Research Journal of Microbiology 7 (1): 13-22, 2012
ISSN 1816-4935 / DOI: 10.3923/jm.2012.13.22

Molecular Phylogenetic Approach for Classification of *Salmonella typhi*

[1]Ramaiah Arunachalam, [2]Balakrishnan Senthilkumar, [3]Duraisamy Senbagam, [2]Ponnusamy Selvamaleeswaran and [4]Moses Rajasekarapandian

[1]Environmental Nanotechnology Division, Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University, Alwarkuruchi-627 412, Tamil Nadu, India

[2]Centre for Biotechnology, Muthayammal College of Arts and Science, Rasipuram-637 408, TN, India

[3]Department of Biotechnology, Vivekanandha College of Engineering for Women, Tiruchengode-637 205, TN, India

[4]Department of Zoology, Arignar Anna Govt. Arts College, Namakkal-637 001, TN, India

Corresponding Author: B. Senthilkumar, Centre for Biotechnology, Muthayammal College of Arts and Science, Rasipuram-637 408, TN, India

ABSTRACT

Salmonella typhi causes a severe systemic illness through intake of contaminated food or water containing bacterium. This kind of pathogen is still residing in asymptomatic carriers which may be considered as a sole source for the outbreak. Thus, the aim of the present investigation was to identify the *Salmonella typhi* strains isolated from asymptomatic typhoid carriers. The cultural characteristics on BSA, Macconkey agar and XLD were used for *S. typhi* identification. The *OmpR* gene regions of these strains were amplified and sequenced. The Maximum Parsimony algorithm tree of *OmpR* was constructed using max-mini branch and bound model. The *OmpR* gene sequences were used for finding similarities and genetic distances with other related bacterial species. The results of similarity and genetic distance analyses were further used for statistical and cluster analyses. The two strains were designated as SS3 and SS5. Both strains were identified as *S. typhi* on the basis of biochemical, phylogenetic, comparative and statistical analyses. Thereby, we conclude that gene such as *OmpR* might be more useful for identifying bacteria at even in species level. However, use of entire genomic sequences is more accurate yet it is more expensive and time consuming process.

Key words: Asymptomatic carrier, *Salmonella typhi*, *OmpR*, phylogeny, classification

INTRODUCTION

Every academicians and scientists gave the impression of creating definition for bioinformatics through their field of interests and were framed various definitions. However, in our point of view, we defined bioinformatics as a combination of biotechnology and information technology. Here, biologist applying information sciences in molecular biology to solve the biological problems, especially in molecules such as DNA, RNA and proteins. The growing quantities of biological data's have initiated the development of huge number of specialized systems in managing and mining aforementioned biological molecules (Higashi *et al*., 2009). Now-a-days availability of enormous

number of genome, gene and protein sequences of various orginism in the public databases initiated the development of numerous statistical method. These methods used to analyze the biological molecules have facilitated quick improvements in phylogenetics to classify and discover evolution of various organisms (Dixit *et al*., 2010; Blanquer and Uriz, 2007). The molecular phylogenetic reconstruction could be given uniqueness to classify the living things even in using of genes (Dixit *et al*., 2010). Hence, bioinformatics is helping in classification of bacteria's efficiently aided by computational methods (Higashi *et al*., 2009). Bacterial analysis by 16S rRNA and *OmpR* has become popular because of these divisions of genes are easy to sequence (Fukushima *et al*., 2002; Chang *et al*., 1997; Malickbasha *et al*., 2010). It is noted that the species delineation based on the 16S rRNA gene sequence analysis is still widely preferred because, when this 16S rRNA gene sequence identity >97% might be specified a specific species. Furthermore, sequencing of this gene has become much cheaper and faster (Pruesse *et al*., 2007; Slabbinck *et al*., 2010).

The both basic and advance approaches such as phenotypic and genotypic characterization were used for bacterial classification. The presences of similarities between microorganisms have derived by numerical taxonomic methods based on a range of present-day observable characteristics (phonetics), usual morphological and physiological test characters. In addition, chemotaxonomic markers such as whole-cell protein profiles, mol% G+C content and DNA-DNA homologies were also used. However, for classification, the powerful technique such as phylogenetic tree reconstruction using nucleotide sequences of conserved genes might acts as molecular chronometers. Both phonetics and phylogenetics was referred to as polyphasic taxonomy. It is suggested that the strategy in report of new species and genus along with organisms taxonomy. The numerical analysis of ribosomal RNA genes leading to the construction of branching phylogeny representing the divergence distance from a common ancestor had provided the foundation of microbial phylogenetics (Owen, 2004).

Salmonella typhi synthesizes three major Outer Membrane Proteins (OMPs) which were greatly rich growth in standard laboratory media. There were three outer membrane proteins such as *OmpC*, *OmpF* porins and *Omp*A (a structural protein). Another major protien was *Pho*E, synthesized under phosphate limitation. In *E. coli*, the expression of *OmpC* and *OmpF* was under the control of *Env*Z and *OmpR*, a two-component signal transduction system coded by the *Omp*B (*OmpR-EnvZ*) locus (Puente *et al*., 1987). There is an interest, in particularly on *OmpR* gene of *S. typhi* is used for finding genealogical relationship (Sam, 2008) with different bacterial species. Thereby, the aim of the present investigation is to classify the bacterial model organism *Salmonella typhi* strains by phylogenetic and comparative analyses.

MATERIALS AND METHODS

Screening of bacterial strains: To screen the *Salmonella typhi* from asymptomatic typhoid carriers, stool samples from fifty food handlers were collected. These samples were subsequently transported to the laboratory using the Cary-Blair transport medium in the screw capped tubes. Then, samples were transferred to the Selenite-F broth (Hi-Media, India) to enhance the growth of organisms. It was incubated at 37°C for 24 h. The colonies were inoculated into the Mac Conkey agar media and Bismuth Sulphite agar media (Hi-media, India). It was also incubated at 37°C for 24 h. Among fifty, the two isolates were identified as *Salmonella typhi* by Gram's staining, motility,

catalase, oxidase, sugar fermentation, indole, methyl red, vogues- proskauer, citrate, triple sugar iron and urease test (Old, 1996; Malickbasha *et al.*, 2010). *Salmonella typhi* SS3 and SS5 strains genomic DNA was isolated (Sambrook *et al.*, 1989) and used for *OmpR* gene amplification described in our previous report (Malickbasha *et al.*, 2010).

Purification of amplified *OmpR* gene product and gel elution: PCR products were separated on low melting agarose (Sigma) and purified using Eppendorf perfectprep gel cleanup kit (Sigma, India) for cycle sequencing process. Nucleotide sequencing was done for the *OmpR* gene of SS3 and SS5 isolates by automated ABI-3100 Genetic Analyser (GeNei, India). Nucleotide sequences were deposited in GenBank under the following accession numbers viz. EU834745 and EU849617, respectively.

Phylogenetic analysis: BLASTN (optimized for megablast) searches were manipulated with the *OmpR* gene sequences of *S. typhi* SS3 and SS5 to obtain a highly identical bacterial species. MEGA5 software (Tamura *et al.*, 2011) programme was started with a set of aligned sequences of *OmpR* gene of *S. typhi* SS3 and SS5 with representative bacterial species (highly identical species) using Clustal W (Thompson *et al.*, 1994). Subsequently search was made for reconstructing phylogenetic trees that are optimal according to Maximum Parsimony (MP) algorithm using Max-Mini Branch and Bound model (Tamura *et al.*, 2011). The phylogenetic trees were reconstructed for each gene of SS3 and SS5 independently. The reliability of evolutionary trees was evaluated by the bootstrap method with 1000 replications. Each tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic trees. The sequence alignments were performed under complete deletion option. The gap open and gap extension penalties in the sequence alignments were 15 and 6.66, respectively.

In pairwise comparison, the *OmpR* sequence of SS3 and SS5 strains along with reference bacterial species were independently used to calculate the sequence similarity and Genetic Distance (GD) using Clustal W2 (Thompson *et al.*, 1994) and MEGA 4.1 (Tamura *et al.*, 2007), respectively.

Statistical analysis: The cluster analysis was carried out for both obtained variables such as sequence similarity and genetic distance of SS3 and SS5 strains using Single Linkage algorithm. This algorithm was optimized under robust and widely applicable Euclidean distance model by PAlaeontological STatistics (PAST) 2.05 software (Hammer *et al.*, 2001).

RESULTS AND DISCUSSION

Identification of bacterial strains: Human's poor personal hygiene and inadequate food handling could potentiate the transmission of *S. typhi*. Several food products kept at room temperature were found to favour the growth of *Salmonella* species. The food handlers prominently played a role in disseminating typhoid bacilli through different food products and water (Lin *et al.*, 1988; Senthilkumar and Prabakaran, 2005). It was suggested that a periodic survey should be made on samples from food handlers and food stuffs. Furthermore, the proper sanitation methods should be followed in hotels and restaurants to avoid food contamination and spread of *Salmonella* sp (Sasikumar *et al.*, 2005). Similarly, the present study was also carried out to screen the typhoid

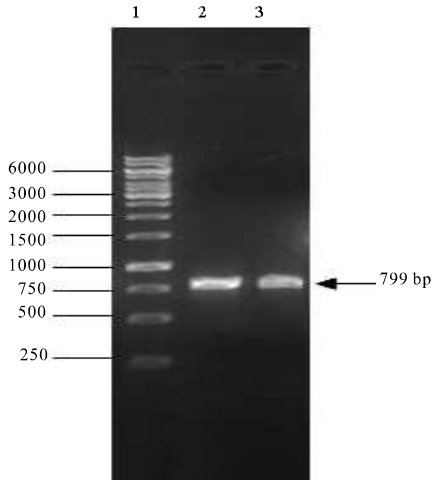

Fig. 1: The amplified product of *OmpR* gene of *S. typhi* isolates SS3 and SS5. Lane 1 indicates DNA marker whereas Lane 2 and 3 shows amplified *OmpR* gene of *S. typhi* SS3 and *S. typhi* SS5. Lane 1: DNA marker, Lane 2: Amplified *OmpR* gene of *S. typhi* SS3, Lane 3: Amplified *OmpR* gene of *S. typhi* SS5

asymptomatic carriers among the food handlers in Salem District, Tamilnadu, India. A total of two *S. typhi* strains were identified out of fifty stool samples of asymptomatic typhoid carriers. These two strains were identified as *S. typhi* by gram's staining, biochemical characters, jet black colonies on BSA and colorless colonies on MacConkay agar medium that were reported by our group (Malickbasha *et al.*, 2010). The PCR products of *OmpR* gene was analyzed by agarose gel electrophoresis for specific size and compared with standard DNA molecular marker. Both the representatives *S. typhi* isolates gave rise to 799 bp for *OmpR*. It is indicated the presence of the wild type gene which encoding for the virulence of the isolates (Fig. 1). The results obtained in this study are similar to those of Pickard *et al.* (1994).

Molecular phylogenetic analysis: A 538 (SS3) and 518 (SS5) bp sequences were amplified from the genomic DNA with MGR 06 (F) 5'AGG GGCGTT TTC ATC TCG-3' and MGR 07 (R) 5'-ACC AGG CTG ACG AACAG-3' primers (Pickard *et al.*, 1994). The *OmpR* genes showed high similarity with *OmpR* genes deposited in the GenBank (Table 1, 2). In this study, *OmpR* gene of different species (different strains of a species) were obtained by BLASTN search. However, 10 species were selected on the basis of high % of sequence identity with good E value for phylogenetic analysis (Table 1, 2). The cumulative results from a limited number of studies to date suggest that 16S rRNA gene sequencing provides genus identification in most cases (>90%) but less so with regard to species (65-83%). With regard to species, 1-14% of the isolates were remaining unidentified even after testing. Fukushima *et al.* (2002) reported that *gyrB* gene based phylogenetic trees could be able to classify some bacteria that could not be classified by their 16S rRNA sequences. Michael and Sharon (2007) reported that, the minimum 500-525 bp essential for phylogenetic analysis and also for species identification, minimum >99% similarity and ideal >99.5% similarity should be desirable.

Table 1: Result of identical sequence searches in GenBank by BLASTN Algorithm (optimized for megablast) using *OmpR* gene of SS3 isolated in the present investigation

GenBank accession No. SS3	Highest identical species	Matched sequence accession No.	Sequence identity (%)	E-value
EU834745	*Salmonella typhi*	EU849617	94	0.0
	Klebsiella pneumoniae	CP000647	92	0.0
	Escherichia coli	CP001509	92	0.0
	Shigella flexneri	CP000266	91	0.0
	Shigella boydii	CP000036	91	0.0
	Shigella sonnei	CP000038	91	0.0
	Shigella dysenteriae	CP000034	91	0.0
	Cronobacter turicensis	FN543093	87	3e-170
	Erwinia tasmaniensis	CU468135	86	7e-162
	Erwinia carotovora	BX950851	85	4e-154

Table 2: Result of identical sequence searches in GenBank by BLASTN Algorithm (optimized for megablast) using OmpR gene of SS5 isolated in the present investigation

GenBank accession No. SS5	Highest identical species	Matched sequence accession No.	Sequence identity (%)	E-value
EU849617	*Escherichia coli*	CP001509	97	0.0
	Shigella flexneri	CP000266	97	0.0
	Shigella boydii	CP000036	97	0.0
	Shigella sonnei	CP000038	97	0.0
	Shigella dysenteriae	CP000034	96	0.0
	Escherichia ferqusonii	CU928158	95	0.0
	Salmonella typhi	EU834745	94	0.0
	Klebsiella pneumoniae	CP000964	87	8e-171
	Salmonella enterica paratyphi A	CP000026	86	2e-157
	Erwinia tasmaniensis	CU468135	84	2e-142

Less than 0.5% similarity and other properties such as phenotype should be considered as final species identification and also, *gyrB* sequence method might be more useful for identifying bacteria to the species level (Fukushima *et al*., 2002). E-value should be below 0.05 would be considered significant; at least they might be worth considering (Lesk, 2005).

According to Michael and Sharon (2007) and Lesk (2005) reports, the both strain SS3 and SS5 had 538 and 518 bp which were more than 500 bp that should be qualified for phylogenetic analysis. In BlastN analysis, isolate SS3 (538 bp) had significant (94%) >90% identity and E value (0.0) <0.05 with *S. typhi* (EU849617). Other species such as *K. pneumoniae* (CP000647) and *E. coli* (CP001509) had 92% identity with 0.0 E value followed by *S. flexneri, S. boydii, S. sonnei* and *S. dysenteriae* had 91% similarities with 0.0 E values (Table 1). Isolate SS5 (518 bp) had significant (97%) >90% similarity and E value (0.0) with *E. coli* (CP001509), *S. flexneri* (CP000266), *S. boydii* (CP000036) and *S. sonnei* (CP000038) whereas *S. dysenteriae* (CP000034), *E. ferqusonii* (CU928158) and *S. typhi* (EU834745) shows 96-94% identity with 0.0 E value (Table 2). It should be noted that SS3 has identical with *S. typhi* (EU849617) than other reference species whereas SS5 highly identical with *E. coli* (CP001509), *S. flexneri* (CP000266), *S. boydii* (CP000036) and *S. sonnei* (CP000038) when compare to *S. typhi* (EU834745) and other reference species. These results indicate that strain SS3 belongs to *S. typhi* (Table 1) whereas SS5 might be belongs to

S. typhi and other six species mentioned in Table 2. The BlastN result of SS5 illustrates that little perplexes to exactly confirm the species from the list of reference bacterial species. It is suggest, there is a need of improved analyses should be performed for bacterial confirmation and classification. For that, the phylogenetic and comparative analyses were carried out to identify the both strains accurately, even in species level.

As the number of sequences available for analysis continues to grow, the structure of phylogenetic trees derived from *OmpR, gyrB* and 16S rRNA sequences becomes both more elaborate and accurate. Chang *et al.* (1997) and Fukushima *et al.* (2002) reported that 16S rRNA and *gyrB* of various *Salmonella, Shigella and Escherichia coli* species were partially sequenced and used for defining all members of the genus, groups of species or individual species. The Fig. 2a had two branches. Among these two branches, one (bottom) divided into two clades contains SS3 along with *S. typhi* (EU849617) and *Shiqella dysenteriae* (CP000034) whereas other reference species were distinct from SS3. These findings were supported by 100 bootstrap values. As shown in Fig. 2b it was clearly observed that the SS5 tree also had two branches and one (bottom) of these was divided into two clades which were supported by 100 bootstrap values. This clade had isolate SS5 along with *Salmonella typhi* (EU834745), *Shigella dysenteriae* (CP000034) and *Klebsiella pneumoniae* (CP000964). Both of these two trees suggest that isolate SS3 and SS5 are belongs to *Salmonella typhi* (Fig. 2). Cilia *et al.* (1996) have reported that 16S rRNA sequences cannot be used to derive phylogenetic tree analyses among closely related bacteria, for example, *Shigella* and *E. coli*, owing to the similarity in these gene regions. However, the present results shown the

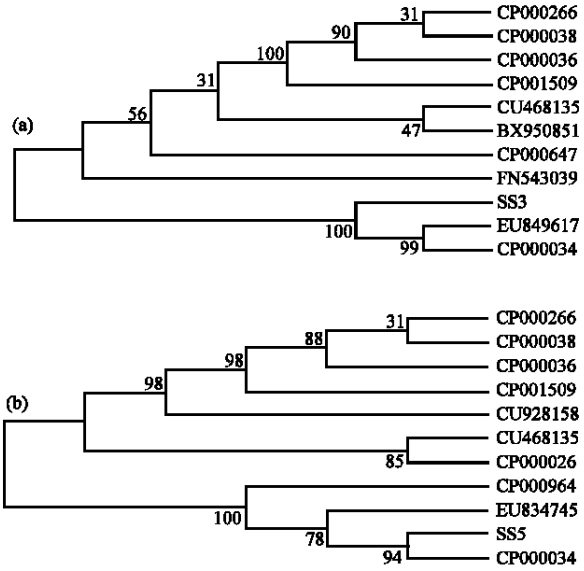

Fig. 2(a-b): Phylogenetic tree of SS3 and SS5 based on the nucleotide sequence of *OmpR* gene with reference sequences obtained from GenBank. The MP trees were constructed by MEGA5 using Max-Mini Branch and Bound model. The reliability of the tree was evaluated by the bootstrap method with 1000 replications. All position containing gaps and missing data were eliminated from the dataset (complete deletion option). (a) SS3 and (b) SS5

possible classification of such closely related bacteria on the basis of *OmpR* gene. It might suggest that the *OmpR* gene is an alternative of 16S rRNA gene to determine the evolutionary relationships of bacteria.

Comparative analysis: The sequences used in this study were considered for further comparative and statistical analyses to confirm the species. The isolates SS3 and SS5 were used as a query to estimate the pairwise GD and similarity with reference bacterial sequences. It shows that the mean of pairwise genetic distances for SS3-0.050-0.570 is obviously identical with SS5-0.034-0.571. The mean of pairwise genetic similarity (%) of SS3 shows 43-95% whereas SS5 had 43-96%, it suggest that interior of the SS3 and SS5 is obviously identical (Table 3). These findings were point out that query sequence of SS3 had very low GD and high similarity with *S. typhi* (EU849617) (0.050; 95%) and *Shiqella dysenteriae* (CP000034) (0.076; 91%) and had moderate GD and less similarity with rest of the species. The strain SS5 had very low GD and high similarity with *S. typhi* (EU834745) (0.050; 95%), *Shigella dysenteriae* (CP000034) (0.034; 96%) and *Klebsiella pneumoniae* (CP000964) (0.122; 87%) and had moderate GD and less similarity with rest of the species (Table 3). These results indicate that the isolate SS3 and SS5 are belongs to *S. typhi* and had an evolutionary relationship with *Shigella dysenteriae* and *Klebsiella pneumoniae*.

Cluster analysis: The similarity and GD values of isolates SS3 and SS5 with reference bacterial species were used for cluster analysis. As shown in Fig. 3a and b, the clusters were joined based on the average distance between all members in the two groups (Hammer *et al*., 2001). It shows that the two rooted trees had two branches which might be typically a site for gene duplication. Bacterial strains from both branches have homologous genes in their genomes which might be reflecting the phylogenetic and statistical relationship of strains as well as generation of genes by duplication(s) and/or mutations. Moreover, isolate SS3 (S. No. 1) was cluster with *S. typhi* (EU849617) and *Shigella dysenteriae* (CP000034) which was represented by serial number 2 and 11. The isolate SS5 (S. No. 1) was cluster with *S. typhi* (EU834745), *Shigella dysenteriae* (CP000034) and *Klebsiella pneumoniae* (CP000964) were represented by serial number 2, 7 and 9 (Fig. 3, Table 3).

Table 3: The pairwise sequence similarity and genetic distance was calculated using Clustal W2 and MEGA4.1 for SS3 and SS5 strain with representative bacterial species

S. No.	Accession No. of representative species with SS3	Similarity (%)	Genetic divergence	Accession No. of representative species with SS5	Similarity (%)	Genetic divergence
1	SS3	100	0.000	SS5	100	0.000
2	EU849617	95	0.050	EU834745	95	0.050
3	CP001509	45	0.538	CP001509	44	0.543
4	CP000266	45	0.538	CP000266	45	0.537
5	CP000036	45	0.538	CP000036	45	0.537
6	CP000038	45	0.538	CP000038	45	0.537
7	CP000647	51	0.554	CP000034	96	0.034
8	FN543093	46	0.528	CU928158	46	0.543
9	CU468135	43	0.548	CP000964	87	0.122
10	BX950851	43	0.570	CU468135	43	0.559
11	CP000034	91	0.076	CP000026	47	0.571

The serial number represents the corresponding species and/or accession number of representative species with SS3 and SS5 isolated in the present study

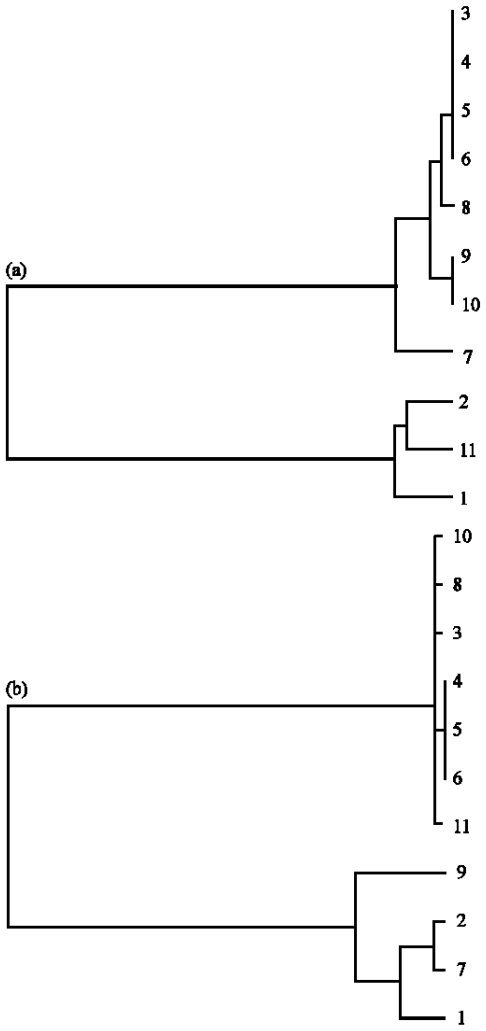

Fig. 3(a-b): The cluster analysis of SS3 and SS5 sequence similarity and genetic distance variables by PAST 2.05 using Single Linkage algorithm under Euclidean distance model. The number 1-11 representing the corresponded species and/or accession number mentioned in Table 3

CONCLUSIONS

Both phylogenetic (Fig. 2) and cluster analyses (Fig. 3) confirm that the strains present in the lower branch might be evolutionarily related to the isolates used in the present study. There is a growing interest in the use of *OmpR* gene instead of 16S rRNA, gyrA and RNAase sequences. We believe that the *OmpR* region could have high reliability for identifying pathogenic bacteria and it is considerately new in phylogenetic analysis. The use of genome sequences will provide a rich source of data for future taxonomic analysis. However, there is a need of extensive study on *OmpR* gene sequences would give more detail for bacterial classification.

ACKNOWLEDGMENTS

Authors thank Muthayammal Educational and Charitable Trust, India for providing wet lab facilities to carryout preliminary work and RA thank Prof. G. Annadurai for providing facility for insilico analysis.

REFERENCES

Blanquer, A. and M.J. Uriz, 2007. Cryptic speciation in marine sponges evidenced by mitochondrial and nuclear genes: A phylogenetic approach. Mol. Phylogenet. Evol., 45: 392-397.

Chang, H.R., L.H. Loo, K. Jeyaseelan, L. Earnest and E. Stackebrandt, 1997. Phylogenetic relationships of salmonella typhi and salmonella typhimurium bases on 16S rRNA sequence analysis. Int. J. Syst. Bacteriol., 47: 1253-1254.

Cilia, V., B. Lafay and R. Christen, 1996. Sequence heterogeneities among 16S ribosomal RNA sequences and their effect on phylogenetic analyses at the species level. Mol. Biol. Evol., 13: 451-461.

Dixit, J., H. Srivastava, M. Sharma, M.K. Das and O.P. Singh *et al.*, 2010. Phylogenetic inference of Indian malaria vectors from multilocus DNA sequences. Infect. Genet. Evol., 10: 755-763.

Fukushima, M., K. Kakinuma and R. Kawaguchi, 2002. Phylogenetic analysis of *Salmonella, Shigella* and *Escherichia coli* strains on the basis of the gyrB gene sequence. J. Clin. Microbiol., 40: 2779-2785.

Hammer, O., D.A.T. Harper and P.D. Ryan, 2001. PAST: Paleontological statistics software package for education and data analysis. Paleontol. Electron, 4: 1-9.

Higashi, S., M. Hungria and M.A.D.C. Brunetto, 2009. Bacteria classification based on 16S ribosomal gene using artificial neural networks. Proceedings of the 8th WSEAS International Conference on Computational Intelligence, Man-Machine Systems and Cybernetics, December 14-16, 2009, Wisconsin, USA, pp: 86-91.

Lesk, A.M., 2005. Introduction to Bioinformatics. 2nd Edn., Oxford University Press, New Delhi, ISBN: 9780199277872, Pages: 360.

Lin, F.Y., J.M. Becke, C. Groves, B.P. Lim and E. Israel *et al.*, 1988. Restaurant-associated outbreak of typhoid fever in Maryland: Identification of carrier facilitated by measurement of serum Vi antibodies. J. Clin. Microbiol., 26: 1194-1197.

Malickbasha, M., R. Arunachalam, B. Senthilkumar, M. Rajasekarapandian and G. Annadurai, 2010. Effect of ompR gene mutation in expression of ompC and ompF of *Salmonella typhi*. Interdisciplinary Sci.: Comput. Life Sci., 2: 157-162.

Michael, J.J. and L.A. Sharon, 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Phrases, perils and pitfalls. J. Clini. Microbiol., 45: 2761-2764.

Old, D.C., 1996. *Salmonella*. In: Mackie and McCartney Practical Medical Microbiology, Mackie, T.J., J.G. Collee and J.E. McCartney (Eds.). 14th Edn., Churchill Livingstone, London.

Owen, R.J., 2004. Bacterial taxonomics: Finding the wood through the phylogenetic trees. Methods Mol. Biol., 266: 353-383.

Pickard, D., J. Li, M. Roberts, D. Maskell and D. Hone *et al.*, 1994. Characterization of defined ompR mutants of *Salmonella typhi*: OmpR is involved in the regulation of Vi polysaccharide expression. Infect. Immun., 62: 3984-3993.

Pruesse, E., C. Quast, K. Knittel, B.M. Fuchs and W. Ludwig *et al.*, 2007. SILVA: A comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res., 35: 7188-7196.

Puente, J.L., V. Flores, M. Fernandez, Y. Fuchs and E. Calva, 1987. Isolation of an ompC-like outer membrane protein gene from *Salmonella typhi*. Gene, 61: 75-83.

Sam, D., 2008. Comparing phylogenetic trees. Connexions module: m15807, Version 1.1., http://cnx.org/content/m15807/1.1/content_info.

Sambrook J., E.F. Fritsch and T. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. 2nd Edn., Cold Spring Harbor Laboratory Press, New York, USA..

Sasikumar, C., V. Kannan and B. Senthilkumar, 2005. Asymptomatic typhoid carriers in Namakkal District, Tamilnadu. J. Environ. Biol., 26: 113-115.

Senthilkumar, B. and G. Prabakaran, 2005. Multiple drug resistant *Salmonella typhi* in asymptomatic typhoid carriers among food handlers in Namakkal district Tamil Nadu. Indian J. Med. Microbiol., 23: 92-94.

Slabbinck, B., W. Waegeman, P. Dawyndt, P. De Vos and B. De Baets, 2010. From learning taxonomies to phylogenetic learning: Integration of 16S rRNA gene data into FAME-based bacterial classification. BMC Bioinform., Vol. 11, 10.1186/1471-2105-11-69

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol. Biol. Evol., 28: 2731-2739.

Tamura, K., J. Dudley, M. Nei and S. Kumar, 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol., 24: 1596-1599.

Thompson, J.D., D.G. Higgins and T.J. Gibson, 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res., 22: 4673-4680.