Indian J Med Res 85, May 1987, pp 496-499

Plasmid mediated enterotoxigenicity in salmonella strains isolated from patients of gastroenteritis

M. Jayasheela, U.K. Sharma, Alok Kumar Mondal* & S.N. Saxena

*National Salmonella & Escherichia Centre, Central Research Institute Kasauli & *Institute of Microbial Technology, Chandigarh*

Accepted November 19, 1986

Transfer of enterotoxigenicity was achieved in 4 of 14 strains of Salmonellae. Use of two different recepients and application of both auto-transfer and mobilization methodologies enhanced chances of transfer of enterotoxigenicity. Comparison of plasmid profiles of the donors and transconjugants with that of enterotoxigenic *Escherichia coli* 10407 revealed the presence of a common plasmid DNA band.

Enterotoxin is known to play an important role in the pathogenesis of Salmonella gastroenteritis[1,2]. The genetic factor controlling *Escherichia coli* heat labile (LT) toxin production has been shown to be transferable[3]. Panhotra *et al*[4] found enterotoxigenicity in Salmonella strains to be non-autotransferable, whereas Sobeh *et al*[5] could successfully transfer enterotoxigenicity from *Salmonella typhimurium* to *Esch. coli* K12 F^-. In our earlier study, 81 strains belonging to 11 serotypes were found to be enterotoxigenic (unpublished data). Of these 14 strains were examined for transferable enterotoxigenicity. The plasmid profile of donor and transconjugants were compared to that of known enterotoxigenic *Esch. coli* 10407.

Material & Methods

Bacterial strains and transfer of enterotoxigenicity : Fourteen enterotoxigenic and multidrug resistant salmonellae (*S. typhimurium* 8; *S. bareilly* 3; and *S. senftenberg* 3) were examined for transfer of their enterotoxigenic property by the method of Anderson and Threlfall[6] which is commonly used to study bacterial R factors. Auto-transfer experiments were carried out in duplicate using two different racepients—*Esch. coli* K12 F^- and *Esch. coli* J 62. Similarly mobilization experiments were also carried out in duplicate using the same final recepients and *Esch. coli* K12△ as intermediate recepient. Transconjugants were examined for their antibiotic resistance pattern by agar dilution method[7] and for enterotoxigenicity by the method of De and Chatterjee[8] using concentrated culture supernates. The concentrated culture supernates were prepared by freeze drying (Edwards Freeze dryer model L22P) the bacteria free supernate of 50 ml syncase broth overnight shake cultures of the strains and redissolving the same in 5 ml of distilled water.

Physical varification of plasmid profiles : Plasmid DNA from donors, recepients and transcojugants were extracted by the rapid small scale alkaline lysis method of Maniatis *et al*[9] and were electrophoresed in 0·7 per cent agarose gels (100 × 75 mm) prepared in tris borate buffer (*p*H 8, 0·1 *M*) using Pharmacia EPS 500/400 power pack in Bio-Rad mini DNA cell at constant voltage of 50 v in TBE buffer till the tracking dye, bromophenol blue, covered approximately 75 per cent of the gel length. The gels were stained by immercing in ethidium bromide (1 μg/ml) in TBE buffer for 10 min. DNA bands were visualised using Fotodyne UV system and photographed on polarbid film. Plasmid DNA preparation of the enterotoxigenic *Esch. coli* 10407 was included for comparison. Eco RI/Hind III digest of λ DNA was used as molecular weight marker.

Results

Only 4 strains of the 14 showed the transfer of enterotoxigenicity. Three strains of *S. typhimurium* with the same antibiotic resistance pattern *viz.*, ACGKSSu-TFz, transferred three different patterns to the recepients (Table) whereas in *S. bareilly* the resistance markers were transferred enblock along with enterotoxigenicity. Auto-transfer was effective in one strain and mobilization was effective in three strains.

Comparison of plasmid profiles of donors, recipients and transconjugants with that of *Esch. coli* 10407 revealed the presence of a common band with electrophoretic mobility similar to that of 21·2 Kbp fragment of Eco RI/Hind III digest of DNA. *Esch. coli* 10407 revealed an

Table. Strains showing Ent+ transfer/mobilization

Serotype	Antibiotic resistance pattern	Autotransfer using recepient		Mobilization using recepient		Character transfered	
		K 12	J 62	K 12	J 62		
S. typhimurium No. 571/84	ACGKSSuTFz	—	+	—	—	SSu	Ent+
S. typhimurium No. 822/84	,,	—	—	+	—	ASu	Ent+
S. typhimurium No. 1402/84	,,	—	—	+	—	AT	Ent+
S. bareilly No. 220/84	AGSuT	—	—	—	+	AGSuT	Ent+

A, ampicillin; S, steptomycin; Su, sulphamethoxazole; G, gentamycin; T, tetracycline

additional slow moving band which was also present in all donors and transconjugants except strain no. 571/84. All donors and transconjugants except no. 220/84 had faster moving components also which were not comparable to the fast moving band of *Esch. coli* 10407.

Discussion

In this study, use of both auto-transfer and mobilization procedures and two receipents seems to have enhanced the transfer of enterotoxigenic property. Using auto-transfer procedure alone Panhotra *et al*[4] could not show transfer of enterotoxigenic property in any of the 12 strains of *S. typhimurium* and Sobeh *et al*[5] could transfer in only one strain amongst 15 strains. Thus, it appears that Ent^+ plasmid in most salmonella strains isolated from patients of gastroenteritis are of non-autotransferable nature, in contrast to that of *Esch. coli* strains[10]. The band with electrophoretic mobility equal to that of 21·2 Kbp fragment is clearly not related to enterotoxigenicity as it is present in the non-enterotoxic recipients also. Fast moving bands are very heterogeneous, are not comparable to that of *Esch. coli* 10407 and are absent in one of the toxigenic strains (220/84). Hence, it appears that only the slow moving band with molecular weight greater than 21·2 Kbp which is present in all the donors (except no. 571/84) and the standard enterotoxigenic strain *Esch. coli* 10407 is likely to be the plasmid encoding for enterotoxigenicity. It is of interest that the strain no. 571/84 is the only strain positive for auto-transfer and differs from the other three strains which are non-autotransferable (but could be mobilized) in plasmid profile also. Molecular weight of enterotoxin coding plasmids in *Esch. coli* has been shown to vary between 42×10^6 to 60×10^6 daltons[10] (63 Kbp to 90 Kbp) whereas, here the plasmid coding for enterotoxigenicity in salmonellae strains have molecular weight greater than 21·2 Kbp. However, no categorical conclusions can be drawn as the plasmid preparations in this study have not been subjected to extensive purification procedures nor have attempts been made to differentiate covalently closed circular (CCC), open circular and linear plasmids. In our strain no. 220/84, a single plasmid appears to be coding for both enterotoxigenicity and drug resistance a phenomenon already shown by Scotland *et al*[11]. Abuse of antibiotics is likely to promote the spread of enterotoxigenic strains because the presence of antibiotic in the bacterial environment may lead to the selection of strains which are both enterotoxic and drug resistant[12].

Acknowledgment

Financial assistance by the Indian Council of Medical Research, New Delhi, for ICMR Unit on Salmonellosis which made this work possible, is gratefully acknowledged.

References

1. Sakajaki, R.K., Tamura, K. and Kurata, T. Enteropathogenic and enterotoxigenic activities on ligated gut loops in rabbits of salmonella and other enteric bacteria isolated from human patients with diarrhoea. *Jpn J Med Sci Biol* 27 (1974) 45.
2. Molina, N.C. and Peterson, J.W. Cholera like toxin released by *Salmonella* species in the presence of mitomycin C. *Infect Immun* 30 (1980) 224.
3. Gowal, D., Saxena, S.N., Mago, M.L. and Rao Bhau, L.N. Plasmid mediated enterotoxin production and drug resistance amongst *Escherichia coli* from cases of infantile diarrhoea. *Indian J Paeditr* 52 (1985) 56.

4. Panhotra, B.R., Mahanta, J. and Agarwal, K.C. Enteropathogenicity of *Salmonella typhimurium*. *Indian J Med Res* 73 (1981) 847.
5. Sobeh, Y.F., Prakash, K. and Sharma, K.B. Plasmid associated enterotoxin production in Salmonella. *Indian J Med Microbiol* 3 (1985) 15.
6. Anderson, E.S. and Threfall, E.J. The characterization of plasmids in enterobacteria. *J Hyg Camb* 72 (1974) 471.
7. Stokes, E.S. *Clinical bacteriology*, 4th ed., (Edward-Arnold, London) 1975.
8. De, S.N. and Chatterjee, D.N. An experimental study of mechanism of action of *V. cholera* on the intestinal mucus membrane. *J Pathol Bacteriol* 66 (1953) 559.
9. Maniatis, T., Fritsch, E.F. and Sambrock, J. *Molecular cloning-A laboratory manual.* (Cold Spring Harbour Laboratory, New York) 1982.
10. McConnell, M.M., Smith, H.R., Willshaw, G.A., Scotland, S.M. and Rowe, B. Plasmids coding for heat-labile enterotoxin production isolated from *Escherichia coli* 078: Comparison of properties. *J Bacteriol* 142 (1980) 158.
11. Scotland, S.M., Gross, R.S., Cheasty, T. and Rowe, B. The occurrence of plasmids carrying genes for both enterotoxin production and drug resistance in *E. coli* from human origin. *J Hyg* 83 (1979) 531.
12. Wachsmuth, I., Falkow, S. and Ryder, F.W. Plasmid mediated properties of a heat-stable enterotoxin producing *E. coli* associated with infantile diarrhoea. *Infect Immun* 14 (1976) 403.

Reprint requests : Dr S.N. Saxena, Director, Central Research Institute, Kasauli 173205