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Molecular Typing of *Escherichia coli* for Detection of Critical Control Points of Farm Shrimp Processing in Sri Lanka

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ABSTRACT. *Farm shrimp and pond water samples were obtained from two harvests of a farm. Further, samples of shrimp were collected from the processing plant during the processing These samples were used to isolate E. coli. Twenty nine E. coli isolates were recovered from first visit and 30 E. coli isolates were recovered from second visit. Of the 59 isolates only 36 (61%) carried plasmids. Most of the plasmid-free strains originate from pond water. Seven strains of E. coli were detected using plasmid profiling. Plasmid-free isolates by chromosomal DNA restriction digestion with BstEII and ribotyping produced 12 strains. With EcoRI, 6 strains were produced. Analytical profile index (API) also showed plasmid-free E. coli were a group of seven strains. Use of ribotyping demonstrated that some strains originated from pond water were carried into early stages of processing. A few other strains were due to contamination in the factory. None of the strains isolated showed any resistance to common antibiotics nor they produced any toxins, heat labile or heat resistant.*

INTRODUCTION

Microbial indicators are commonly employed to assess food safety and sanitation. Use of Enterobacteria, coliforms, and *E. coli* as microbiological parameters of sanitary quality have a long history over a century (Schardinger, 1892).

Many early workers accepted the presence of *E. coli* and coliforms as an index of insanitary conditions in food and therefore of potential pathogenicity (Thatcher and Clark, 1968) but many were opposed to any

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correlation between pathogens and enteric organisms in foods (McCoy, 1962). The habitat of the animal providing raw material (Todd, 1980) and food handling and processing (Wentz *et al.*, 1985) are believed to lead to contamination of foods with such organisms and public health problems. This has resulted in their inclusion in microbiological standards to assess the safety of foods.

Differentiation of microorganisms into strains has been used for many years for investigations in clinical microbiology (Meitert and Meitert, 1978). Such techniques, especially molecular ones, are now being used to investigate problems in food systems (Dodd *et al.*, 1987).

This study was undertaken with a view to investigate the critical control points of shrimp processing using molecular typing methods namely, plasmid profiling and ribotyping to determine the presence of some virulent strains and the sources of contamination of such strains.

MATERIALS AND METHODS

A total of 100 samples of farm shrimp and 10 samples of pond water were collected for testing for *E. coli* during two harvests and subsequently during processing of these harvests at the factory. Pond water samples were obtained prior to shrimp harvest. Five samples of shrimp were collected immediately after each harvest. Forty five further samples were collected at nine different stages of processing (5 at each site) namely, Receiving; Deheaded (Before Wash); Deheaded (After Wash);Peeled and Undeveined (After Wash); Peeled and Deveined (After Wash); Headless Shell-on (Frozen); Cooked (Unpeeled); Cooked (Peeled and Undeveined); cooked(Peeled and Deveined). This process of sampling was repeated after three months.

Bacteriological testing

Samples were blended and serial dilution were prepared using peptone water (Oxoid). Analysis of samples for *E. coli* was done using of MacConkey broth (MB) and Brilliant green bile broth (BGBB)(Oxoid). The positive sample were streaked on MacConkey agar (MA), for isolation of presumptive positive *E. coli* isolates. These isolates were confirmed as *E.*

coli using Indole, methyl red, Voges-Proskauer and Citrate tests. Methods used were those described in Collins and Lyne (1984).

Plasmid profiling

E. coli isolates were grown at 37 C on Brain Heart Infusion (BHI) agar and two or three well isolated colonies were used for plasmid profiling using the method of Birnboim and Doly (1979). Plasmid molecular weights were compared using with *Hind*III digest of Lambda DNA as the size marker.

Ribotyping

Thirty six plasmid free isolates of *E. coli* were probed using DNA encoding rRNA (ribotyping). The characteristic patterns, ribo patterns or fingerprints were used to differentiate strains.

Extraction, digestion and electrophoresis of bacterial genomic DNA

Total chromosomal DNA was extracted by the method of Pitcher *et al.*(1989). DNA samples (1 μg) were digested with three restriction enzymes (*Eco*RI, *Hind*III and *Bst*EII) according to the manufacturers instructions (Boehringer Mannheim Biochemicals). Samples of digested DNA were electrophoresed in 0.8% (w/v) agarose gels in Tris acetate buffer at 30V for 16-18h. A kilobase (Kb)Ladder (Gibco BRL) and *Hind*III digest of Lambda DNA was used as size markers.

Southern hybridization and detection

The restriction fragments were then denatured with sodium hydroxide nitrocellulose papers using the method of Southern (1975). DNA hybridization was carried out using the digoxygenin probes (Boehringer Mannheim Biochemicals). Probes were prepared by labelling 16S and 23S rRNA (Boehringer Mannheim Biochemicals) using AMV reverse transcriptase. Hybridization was performed in water phase buffers at 68 C overnight as described by the manufacturers. Bound probe was detected by an enzyme labelled antibody directed against the modified base (Boehringer-Mannheim Biochemicals).

Analytical profile index (API) test

API 20E is designed for analytical profile index of *Enterobacteriaceae*. Seventy five percent of the *E. coli* isolates were tested by the API 20E System (bioMeriux UK Ltd) to differentiate into API profiles using manufacturers instructions. The computer data base was used for detection.

Antibiotic resistance

Resistance to the following antibiotics was tested using antibiotics discs (Mast Diagnostics) containing ampicillin 10(μg), cephaloridine (5μg), colistin sulphate (25μg) gentamicin (10μg), streptomycin (10μg), sulphatriad (200μg), tetracycline (25μg) and cotrimoxazole (25μg). Seeded BHI agar plates showing presence or absence of clear zone was used to check the resistance towards the antibiotics.

Enterotoxin production and screening for *E. coli* O157

VET-RPLA (Oxoid) toxin detection kit, and (Oxoid) immunoassy kit were used for detection of heat labile and heat stable toxin according to manufacturers instructions.

The isolates of *E. coli* were grown on sorbitol MacConkey agar (Oxoid)(SMA). Colourless colonies on SMA were examined using *E. coli* O157 latex test (Oxoid) for agglutination according to the manufacturers instructions.

RESULTS

Plasmid profiling

Of the total of 59 *E. coli* isolates analyzed for plasmid profiles, 36(61%) of the isolates had no plasmids (Profile A) indicating a low plasmid carriage rate. The proportion of plasmid-carrying *E. coli* from pond water was relatively low (10%) while isolates associated with shrimp had a slightly higher carriage rate of 44%.

The plasmid bands varied in size from approximately 4Kb to 1.8Kb. Table 1 gives the distribution of plasmid profiles of *E. coli* isolated from farm shrimp and pond water. Apart from the plasmid free isolates (A) seven plasmid profiles were observed in this study (B-H). Seven of eight profiles detected in this study are presented in Figure 1. During the first visit plasmid profiles B,C,D,F,G & H were found. Except for profile F, all these profiles were present in samples taken on the second visit when an additional profile E, was found (Table 1). Since the profiles B,C,G and H were recovered during both visits it appeared that the same sources of contaminations were present over a period of 3 months. With the exception of plasmid profile H in the first visit which had an incidence of 10%, all the other plasmid carrying strains had a very low level of incidence (Table 2).

Plasmid profile F displayed the highest number of DNA bands (three). Two profiles D & G had two DNA bands while the other four profiles B,C,E and H had only one band.

Ribotyping

Preliminary screening using the enzymes revealed *Bst*EII ribotyping patterns to be the most useful in discrimination of strains but a few strains did not produce very clear bands mostly in visit 1. Digestion of chromosomal DNA with *Hind*III produced many faint bands in the fingerprints compared to the other two restriction enzymes. The fingerprints produced with *Hind*III were not very useful in this study for strain differentiation.

Digestion with *Bst*EII generated an average of 14 fragments per strain ranging in size from 1.5 Kb to 23 Kb. Out of total of twelve patterns detected among all the isolates nine patterns are shown in Figure 2. Tables 3 and 4 give the distribution of these patterns at different sampling locations. A few isolates, could not be differentiated due to faint patterns produced.

Most strains displayed a set of five common fragments of which the largest two are approximately 4Kb. These were followed by a set of three smaller fragments evenly spaced in the range of 2-2.3 Kb.

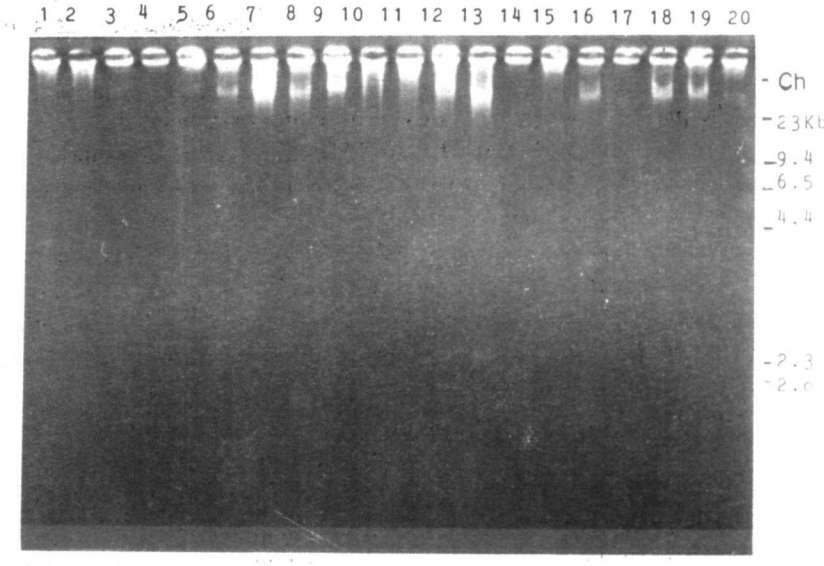

Figure 1. Plasmid profiles of *E.Coli.* strains isolated shrimp profile A, (Plasmid-free isolates) (Lanes 3, 6 & 7, 9-12, 14 & 15, 17-18 & 20); profile B, (Lanes 4,5 & 19); profile C, (Lane 8); profile D, (Lane 2); profile F, (Lane 16); profile G, (Lane 13); profile H, (Lane 1). Ch refers to chromosomal band. Fragment sizes are given in the left hand margin.

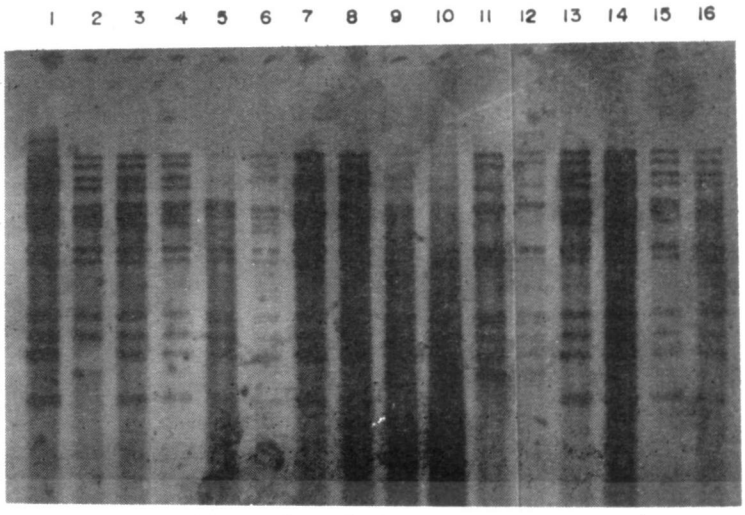

Figure 2. Ribotyping of plasmid - free *E. coli* isolates from shrimp and pond water (visit 2) using *Bst*EII hybridization with digxigenin-labelled probe. Pattern.1, (Lanes 3, 4, 8, 13, 14, 15, & 16); Pattern 2, (Lane 10) Pattern 4,(Lane 12); Pattern 6,(Lane 7), Pattern 7, (Lane 11); Pattern 9, (Lane 1); Pattern 10, (Lane 12); Pattern 11, (Lane 5); Pattern 12, (Lane 2).

Table 1. Distribution of plasmid profile of *E. coli* on shrimp and pond water in two sampling visits

Sampling site	Sampling visit	Plasmid profile								Total
		A	B	C	D	E	F	G	H	
1) Pond water	V1	5	-	-	-	-	-	-	-	5
	V2	4	-	-	-	1	-	-	-	5
2) Shrimp	V1	0	-	-	-	-	-	-	-	0
(after harvest)	V2	0	-	-	-	-	-	-	-	0
3) Deheaded	V1	-	1	-	-	-	-	-	-	1
(before washed)	V2	5	-	-	-	-	-	-	-	5
4) Deheaded	V1	0	-	-	-	-	-	-	-	0
(after washed)	V2	0	-	-	-	-	-	-	-	0
5) Peeled & undeveined	V1	2	-	1	1	-	-	-	-	4
(after washed)	V2	2	1	-	1	-	-	1	-	5
6) Peeled & deveined	V1	4	-	1	-	-	-	-	-	5
(after washed)	V2	3	-	1	-	1	-	-	-	5
7) Headless Shellon	V1	-	-	-	-	-	1	-	-	1
(Frozen)	V2	-	-	-	-	-	-	-	-	0
8) Cooked (unPeeled)	V1	1	-	-	-	-	-	1	1	3
	V2	-	-	-	-	-	-	-	-	0
9) Cooked (Peeled &	V1	2	1	-	-	-	-	1	1	5
undeveined)	V2	3	-	-	-	1	-	-	1	5
10) Cooked (Peeled &	V1	3	1	-	-	-	-	-	1	5
deveined)	V2	2	-	1	-	-	-	1	1	5
Total		36	4	4	2	3	1	4	5	59

V1= First samples V2= Second samples

Table 2. Percentage incidence of *E. coli* of different plasmid profiles during first and second sampling visits

Plasmid profile	First Sampling 1	Second Sampling
A	58	63.6
B	3.4	3.3
C	6.9	6.6
D	3.4	0
E	0	10.0
F	3.4	0
G	6.9	13.3
H	10.3	3.3
	100	100

On the basis of fragments produced by *Bst*EII six strains were distinguishable among the plasmid-free isolates from the first visit (not presented) and two other isolates did not produce very clear bands whilst 9 strains were distinguishable amongst those isolates from the second visit (Figure 2) and in total 12 patterns were displayed.

Six of the patterns were produced by plasmid free *E. coli* from the second visit using *Eco*RI as the digestion enzyme (results not presented). This process generated on average 11 fragments per strain with their molecular weight ranging from 1.0 Kb to 23 Kb. A variety of patterns was also obtained for first visit but their relationship to those of the second visit is not clear due to faint patterns obtained for first visit. Some of the isolates of *E. coli* as revealed by *Bst*EII and *Eco*RI showed complete correspondence between the types identified except for *Eco*RI pattern B which gave patterns 9 & 11 by *Bst*EII (Table 4).

Strains with pattern 1 were detected on both visits and were found to be the most common type amongst the isolates. Over 30% of the isolates from the first visit and over fifty percent of the isolates from the second visit showed this pattern, while none of the other isolates from the same visit shared the same ribo profile.

Table 3. Distribution of BstE II ribo pattern of nine *E. coli* in relation to sampling sites (First visit)

Isolation Site	Pattern Number											
	1	2	3	4	5	6	7	8	9	10	11	12
1) Pond water	2	-	-	-	-	1	-	-	-	-	-	-
2) Deheaded	-	-	-	-	-	-	-	-	-	-	-	-
3) PUD	1	-	1	-	-	-	-	-	-	-	-	-
4) PAD	1	-	-	-	-	-	-	1	-	-	-	-
5) Cooked unpeeled	-	1	-	-	-	-	-	-	-	-	-	-
6) Cooked PUD	-	-	-	-	-	-	-	1	-	-	-	-
7) Cooked (PAD)	-	-	-	-	-	-	-	-	-	-	-	-
Total	4	1	1	0	0	1	1	1	0	0	0	0

PUD = Peeled and undeveined; PAD = Peeled and deveined

Table 4. Distribution of *Bst*E II ribo pattern of seventeen *E. coli* in relation to sampling sites (Second visit)

Isolation Site	Pattern Number											
	1	2	3	4	5	6	7	8	9	10	11	12
1) Pond water	3	-	-	-	-	-	-	-	1	-	-	-
2) Deheaded	3	-	-	-	-	-	1	-	-	1	-	-
3) PUD	1	1	-	-	-	-	-	-	-	-	-	-
4) PAD	2	-	-	-	-	-	-	-	-	-	-	-
5) Cooked unpeeled	-	-	-	-	-	-	-	-	-	-	-	-
6) Cooked PUD	-	-	-	1	-	-	1	-	-	-	1	-
7) Cooked (PAD)	-	-	-	-	1	-	-	-	-	-	-	-
Total	9	1	0	1	1	0	1	0	1	1	1	1

PUD = Peeled and undeveined PAD = Peeled and deveined

This generated on average 11 fragments per strain with a range in their molecular weights, from 1.0 Kb to 23 Kb. *Eco*RI ribo pattern A was shared by eight isolates. A variety of ribo patterns was also found amongst strains of the first visit but their relationship to those of the second visit was not clear due to the faint patterns obtained for visit 1.

A comparison of the grouping of some of the isolates of *E. coli* as revealed by *Bst*EII and *Eco*RI. This shows complete correspondence between the types identified by each digestion method with the exception of *Eco*R1 pattern B which gave 2 patterns (9 & 11) by *Bst*EII digestion.

Detection of 12 fingerprints for 28 isolates of *E. coli* free of plasmids is clearly an indication of the widely varying sources of contamination of the product with *E. coli*. Pattern 1 was very common amongst isolates from pond water in both visits and this probably indicates that this strain is more resistant to brackish water for longer periods than the other strains. This strain was also recovered in the initial stages of shrimp processing suggesting that some strains can be carried to the processing line. The non-recovery of this strain in the cooked product may suggest a low incidence or the absence of this strain amongst the processing staff and this part of the processing line.

Ribo types 1, 2 and 7 were detected during both visits which shows that the contaminating sources of these *E. coli* were still there after three months. Apart from strains 1, 6 & 9 all other strains appeared to arise due to contamination at the factory and more likely from processing workers. Three ribo patterns detected in the first visit were also found in the second visit, again indicating the persistence of common sources of contamination.

Nutritional profile (API) typing

Forty four strains of *E. coli* were tested for API profile. There were eight profiles detected during this investigation. Of these five, were shown by the isolates recovered from both visits. API profile I was found to be the most common profile (29.5%) followed by API profile II (22.7%). The least frequent profile (VIII)(2.3%) was recovered only during the first sampling visit.

Antibiotic resistance typing

All strains were sensitive to ampicillin, cephaloridine, colistin sulphate, gentamicin, streptomycin and tetracycline. All the isolates showed a high level of sensitivity. Most of the strains showed no inhibition zone for sulphatriad and cotrimoxazole suggesting that they were resistant to these two antibiotics. Occasional strains showed a clear zone initially (24 h incubation) which disappeared between 24-48 h incubation, indicating the limited susceptibility of these strains.

There was no observable relationship between possession of plasmids and resistance to the eight antibiotics used in this study. Plasmid-bearing strains as well as the plasmid-free strains showed similar sensitivity and resistance to the antibiotics tested.

Enterotoxin detection and *E. coli* 0157

None of the *E. coli* strains isolated in this study produced heat labile or heat resistant toxins and they all failed to produce any agglutination with *E. coli* 0157 antisera.

DISCUSSION

The results of the present study of over 50 *E. coli* strains proved that plasmid profiling is a good method for differentiation of strains which carry plasmid DNA however, the low carriage rate displayed by many strains made it very limiting in this situation.

Plasmids can be transferred from cell to cell at a varying frequency (Howell and Martin, 1978) thus resulting in variable plasmid carriage rates for different organisms. Even in the same organism, plasmids are mainly responsible for variations leading to adaptations (Reanney, 1976). Hence the plasmid content for a bacterial population in an environment is greatly influenced by ecological factors.

Any cell which fails to inherit a copy of a plasmid is reported to have a reduced energy expenditure and hence under non-selective conditions will generally out-grow plasmid containing strains (Day, 1982). The low plasmid carriage rate of *E. coli* in brackish water suggests that growth in this

environment may have some effect in curing plasmids. The work of Hill and Carlisle (1981) showed that enrichment with a surface active agent and a high temperature of incubation (44.5 C) decreased the probability of recovering *E. coli* strains which harbour plasmids. Hill *et al.*, (1985) reported that 50% of the *E. coli* from human sources did not grow, even though large inocula were used, while about half of the plasmid-containing strains lost plasmids during enrichment, with a plasmid loss of about 96% in pure cultures.

In the present study, due to the low numbers of lactose positive colonies on MacConkey agar (MA) found after direct plating, enrichment procedures were adopted using MacConkey broth (MB) and Brilliant green bile broth (BGBB) before confirmation for *E. coli* using IMViC tests. The enrichment procedure so adopted may have contributed to some loss in plasmids.

In this study, a low plasmid carriage rate amongst the shrimp isolates was coupled with a low number of plasmids per strain while none of the strains showed any drug resistance towards common antibiotics. Nakamura *et al.*, (1985) observed a very high incidence of plasmids in drug resistant *Salmonella typhimurium*. Investigators believe that some pathogenic determinants are carried on plasmids (Smith and Hall, 1968) and any procedure in which plasmids are lost would tend to reduce the efficiency of recovering virulent strains.

A diverse range of drugs have been used for control of fish disease (Snieszko, 1978). Modern agricultural practices have always been based on the most efficient production methods in which the use of antibiotics in feed is common. Although various authorities have tried to discourage the use of antibiotics in fish and shrimp feed, DePaola *et al.*, (1988) reported that 89% of Aeromonas hydrophila isolates from cultured catfish in the USA were resistant to tetracycline while market catfish demonstrated resistance of 1% only.

Non-recovery of resistant *E. coli* isolates from shrimp may be mainly due to the adoption of traditional farming practices and non-exposure of animals to antibiotics as growth promoters or as prophylactic agents. Any decrease in the detection the plasmid-harbouring strains cannot be disregarded in this respect, as plasmids commonly convey antibiotic resistance.

The origin of enteric bacteria in foods may be via two main routes, mainly from domestic and farm animals, and also by humans, rainwater run off and sewage. Enterotoxigenic (ETEC) strains of *E. coli* from such sources can cause a variety of different diseases. They are believed to cause diarrhoea among travellers to tropical countries (Gross and Rowe, 1985). The strains of ETEC that produce heat labile (LT) and heat stable (ST) toxin and also enteropathogenic (EPEC) strains producing ST are commonly reported to cause gastroenteritis (Jay, 1992). Plasmids have been reported to code for these toxins (Hardy, 1986).

In a study conducted in the USA, 8% of *E. coli* isolated from foods were reported to produce either ST or LT enterotoxin (Sack *et al.*, 1977). In the present study none of the strains produced agglutination with *E. coli* 0157:H7 antisera nor did they produce any enterotoxin. It may seem that the shrimp products used in this study were free of public health problems associated with these *E. coli* strains.

However, Mehlman and Romero (1982) found that only 31% of the pathogenic *E. coli* strains could be quantitatively recovered by AOAC methods (AOAC, 1980) and 30% of the strains were inhibited by MA. Unlike most typical *E. coli* strains, *E. coli* 0157:H7 does not grow, or grows poorly at elevated temperatures (Doyle and Schoeni, 1984).

Lack of selective pressure during enrichment and other culture procedures may lead to loss of plasmids which carry toxin production coding DNA sequences (Evans and Evans, 1978). Enrichment and other cultural procedures could decrease the efficient recovery of virulent strains. Non-recovery of these strains in this study may be partly due to the adoption of such methods. The inability of the standard procedures for isolation of faecal *E. coli* to allow isolation of *E. coli* 0157:H7 raises the question of the usefulness of such methods.

Species of an organism can be characterised by a specific pattern of rDNA restriction fragments (Tompkins *et al.*, 1986). In some cases where several strains from the same species were studied more than one pattern was obtained (Grimont and Grimont, 1986). Williams and Collins (1991) used ribotyping to differentiate Streptococcus species. Ribotyping results in a recent study on *E. coli* (Wachsmuth *et al.*, 1991) revealed that an NcoI digest can distinguish epidemic *E. coli* strain 0157:H7 and may provide a more stable marker than plasmid profiles.

In this study, the strains with a particular plasmid isolated in the first visit were also recovered during the second visit. Since there were about 20 workers engaged in the shrimp processing operation, recovery of four plasmid profiles in both visits suggests that these strains are commonly carried by many factory workers or that some workers, less careful about their personal hygiene, were contributing to them during both visits.

Cox *et al.*, (1988) in a critical assessment of use of Enterobacteriaceae in food microbiology believed *E. coli* could establish themselves as a part of a resident flora of the factory environment, especially under wet conditions. Many workers have reported such indigenous strains of *Salmonella aureus* in poultry processing plants (Adams and Mead, 1983). Unlike modern poultry processing which involves complicated machinery, manual shrimp processing adopted at this plant had only simple equipment which can easily be cleaned, disinfected and dried regularly.

Using restriction fragment length polymorphism of rRNA, 30 *E. coli* strains were examined. Chromosomal DNA of *E. coli* digested with *Bst*EII produced highly discriminatory riboprofiles and 12 strains could be readily distinguished by characteristic banding patterns although the bands of a few strains were not well produced. *E. coli* strains which were not typable by plasmid profile analysis could not be discriminated by antibiotic resistance patterns. API profiles produced some strain differentiation, although they showed a high degree of overlap of plasmid-free and plasmid bearing strains but they could be used to subdivide some plasmid profile strains. On the other hand, strain differentiation by ribotyping appeared to be particularly useful in understanding the contamination patterns in shrimp processing. Ribotyping revealed that the plasmid-free group of *E. coli* was a highly diverse group of 12 strains. Although a large proportion of plasmid-free *E. coli* were associated with pond water, they were only represented by three ribo patterns. Of these three patterns, riboprofile 1 was associated with the majority of strains and was detected during both visits suggesting that it was a common *E. coli* strain in pond water. This strain was also detected more in the early stages of shrimp processing than in the later stages and was not in the cooked products. This clearly indicates that this strain originated from pond water. Two other strains were displayed by the shrimp associated with isolates from both visits. Both these strains were detected after cooking during at least one of the visits indicating that they were introduced from the factory and not from the farm.

Four plasmid-bearing strains were common to both visits. Among the plasmid-free strains, three ribo profiles were also isolated during both visits. This clearly indicates the persistence of the source of *E. coli* contamination at the plant.

Pond water has a greater chance to receive enteric contaminants from various sources. However failure to recover a wider range of strains in pond water is hard to understand. This raises an important question; are certain strains selected in this environment? When the level of contamination and incidence is high, obviously it should be expected that a large number of strains would be obtained, but at low levels of incidence, artificial aeration and brackish water in the pond might be selective for certain *E. coli* strains as well as the consequence of the enrichment procedure used in isolation. It seems very likely that under these non-selective growth conditions in the environment, as reported by others, plasmid-free isolates which need less energy for growth may out-grow plasmid harbouring ones.

CONCLUSIONS

E. coli isolates from pond water and shrimp had low plasmid carriage rate. Plasmid examination of *E. coli* showed that pond water *E. coli* are mostly plasmid free. The frequent occurrence of plasmid free strains among shrimp *E. coli* suggested their origin as being the pond water. Using ribotyping, it was shown that not all *E. coli* free of plasmids originated from pond water and some may have been introduced by other sources in the factory. Presence of many common strains during two visits three months apart illustrates the persistence of these strains. More work needs to be carried out trace them to the factory environment or to individual operatives. In spite of the enormous potential of DNA-probe technology for diagnostic microbiology and hazard analysis critical control point (HACCP) related work, ribotyping is a time consuming laborious and expensive exercise. However there are signs that user friendly kits would soon appear in the market in such a situation could revolutionize quality control in food industry.

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