Genome DNA Sequencing, Phylogenetic Dendrogram and Outer Membrane Protein Separation Studies of Local Najaf S. Typhi Isolates/ Iraq

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Abstract
From a clinically proven human typhoid cases, five S. typhi local isolates were VITIC identified and designated as: S1, S2, S3, S4 and S5. PCR for the S. typhi specific 16s rRNA genes were performed on the genomic DNA of the five isolates. Gene sequences were found as 1500 bps for the isolates S1-S4 and 1470 bps for S5. The genomic DNA similarities to standard NCBI S. typhi isolates were showing 97%, 94%, 82%, 98% and 99% corresponding to S1, S2, S3, S4 and S5 respectively. The phylogenetic dendrogram showed S5 is more identical to S3 by genetic distance 5.284 and combined into one root. The outer membrane protein was separated, purified and identified as 55 kDa and 62 kDa on SDS – PAGE. Such molecular weights were comparable to S. typhi OMP. The pure made OMP preparation is being suggestive for the; sero-diagnosis , vaccine candidate and herd immunity studies of human typhoid.
Keywords: DNA Sequencing, Membrane Protein separation, S. typhi

1. Introduction

The outer membrane protein OMP of gram-negative bacteria including *S. typhi* is a highly asymmetric lipid bilayer. The outer layer is occupied by lipo-polysaccharide LPS forming a unique constitute OMP, whereas the inner layer is covered by phospholipids. The chemical composition of OMP consist of 50% protein either in form of integral membrane protein or as lipoprotein that are anchored to the membrane through N terminally attached lipid as well as pore forming protein, porins. The general functions of *S. typhi* OMP are;dynamic interface between the bacterium and its environment, forming cell structure, adhesion to other cells and regulation of nutrient and drug transport [1].While the immune functions are; antigenic immunogenic[2],vaccine candidate [3] and virulence associated and diagnostic antigen[4]. Porins are antigenic immunogens and immune protective against live *S. typhi* challenge [4] [5] [6] [7]. The objective of present work was to trace 16s rRNA genes of *S. typhi*, genetic dendrogram and separate OMP from *S. typhi* isolate S5.

2. Materials & Methods

A gram negative rod-like bacteria was isolated from clinical typhoid cases. The isolates were identified by VITIC system as S. typhi and subjected to molecular identification via DNA isolation, purification and identification by gel electrophoresis then by 16s rRNA genes PCR studies. The isolate S5 was grown in BHIB, centrifugated, washed, bursted by glass-beads, centrifugated and protein was separated by saturated ammonium sulfate. Crude protein preparation was purified by ultrafiltration & SDS-PAGE [8] [9] [10].

3. Results

Molecular Identification

The obtained clinical isolates were tested by VITIC system showed : 95%, 99%, 99%, 99% and 95% respectively to S1, S2, S3, S4 and S5.The genomic DNA of five obtained local isolates were PCR tested for 16s rRNA genes which were used along with a primer in each isolate. The presence of specific genes confirmed by gel electrophoresis. Figure (1) showed that S1 to S4 were 1500 bps and S5 was at 1470 bps.

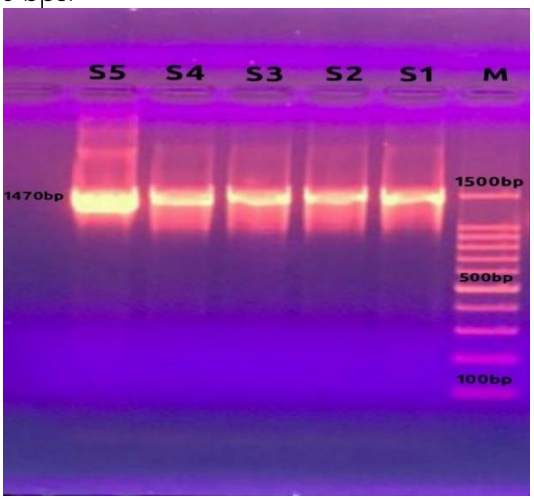

Figure (1): Gel electrophoresis of 16s rRNA genes of *Salmonella typhi* isolates, where M: Marker, S1: First *Salmonella typhi* isolate, S2: Second *Salmonella typhi* isolate, S3: Third *Salmonella typhi* isolate, S4: Fourth *Salmonella typhi* isolate

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and S5: Fifth *Salmonella typhi* isolate.

Phylogenetic Dendrogram

The complete genomic DNA sequencing were performed in South Korea. The sequencing results were analyzed through insert into NCBI and through that analysis, it was found that the five genomic DNA samples were matching with Salmonella enterica subsp. enterica serovar typhi .

Table (1): The genomic sequence similarities of Salmonella typhi.		
Bacterial isolate	Identity	Identity percent
S1	Salmonella enterica subsp. enterica serovar Typhi	97%
S2	Salmonella enterica subsp. enterica serovar Typhi	94%
S3	Salmonella enterica subsp. enterica serovar Typhi	82%
S4	Salmonella enterica subsp. enterica serovar Typhi	98%
S5	Salmonella enterica subsp. enterica serovar Typhi	99%

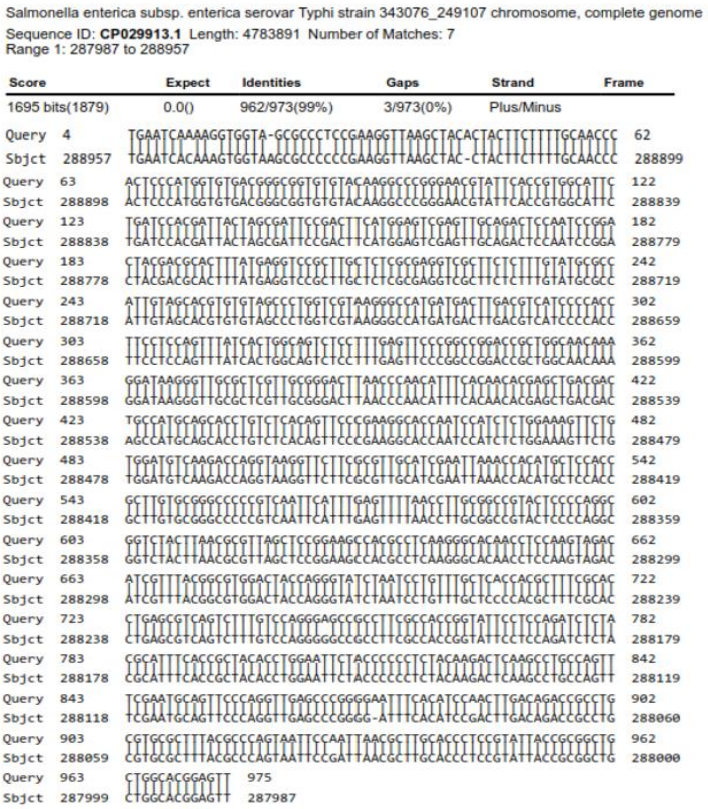

Figure (2): Sequencing result of fifth Salmonella typhi isolate (S5). Pair-wised alignment of partial nucleotide sequence of 16S ribosomal rRNA gene (Query) to that of Salmonella typhi strain strain 343076_249107 chromosome16S ribosomal RNA gene whose sequence producing highest score of homology during BLASTn esarch.

	1	2	3	4	5
1. Salmonella typhi 1					
2. Salmonella typhi 2	11.429				
3. Salmonella typhi 3	10.935	8.458			
4. Salmonella typhi 4	11.408	9.780	9.518		
5. Salmonella typhi 5	11.181	8.467	5.248	13.528	

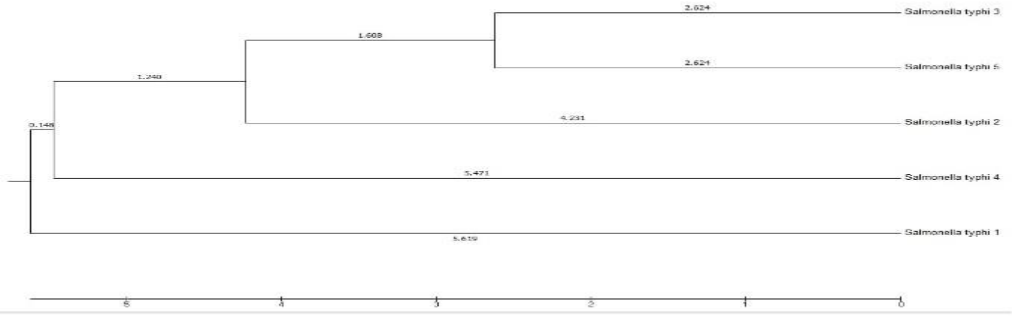

Figure (3): Phylogenetic dendrogram of local Salmonella typhi isolates.

Phylogenetic tree is illustrated in figure (3), it shows that third Salmonella typhi isolate (S3) is more identical with fifth Salmonella typhi isolate (S5) by 5.248 genetic distance and combined into one root, and these two isolates are more identical and combined with second Salmonella typhi isolate (S2), and these three isolates are more identical and combined with fourth Salmonella typhi isolate (S4), and all these four isolates are combined with first Salmonella typhi isolate (S1). It is noted that fourth Salmonella typhi isolate (S4) and fifth Salmonella typhi isolate (S5) are the most different isolates by 13.528 genetic distance.

Outer Membrane Protein (OMP)

The isolate S5 was elected for separation of OMP. The separated, purified and identified proteins from BHIB bulk culture patch were analyzed by protein identification through SDS – PAGE method. Two protein bands at 55kDa and 62kDa as compared to ladder protein which have molecular weight of 245kDa confirming the presence of OMP. Figure (4).

Figure (4): SDS-PAGE for outer membrane protein of Salmonella typhi

4. Discussion

The identification results of VITIC system to the analyzed isolates S1, S2, S3, S4 and S5 are in line with that Kadhum (2019) [11]. The genomic DNA PCR for 16s rRNA genes and DNA electrophoresis results were confirming the presence of 1500bps for S1 – S4 and 1470 bps for S5 [12]. The phylogenetic dendrogram studies of the five isolates were showing three similarity clades of the isolates 2 to 5. Isolate 4 and 5 seemed quite different than the other isolates by genetic distance of 13.528. These results are in line with [13].

The molecular weight studies of S. typhi local isolate OMP were inline of other workers, Table 2.

Table (2): Molecular weight of *S. typhi* OMP.

Molecular weight in KDa	Reference
Crude 25, 35, 55KDa Pure 36KDa	[9]
49 KDa	[14]
50KDa	[15]
17 to 80 kDa	[16]
17 to 70 kDa	[17]
55, 62KDa	This study

The OMP of S. typhi valid for immunodiagnosis of typhoid [4], vaccine candidate [3] [8] [9].

5. Conclusion

16s rRNA genes of Salmonella typhi were found at 1500 and 1470 bps sequences. Phylogenetic dendrogram studies have shown three similarity clades. S4 and S5 were of marked difference with genetic distance of 13.528. Other isolates were similar. OMP was of 55KDa and of 62KDa. OMP valid as immunodiagnostic and vaccine candidate.

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