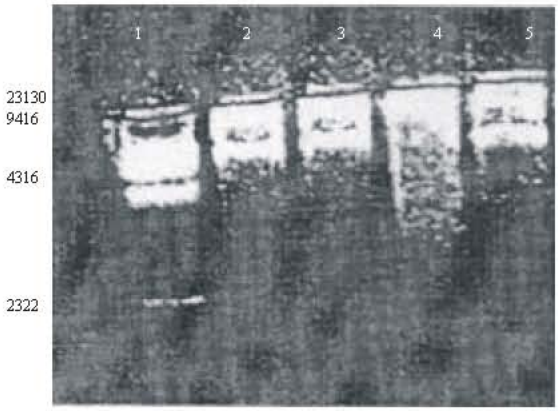

Fig. 5: Restriction endonuclease digestion of *Escherichia coli* plasmid DNA
1. DNA-Hind III digest
2. *Eco* RI digested plasmid DNA (Fly sample)
3. *Eco* RI digested plasmid DNA (Garbage sample)
4. *Sma* I digested plasmid DNA (Fly sample)
5. *Sma* I digested plasmid DNA (Garbage sample)

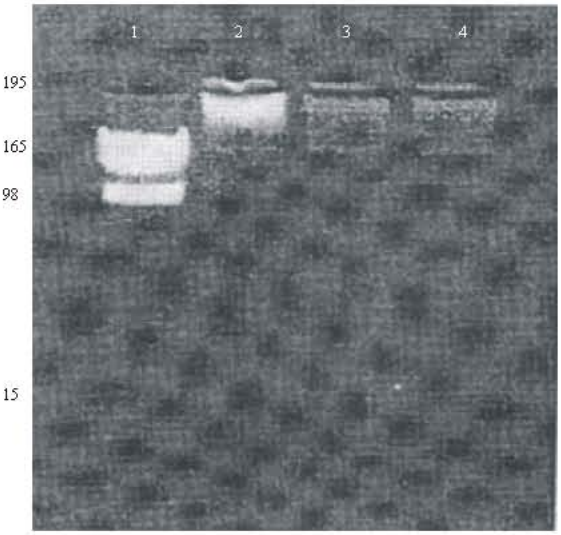

Fig. 6: DNA plasmid profile of two undigested isolates of *Staphylococcus xylosus*
1. Standard plasmid
2. Undigested plasmid DNA (Garbage sample)
3. Undigested plasmid DNA (Fly sample)
4. Undigested plasmid (Fly sample)

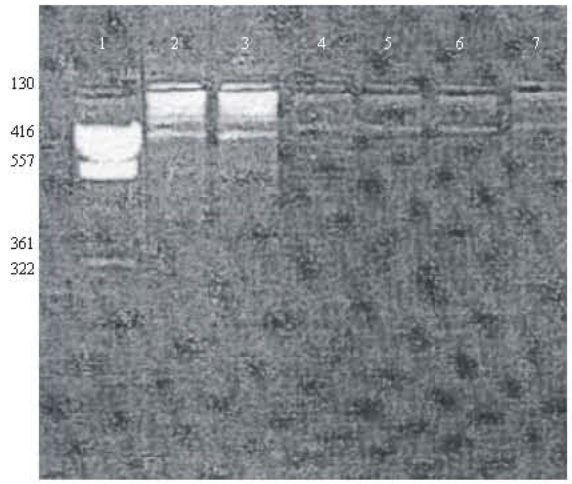

Fig. 7: Restriction endonuclease digestion of *Staphylococcus xylosus* plasmid DNA
1. λ DNA-Hind III digest (marker)
2. *Eco* RI digested plasmid DNA (Garbage sample)
3. *Eco* RI digested plasmid DNA (Fly sample)
4. *Sma* I digested plasmid DNA (Fly sample)
5. *Sma* I digested plasmid DNA (Fly sample)
6. *Sma* I digested plasmid DNA (Garbage sample)
7. *Sma* I digested plasmid DNA (Garbage sample)

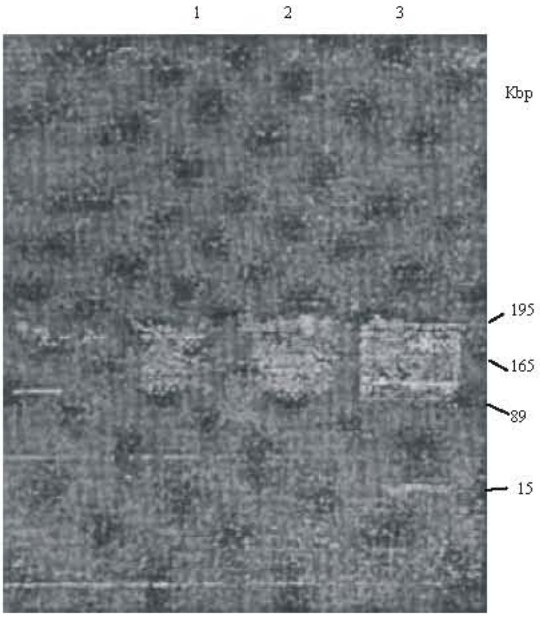

Fig. 8: DNA plasmid profile of two undigested isolates of *Klebsiella pneumonia*
1. Undigested plasmid DNA (Fly sample)
2. Undigested plasmid DNA (Garbage sample)
3. Standard plasmid

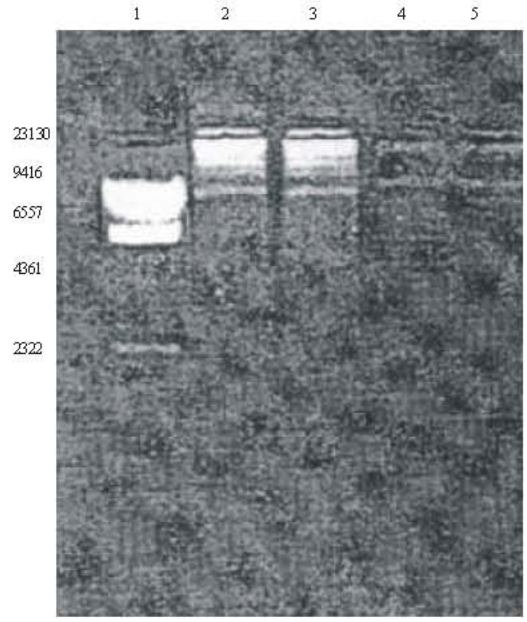

Fig. 9: Restriction endonucleas e digestion of *Klebsiella pneumonia* plasmid DNA
1. λ DNA-Hind III digest (marker)
2. *Sma* I digested plasmid DNA (Fly sample)
3. *Sma* I digested plasmid DNA (Garbage sample)
4. *Eco* RI digested plasmid DNA (Fly sample)
5. *Eco* RI digested plasmid DNA (Garbage sample)

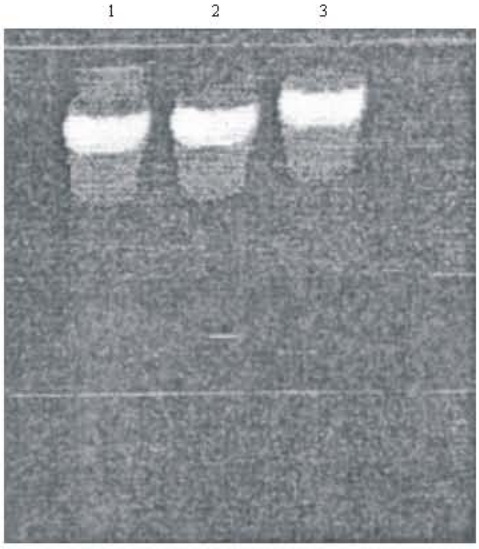

Fig. 10: DNA plasmid profile of two undigested isolates of *Enterobacter sakazakii*
1. Standard plasmid
2. Undigested plasmid DNA (Garbage sample)
3. Undigested plasmid DNA (Fly sample)

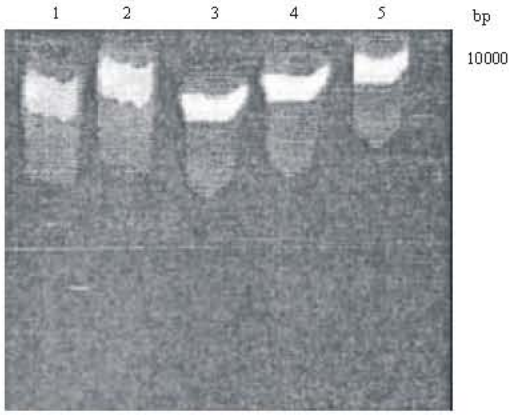

Fig. 11: Restriction endonuclease digestion of *Enterobacter sakazakii* plasmid DNA
1 *Eco* RI digested plasmid DNA isolated from *Enterobacter sakazakii* (Fly sample)
2 *Eco* RI digested plasmid DNA isolated from *Enterobacter sakazakii* (Garbage sample)
3 *Sma* I digested plasmid DNA isolated from *Enterobacter sakazakii* (Fly sample)
4 *Sma* I digested plasmid DNA isolated from *Enterobacter sakazakii* (Garbage sample)
5 Molecular weight of 1 kb ladder digested (DNA marker)

isolates have one plasmid array of different molecular weight (Fig.10, Table 2). Treatment of plasmids with *Eco* RI showed one cut at 9744 bp (fly bacterial isolates) and at 9949 (garbage bacterial isolate). *Sma* I enzyme cut the bacterial DNA from flies at 9590 and 8513 bp while that of garbage at 9897 and 8821 bp (Fig. 11 and Table 2).

DISCUSSION

Many pathogenic bacterial species could be isolated from flies and its breeding wastes Foetdar *et al.* [4, 17] In the present study, five bacterial genera were found identical when isolated from garbage samples and Associated flies. Pathogenicity of such isolates was confirmed by [14, 18] these five bacterial genera were identified according to their microscopical and biochemical properties. Protein and DNA analysis proved that, three genera showed similarity: *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus xylosus*. They are previously isolated from EL-Demerdash hospital and Fifteenth of May city [5]. Protein profile of *E. coli* has four fractions identical in molecular weight basis when this bacterium isolated from waste site or its breeding flies. For *Pseudomonas aeruginosa* the protein fraction of 39.06 KD sharing its two isolates from waste and flies. *Staphylococcus xylosus* show the similar protein fractions in each isolate. The first fraction from garbage estimated to be 181.84, while the first fractions extracted from flies has MWt181.14. The second fraction has MWt 101.39 from garbage baceria and 101.83 from fly bacteria. This slight variation in molecular weights is not significant and may be due to variation in loading specimens during electrophoretic run. On the other hand protein analysis proved dissimilarity between other bacterial species as *Enterobacter* and *Klebsiella* spp.

Protein analysis using SDS polyacrylamide gel technique is successfully used to characterize bacterial toxin proteins [6, 7, 12, 19, 20]. Plasmid DNA characterization confirmed the aforementioned results. Alkaline lysis method for plasmid isolation and endonuclease digestion are widely used for bacterial characterization [18, 21-26]. *E. coli* showed the same DNA bands for both isolates from bacteria collected from garbage or fly samples. Digestion of plasmid DNA with both *Eco* RI and *Sma* I gave the same figure for both *E. coli* isolates. The same results are obtained for characterization of DNA plasmids of *Staphylococcus* and *Pseudomonas* spp.

CONCLUSION

We can concluded that, protein characterization and DNA fingerprints are decisive and accurate methods when testing similarity of isolated bacterial samples. Biochemical figures are not enough. Results give solid incrimination of house fly in transmission and dissemination of solid waste contaminations. This results may be a helpful tool for other pathogenic microorganisms, which contaminate types of solid wastes such as viruses and parasitic agents.

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