Horizontal Transfer of Domains in *ssrA* gene Among Enterobacteriaceae

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Abstract

Background

The tmRNA (transfer messenger RNA), encoded by *ssrA* gene, is involved in rescuing of stalled ribosomes by a process called *trans*-translation. Additionally, regions of the *ssrA* gene act as recognition sites for various integrases. Variations in *ssrA* genes were widely reported among the members of Enterobacteriaceae, but the functional relevance in the course of evolution are not well understood. In this study, we investigated the horizontal gene transfer of tmRNA among the members of Enterobacteriaceae.

Methods and Results

Horizontal gene transfer in tmRNA was found by predicting recombination signals in the tmRNA belong to Enterobacteriaceae using recombination detection program (RDP5). Our results revealed 7 recombination signals in tmRNA among different species. We further showed that the recombination signals was more in the domains present in the 3' end than the domains in the 5' end of tmRNA. Of note, the mRNA region, which codes for the peptide tag was reported in many recombination signals. Further, members belonging to genera *Yersinia*, *Erwinia*, *Dickeya*, and *Enterobacter* were highly represented in the recombination signatures.

Conclusions

Taken together, our results revealed a high level of recombination among specific regions of tmRNA of Enterobacteriaceae and suggest the possible role of recombination in the diversification of SsrA function in proteolysis and other pathways.

1 Introduction

tmRNA (transfer messenger RNA) is a small stable RNA coded by the gene *ssrA*. It serves both as tRNA and mRNA and rescues ribosomes that are stalled during translation. tmRNA is also recognized for cellular homeostasis by regulating the levels of various proteins through ribosome rescuing. *ssrA* gene has been implicated in functions related to viability, pathogenesis, development and stress response [1]. *ssrA* gene is ubiquitously distributed among all eubacterial species, and in eucaryotic organelles like mitochondria, chloroplast and in few bacteriophages as well [2]. Surprisingly *ssrA* gene serves as a major site for the integration of mobile genetic elements. In members of Enterobacteriaceae, *ssrA* gene was over represented on genome-wide analysis of integration sites for mobile genetic elements [3]. Thus, identification of horizontal gene transfer (HGT) in *ssrA* could help us to identify the possible mode of evolution of tmRNA among the members of Enterobacteriaceae. Integration of mobile genetic elements was reported to excise *ssrA* gene during the process of HGT [4]. One way to decipher HGT in tmRNA is to identify recombination signals among different species. Thus, prediction of recombination signals in mature tmRNA may provide evidence about HGT events it came across. The present investigation aims to predict the recombination signals among selected 53 members of Enterobacteriaceae through RDP5 (Recombination detection program 5). The results revealed that recombination signals were identified in pseudoknot 1 (PK1) mRNA domain, pseudoknot 2 (PK2), pseudoknot 3(PK3), pseudoknot 4 (PK4) and T-arm, except D-loop, which is present in the 5'end of tmRNA. Recombinant tmRNA was present in the species belong to the genera *Erwinia*, *Yersinia*, *Enterobacter*, *Dickeya*, *Blochmannia* and *Pantoea*. The recombination signals revealed a donor-recipient relationship among the species of Enterobacteriaceae. These results highlighted the role of HGT in evolution of tmRNA belonging to Enterobacteriaceae.

2 Methodology

2.1 Sequence Alignment

Mature tmRNA sequence from Enterobacteriaceae was retrieved from the tmRNA website [2]. The repeated sequence was removed manually. Sequence alignment was done by Clustal Omega [5]. The Fasta formatted alignment file was downloaded and used as an input file for the recombination analysis.

2.2 Identification of Recombination Signals

RDP5 program was downloaded from <http://web.cbio.uct.ac.za/~darren/rdp.html> and installed in windows based operative system [6]. Putative recombination signals in a given alignment file were analyzed by 9 programs: RDP, GENECONV, BOOTSCAN, MAXCHI, CHIMAERA, SISCAN, 3SEQ, PHYLPPO and LARD as implemented in RDP5 package using default settings with Bonferroni corrected p-value cut-off of 0.05 and 0.01. The resulting file was downloaded and the absolute recombination nucleotide coordinate positions in the alignment were assessed manually for analysis.

2.3 Phylogenetic analysis

The aligned sequences were used for Phylogenetic analysis. The Phylogenetic trees were constructed using the maximum-likelihood method using MEGA X with default settings [7]. The numbers at each node represent percentage bootstrap values with 1000 replicates.

2.4 Neighbor Net and detection of recombination

SplitsTree4 was used for Neighbor Net analysis [8]. A p-value of less than 0.01 was set to determine the significance of the recombinant segments; this was done via a bootstrap test with 100 replicates.

3 Results

In this investigation, we have predicted putative recombination signals in mature tmRNA of species belong to Enterobacteriaceae. A total of 53 species from 16 genera were taken for analysis. Supplementary Table 1 summarizes the name of species taken for analysis and information about the total length of tmRNA and sequence of peptide tag. The length of tmRNA among the species did not show significant variation except a few. The size of the smallest tmRNA is 312 nts, which belongs to *Enterobacter aerogenes* whereas the length of the largest tmRNA is 408 nts and it belongs to *Erwinia pyrifoliae* (Supplementary Table 1). Variations in the length of mature tmRNA largely arise due to the sequence difference in the mRNA region, pseudoknot 2 (PK2) (Fig. 1b), however differences in pseudoknot 3 (PK3) were observed in *Blochmania* spp. The nucleotide coordinates of the HGT events were summarized in Table 1 and Fig. 1. Putative recombination signals were analyzed in the following ways. Primarily the recombination signals were analyzed in the context of tmRNA domains (i.e. the regions which are involved in HGT and the regions which are not). Secondly, recombination signals were analyzed as individual events with their donor and recipient status. Thirdly, phylogenetic analysis to access the closeness of minor and major parents towards the recombinant in a segmented alignment files which are specific to the seven recombination events.

Table 1
Recombination events predicted by RDP5 and their details.

Recombination event	Nucleotide co-ordinates	Potential Recombinant	Minor parent	Major parent	Programs predicted and their E-values	Domains of tmRNA in the co-ordinates	Species having sequence evidence of the same Recombination signature
Event 1	101–153	<i>D. dadantii</i>	<i>B. pennsylvanicus</i>	<i>D. zeae</i>	GENECONV (1.94 X 10 ⁻⁹), Maxchi (4.88 X 10 ⁻⁶), SiSscan (8.37 X 10 ⁻²⁷), 3Seq (1.65 X 10 ⁻⁴)	Partial region of ORF and partial region of PK2	<i>E. amylovora</i> , <i>E. tasmaniensis</i> , <i>D. chrysanthemi</i> , <i>Y. intermedia</i> , <i>Y. bercovieri</i> , <i>Y. mollaretti</i> , <i>Y. rohdej</i> , <i>Y. enterocolitica</i>
Event 2	81–131	<i>P. vagans</i>	<i>S. boydii</i>	-	SiSscan (2.07 X 10 ⁻⁹), 3Seq (4.12 X 10 ⁻³)	ORF	<i>P. stewartii</i> , <i>P. atrosepticum</i> , <i>P. wasabiae</i>
Event 3	60–84	<i>B. floridanus</i>	<i>D. zeae</i>	-	GENECONV (1.59 X 10 ⁻⁵)	Partial region of PK1	-
Event4	252–345	<i>E. amylovora</i>	<i>Y. intermedia</i>	<i>P. stewartii</i>	Maxchi (4.66 X 10 ⁻³), Chimaera (6.08 X 10 ⁻⁴), SiSscan (1.54 X 10 ⁻⁵), 3Seq (4.20 X 10 ⁻⁵)	Partial region of PK4 and T-arm	<i>E. tasmaniensis</i>

Recombination event	Nucleotide co-ordinates	Potential Recombinant	Minor parent	Major parent	Programs predicted and their E-values	Domains of tmRNA in the co-ordinates	Species having sequence evidence of the same Recombination signature
Event 5	89–179	<i>P. ananatis</i>	<i>Y. frederiksenii</i>	-	Maxchi (1.41 X 10 ⁻³), Chimaera (4.89 X 10 ⁻²), SiSscan (5.97 X 10 ⁻⁵), 3Seq (7.89 X 10 ⁻⁴)	ORF and Partial region of PK2	<i>E. billingiae</i> , <i>P. stewartii</i>
Event 6	246–359	<i>Y. intermedia</i>	<i>P. vagans</i>	<i>P. rettgeri</i>	Maxchi (4.55 X 10 ⁻²), SiSscan (9.67 X 10 ⁻⁴), 3Seq (3.04 X 10 ⁻²)	PK4 and T-arm	<i>Y. bercovieri</i> , <i>Y. mollaretti</i> , <i>Y. enterocolitica</i>
Event 7	167–211	<i>E. amylovora</i>	<i>Y. frederiksenii</i>	-	SiSscan (5.18 X 10 ⁻²), 3Seq (9.32 X 10 ⁻³)	Partial region of PK2 and partial region of PK3	<i>E. tasmaniensis</i>

3.1 Domain-specific gene traffic in mature tmRNA

Out of 7 recombination events predicted, putative recombination signals were identified in domains of tmRNA like PK1, mRNA domain, PK2, PK3, PK4, and T arm except D-arm (refer Fig. 1c). The gene traffic tends to cluster near mRNA domain (Events 1, 2, and 5). Other domains showed one (PK1 in Event3 and PK3 in Event 7) or two (PK2 in Event 5 and 7; PK4 and T-arm in Event 4 and 6) events where as the D arm is free from the genetic traffic. This shows domain-specific nature of tmRNA genetic traffic.

3.2 Phylogenetic and Neighbor net analysis

For each event, the sequence alignment was split into two in such a way that one has the recombined region (derived from the minor parent) and the other has the rest of the sequence (sequence in recombinant donated by the major parent). All the files were subjected to the phylogenetic tree construction. The recombinant, major and minor parent sequences were highlighted with different color (Supplementary Fig. S1-S7). Phylogenetic trees constructed for the events 1, 6 and 7 showed the predicted major parent and minor parent sequences shared the nearer clad with the recombinant sequence. This also adds additional weightage for our claim. The middle portion of the *ssrA* is more prone to recombination than the flanks.

The neighbor net analysis was performed to analyze the genetic relationship among species. Reticulation reflects the evidence of recombination. The PhiTest gave significance signals for recombination with a p-value of 0.006016. The recombinants are highlighted in red (Fig. 3). Except for *B. floridanus*, all the recombinants are found in the main group. Species belong to *Yersinia* form two different groups which are incomparable with recombination event 1 and 6. Specieslike *B. floridanus*, *B. pennsylvanicus*, *P. rettgerii*, *P. vulgaris*, *P. mirabilis* clustered together as individual groups and well separated from the rest of the strains. These suggest a divergent evolution in the tmRNA from the rest of the population.

3.3 Recombination events predicted a donor-recipient relationship

tmRNA of *D. dadantii* (refer Fig. 2a), *P. vagans* (refer Fig. 2b), *B. floridanus* (Fig. 2c), *E. amylovora* (refer Fig. 2d), *P. ananatis* (refer Fig. 2e) and *Y. intermedia* (refer Fig. 2f) were found to be recombinants (i.e. act as recipients) (refer Table 1). Species like *B. pennsylvanicus*, *S. boydii*, *D. zea*, *Y. intermedia*, *Y. frederiksenii* acts as donors in various recombination events. The details of each of the recombinant signals were discussed below in detail. Interestingly *Y. frederiksenii* was found to donate different regions corresponding to (89 – 179 and 167 - 211) to *P. ananatis* and *E. amylovora*. Two recombinant signals were found in different domains of *E. amylovora* tmRNA (corresponds to Event 4 and Event 7) in which *Y. intermedia* and *Y. frederiksenii* act as donors, respectively. Species like *P. vagans* and *Y. intermedia* acts both as donor and recipient in different recombination events (refer Table 1). Sequences of recombinant tmRNA belong to *Y. intermedia* and *E. amylovora* also showed evidence for recombination event 1.

Event 1

In Recombination event 1, the recombination breakpoints were between 101 - 154 nts. Putative recombinant tmRNA was found in *D. dadantii*, (refer Fig. 2a) which is a plant pathogen formerly called *Erwinia Chrysanthemi*. It infects tubers, bulbs, and ornamental crops [9].

B. pennsylvanicus acts as a donor in this event which lives as a symbiont in insects [10]. Surprisingly evidence of this recombination event was also found in *E. amylovora*, *E. tasmaniensis*, *D. chrysanthemi*, *Y. intermedia*, *Y. bercovieri*, *Y. rhodei* and *Y. enterocolitica*.

Event 2

In Recombination event 2, the recombination breakpoints were between 81 - 131 nts. Putative recombinant tmRNA was found in *P. vagans* (refer Fig. 2b) which is an epiphyte [11]. Human pathogen *S. boydii* [12] acts as a donor in this event. Evidence for similar recombination event was found in *P. stewartii*, *P. atrosepticum* and *P. Wasabiae*.

Event 3

In Recombination event 3, the recombination breakpoints were between 60 – 84 nts. Putative recombinant tmRNA was found in *B. floridanus* (refer Fig. 2c) which is an endosymbiont of carpenter ant [13]. Plant pathogen *D. zea* acts as a donor in this event.

Event 4

In Recombination event 4, the recombination breakpoints were between 252 -315 nts. Putative recombinant tmRNA was found in *E. amylovora* (refer Fig. 2d) which is a plant pathogen [14]. Donor for this event is *Y. intermedia* which is a Human pathogen [15]. Evidence for similar recombination event was found in tmRNA of *E. pyrifoliae* and *E. tasmaniensis*

Event 5

In Recombination event 5, the recombination breakpoints were between 89 -179 nts. Putative recombinant tmRNA was found in *P. ananatis* (refer Fig. 2e) which is both human and plant pathogen [16]. Insect pathogen, *Y. frederiksenii* [17] acts as a donor. Evidence for similar recombination event was found in *E. billingiae* and *P. stuartii*.

Event 6

In Recombination event 6, the recombination breakpoints were between 246 -359 nts. Putative recombinant tmRNA was found in human pathogen *Y. intermedia* (refer Fig. 2f). *P. vagans* acts as a donor. Evidence for similar recombination event were found in species *Y. intermedia*, *Y. bercovieri*, *Y. mollaretii*, *Y. rohdei*, *Y. enterocolitica* and *Y. frederiksenii*

Event 7

In Recombination event 7, the recombination breakpoints were between 167 -211 nts. Putative recombinant tmRNA was found in *E. amylovora* (refer Fig. 2d). None of the tested tmRNA have similarity in this region. Evidence for similar recombination event was found in *E. amylovora*, *E. pyrifoliae* and *E. tasmaniensis*.

The recombination events occurred between a functionally diverse set of species. tmRNA of many species were reported to have evidence for recombination event. These results depicted a diverse nature of gene traffic in tmRNA of Enterobacteriaceae.

4 Discussion

Rescuing stalled ribosomes is vital for the proper progression of translation as well as transcription in Prokaryotes. Bacterial cell evolved with multiple strategies for rescuing stalled ribosomes [18, 19, 20]. Among them tmRNA mediated *trans*-translation is the primary way. *ssrA* was well recognized for variations. Integration sites of various HGT elements were reported in *ssrA* gene. Even though the fact that *ssrA* gene is more prone to HGT was well established, regions involved in transfer and the genetic traffic are hard to come by. The present investigation aims to find the answer for the above points by identifying the recombination signals in mature tmRNA belong to the members of Enterobacteriaceae. A total of 7 recombination events were identified by RDP5. Among them, recombinant tmRNA was reported in six different species. Domain-specific recombination nature of tmRNA highly correlated with the structure and function of tmRNA. Normally domains in 5' end of tmRNA are indispensable for *trans*-translation and the domains at the later part of tmRNA were robust. Also, bases in the D-loop of tmRNA are conserved and play a crucial role in structural stability and interaction with Small protein B (SmpB) makes tmRNA-SmpB complex to resemble tRNA^{Ala} [21], which is essential for the process for *trans*-translation. Moreover, the interaction between D-loop and T-arm in tmRNA is similar to that of in canonical tRNA and makes tmRNA structurally more similar to tRNA [22]. *In-vitro* studies suggest that PK1 is indispensable than the rest of the pseudoknots. The hairpin structure of PK1 supports *trans*-translation by interacting with the ribosome, and also involved in the positioning of template in ribosomes properly. But the domains at the distal part of tmRNA (3' end) are dispensable [23, 24, 25, 26]. Even though other pseudoknots (like PK2 and PK4) are proposed to be involved in various stages of *trans*-translation, discrepancy, and deviation in their structure have been reported [24]. Interestingly the species which have long tmRNA (in this investigation) have variation in ORF and PK2 and PK3 (*Erwinia* spp.) which correlates with present results. tmRNA's without mRNA region (degradation tag) were reported in mitochondria of jakobids (flagellated protists) which portray the robustness of ORF than other domains [27]. These results are incomparable with our results that the indispensable domains are underrepresented in recombination signals than other regions. The recombination signals were not detected in the proximal region of tmRNA. This could be since the recombinants that arose in those domains could be eliminated from the population. These results revealed domain specificity in the evolution of the *ssrA* gene.

Parental analyses of each of the recombination signals reveal the diversity of HGT in the family Enterobacteriaceae. Out of the 53 species tested, 6 (~ 3%) species were found to have recombinant tmRNA. Transfer of segments of tmRNA was found

among a wide range of species belong to different niche and functions (ex: Event 1 and 2). Eventhough this investigation does not point out any details about HGT (i.e. the niche in which the participants can coexist, mode of gene transfer among them), it clearly indicated that under unfavorable conditions, nature will not restrict these recombination events [28, 29, 30, 31]. Also, there is no limitation in nature for a horizontally transferred gene to get further transferred *via* another event. Species found to have sequences similar to the recombinant one could be due to the above-mentioned notion. Even though the HGT and genetic variations associated with *ssrA* gene were so evident; it is this study that leads to the identification of the recombinant tmRNA and established the diversity like HGT among the species of Enterobacteriaceae. The results further revealed that recombination events could supply the necessary platform for the evolution of the *ssrA* gene and further played a major role in shaping the structure and function of tmRNA.

5 Conclusion

To conclude, this work has led to the identification of recombination signals in tmRNA of Enterobacteriaceae members. This research further emphasizes the highly conserved regions are less likely to undergo lateral transfer and regions which are robust frequently undergo lateral transfer as per the need of time, environment, and function etc. Meanwhile, the limitations of the present investigation are that the forecast results were obtained based on computational analysis on existing limited resources.

Declarations

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Author contribution

TN and SR conceived and designed the study. MHM was in charge of overall direction, planning and supervision. TN, SR and MHM analyzed the data and wrote the manuscript. PS helped in analyzing the data and writing the manuscript. All the authors read and approved the final manuscript.

Consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors

Consent to publish

This article does not contain any studies with human participants or animals performed by any of the authors

Availability of data and material

All supporting data are included in the additional files. The materials generated during the current study are available upon request

Compliance with ethical standards

Conflict of Interest

We hereby declare that there is no conflict of interest between the authors of this manuscript.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors

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Figures

Fig. 1

Figure 1

a) Cartoon representation of linearized tmRNA (linearized). Different domains are highlighted with different colors. b) Recombinational events are labeled in a linearized tmRNA. c) The alignment overview of tmRNA. The color code indicates the sequence conservation status

Fig 2.

Figure 2

Putative recombinant tmRNAs predicted by RDP5. The region acquired through Horizontal gene transfer is highlighted Fig. 2a tmDNA of *D. dadantii* Fig. 2b tmDNA of *P. vagans* Fig. 2c tmDNA of *B. floridanus* Fig. 2d tmDNA of *E. amylovora* Fig. 2e tmDNA of *P. ananalis* Fig. 2f tmDNA of *Y. intermedia*

Figure 3

Neighbor Net analysis of Enterobacteriaceae. a) Neighbor net based on tmRNA sequences, the uncorrected P distance was used and the network was drawn employing equal angle method. b) A considerable reticulation was observed in the network. This indicate that recombination affected the sequence of tmRNA. Phi Test was conducted and found favorable for recombination ($p = 0.006016$). The recombinants were highlighted

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [05SupplementaryData01.docx](#)
- [06SupplementaryData02.txt](#)
- [SupplementaryFigSI.pptx](#)