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Phylogenetic analysis reveals the coexistence of interfamily and interspecies horizontal gene transfer in *Streptococcus thermophilus* strains isolated from the same yoghurt

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ABSTRACT

Horizontal gene transfer (HGT) is an important evolutionary mechanism that has shaped prokaryotic genomes. For *Streptococcus thermophilus*, there is no direct evidence that the bacteria might acquire a second paralog from a different origin in the same niche. In this study, we found that four isolates of *S. thermophilus* (B, C, E and F) from the same yoghurt contained two putative homologs of the *eno* genes (*eno-1* and *eno-2*) and two putative homologs of the *guaB* genes (*guaB-1* and *guaB-2*). Both *eno-1* and *guaB-1* shared 100% nucleotide identity among the four isolates, and with isolate A and *S. thermophilus* ND03. Phylogenetic and nucleotide divergence analyses indicated that *guaB-2* of these isolates may have been acquired from species in the genus *Streptococcus*, while *eno-2* of isolates B and C may have been acquired from a donor in the genus *Streptococcus*. The *eno*-2 genes of isolates E and F may have been acquired from a donor in the *Enterococcus* genera. Relative synonymous codon usage analysis confirmed the *eno*-2 genes of isolates E and F as being acquired from a donor in genus *Enterococcus*. This study provides evidence that interfamily and interspecies HGT occur in *S. thermophilus* strains isolated from the same niche.

1. Introduction

HGT is the collective name for processes that allow the exchange of DNA among organisms of different species (Jain et al., 2003). This occurs mainly in two ways. Either the new sequence replaces a homologous sequence through the process of homologous recombination (replacing HGT) or it is acquired through an additive integration (additive HGT) process (Thomas and Nielsen, 2005; Choi et al., 2012), via transformation, transduction and conjugation (Feil et al., 2000; Tettelin et al., 2005; Delorme et al., 2007; Brochet et al., 2008; Liu et al., 2009; Eng et al., 2011). There have been frequent reports of both additive HGT of genes that are involved in antibiotic resistance and antigenic diversity (Houndt and Ochman, 2000; Weigel et al., 2003; Wildschutte et al., 2010; Knight et al., 2012; Varga et al., 2012; and see reviews by Heuer et al., 2011; Blázquez et al., 2012), and replacing HGT of highly conserved genes that occurred within species, interspecies, cross-family, and interkingdom (Andersson and Roger, 2003; Chen et al., 2010; Crémet et al., 2012; Fitzpatrick et al., 2008; Lawrence et al., 2011; Liu et al., 2012; López-García et al., 2004; Macheras et al., 2011; Rödelsperger and Sommer, 2011; Rot et al., 2006; Saunders et al., 2005; Zhong et al., 2012).

In *Streptococcus*, HGTs have been frequently reported (Dowson et al., 1989; Feil et al., 2000; Tettelin et al., 2005; Delorme et al., 2007; Brochet et al., 2008; Liu et al., 2009; Hiller et al., 2010; Eng et al., 2011), along with evidence that even highly conserved genes involved in transcriptional processes can be transferred through HGT between species (Schouls et al., 2003; Kamikawa et al., 2008). To date, no direct evidence exists to show that *Streptococcus* can acquire conserved housekeeping genes from multiple sources within the same niche. In this study, we showed that strains of *Streptococcus thermophilus* from the same niche acquired their additive eno and guaB homologous genes (Eno-2, GuaB-2). The most interesting observation is that both interspecies and cross-family additive HGT of the *Eno-2* gene took place in the same organism.

2. Materials and methods

2.1. Bacterial strains

S. thermophilus LVRI TZ isolate A (isolate A), *S. thermophilus* LVRI TZ isolate B (isolate B), *S. thermophilus* LVRI TZ isolate C (isolate C),

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S. thermophilus LVRI TZ isolate E (isolate E) and *S. thermophilus* LVRI TZ isolate F (isolate F) were isolated from the same sample of yoghurt in our laboratory. Based on the analysis of 16S RNA and partial *tuf* gene sequences, we found that all the isolates have 100% nt identity with that of *S. thermophilus* ND03. *Streptococcus* sp. LVRI_193, which was also isolated in our laboratory, was included in the analysis. JM109 competent cells were purchased from Takara-Biotechnology (Takara-Biotechnology (Dalian) Co. Ltd., Dalian, China).

2.2. DNA extraction, primers, and PCR conditions

Genomic DNA was extracted from cultured material using a GenElute™ Bacterial Genomic DNA Kit (Sigma–Aldrich Co. Ltd., St. Louis, MO, USA) according to the manufacturer's instructions. The sequences of *eno*, *guaB* and other genes were amplified using a Quick Taq HS DyeMix (Toyobo Biotechnology, Co. Ltd., Shanghai, China) and a Bio-Rad (Hercules, CA, USA) Mini™ personal thermal cycler. The primers and PCR cycling conditions are listed in Table 1. The PCR products were ligated into the pMD18 vector (Takara-Biotechnology (Dalian)).The recombinant plasmids were then transformed into JM109 competent cells according to the manufacturer's instructions. Positive isolates were sent to the Shanghai Sangon Biotechnology Company for sequencing. The PCR products were sequenced using a BigDye Terminator v3.1 cycle sequencing kit using ABI-PRISM3730 Genetic Analyzer.

2.3. Phylogenetic analysis

The phylogenetic trees were generated by both the neighbor-joining (NJ) method and the maximum likelihood (ML) method using Mega 5.10 (Felsenstein, 1985; Kimura, 1980; Tamura et al., 2011). The Kimura 2-parameter in Mega 5.10 was used to construct the NJ tree and to calculate the distance. ML model testing was performed to find the most suitable ML model for tree construction. Relative synonymous codon usage (RSCU) was calculated using CodonW (http://mobyle.pasteur.fr/cgi-bin/portal.py?#forms::codonw).

Nucleotide sequence accession numbers. The *eno* and *guaB* gene sequences from the isolates were deposited in GenBank with accession numbers JX436498 to JX436505. The details of other eno genes and guaB genes accession numbers are listed in Additional Table 1.

3. Results

3.1. Sequence analysis revealed that isolates B, C, E and F contained two eno and two guaB genes

The expected fragments of the partial *eno* and *guaB* genes of all the isolates were amplified and sequenced. A BLAST search revealed that only isolate A contained the same *eno* and *guaB* genes as that of other *S. thermophilus* strains, and for the other isolates, both the *eno* and *gua B* genes were different from those of *S. thermophilus* strains. For the *eno* gene, isolates B and C have high sequence similarities with members of the genus *Streptococcus*, while isolates E and F have high sequence similarities with members of the genus *Enterococcus*. For the *guaB* gene, all sequences were highly similar to those of members of the genus *Streptococcus* (Table 2).

To find out if the *eno* and *guaB* genes that had high nucleotide identity with *S. thermophilus* strains exist in isolates B, C, E, and F, different PCR primers were used to amplify the *eno* and *guaB* genes (Table 1). The sequencing results of the PCR products revealed that all the sequences have 100% nucleotide identity with those of *S. thermophilus* ND03.

Thus, isolates B, C, E and F contained two *eno* homologs and two *guaB* homologs. One *eno* gene (*eno-1*) and one *guaB* gene (*guaB-1*) were the same as those of other *S. thermophilus* strains; the other *eno* homolog (*eno-2*) and *guaB* homolog (*guaB-2*) may have been acquired from other bacteria.

Table 1
Primers used in this study.

Primer name	Primer sequence	Length	Annealing temperature	
Eno-F	5′-CTGATGTTTACGCTCGCG-3′	607 bp	50.0 °C	a
Eno-R	5′-CCACCTTCGTCACCAACA-3′			
guaB-F	5′-GTTATTGAGTTTCCAAATGCTGC-3′	722 bp	50.0 °C	a
guaB-R	5′-CCCATACCAGAGCGAATACCACC-3′			
Eno1-F	5′-GTCGGGGATTGATTTTGTACTGGAT-3′	1585 bp	50.0 °C	b
Eno1-R	5′-CCAACGAAGACCTTCTTTGAAAGTT-3′			
Eno2-F	5′-AGGTAAATTAGGTGCAAACGC-3′	1855 bp	50.0 °C	b
Eno2-R	5′-GGTGATAGCAAACTAGCTCG-3′			
GuaB1-F	5′-AGCTGATGGACATCATAGTATTCTC-3′	1917 bp	50.0 °C	b
GuaB1-R	5′-CCAACTTATTCGCTTCATTGACAGA-3′			
GuaB2-F	5′-TCATGGTCATTCAGCAGGAGTTCTT-3′	1313 bp	50.0 °C	b
GuaB2-R	5′-CAGCTGCCTGAGAAATTGGATAAAC-3′			

a: Primers used to amplify partial sequences for the first eno and first guaB gene of isolate A, and the second eno and second guaB gene of isolates B, C, E, F.
b: Primers used to amplify the first eno and first guaB gene of isolates B, C, E, F. Primer Eno1 amplifies a partial eno gene and its adjacent upstream gene; primer Eno2 amplifies a partial eno gene and its adjacent downstream gene; primer GuaB1 amplifies a partial guaB gene and its adjacent upstream gene; and primer GuaB2 amplifies a partial guaB gene and its adjacent downstream gene.

Table 2
Highest similarity scores of partial eno and guaB gene sequences with the published data from NCBI.

Isolates	eno	guaB
A	100% nt Similarity with that of *S. thermophilus* ND03	100% nt Similarity with that of *S. thermophilus* ND03
B	94% nt Similarity with that of *Streptococcus* sp LVRI_193	85% nt Similarity with that of *S. pseudopneumoniae* IS7493
C	99.7% nt Similarity with that of *Streptococcus* sp LVRI_193	85% nt Similarity with that of *S. pseudopneumoniae* IS7493
E	96% nt Similarity with that of *Enterococcus hirae* ATCC 9790	83% nt Similarity with that of *Streptococcus parasanguinis* FW213
F	96% nt Similarity with that of *Enterococcus hirae* ATCC 9790	85% nt Similarity with that of *Streptococcus oralis* Uo5