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# The Horizontal Transfer of Genetic Elements of Antibiotic Resistance in *Streptococcus thermophilus*

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**One strain of *streptococcus thermophilus* was tested to be resistant to most current antibiotics and has 100% identical sequence with LMD-9 strain after 16s rRNA analysis. Horizontal gene transfer is a significant phenomenon for the transfer of antibiotic resistance between different bacteria. Thus, the phenomenon was investigated at genome-wide level. The results indicated that a transposase gene was transferred from *enterococcus* to *stremptococcus thermophilus*, and plasmid2 was transferred from *streptococcus thermophilus* to *sreptococcus pyrogenes*, *enterococcus faecium* and *streptococcus pneumoniae*. Furthermore, exopolysaccharide operon gene and glycopeptide antibiotics resistance protein gene (GARPG) were transferred from *streptococcus thermophilus* to *Streptococcus salivarius*. GARPG was also found to be transferred from *streptococcus thermophilus* to *Lactococcus lactis*. Cell wall thickening was also a feature of broad-range antibiotics resistance in *strepotococcus thermophilus*. These warned us that the safety status of the dairy bacteria should be taken an all-around consideration. Creating the dairy bacteria without the resistance for common antibiotics will be extremely demanded.**

**Key words**: *Streptococcus thermophilus*, Antibiotics resistance, Horizontal gene transfer, Cell wall thickening.

Nowadays, *Streptococcus thermophilus,* has globally become the main foodstuffs as the dairy bacterium. The discovery and development of antibiotics has led to dramatic improvements in treating infectious diseases and significantly increased yogurt production. However, widespread use of antibiotics has accelerated emergence of multidrug-resistant pathogens[1]. Therefore, it is very important to guarantee the safety status. However, *S. thermophilus* from yogurt culture has been found resistant to a few current antibiotics when a wide range of antimicrobial compounds are used for *S. thermophilus*[2].

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Horizontal gene transfer (HGT) is supposed to play an important role in the spread of the resistance[3]. Furthermore, transposons might speed up the dissemination of the resistance as mobile genetic elements[4]. Once the pathogens are armed with such a new weapon, they will acquire antibiotics resistance easily. Multidrug transport system genes also make a major contribution to this intrinsic antibiotic resistance[5]. Parallelly, glycopeptide antibiotics resistance protein gene is related to both acquired and intrinsic resistance mechanisms[6]. Here, the mechanism for disseminating antibiotics resistance should be clear and an alternative to provide the dairy bacteria without resistance is urgently requested to maintain consumers' health.

Thus, we tried to find some clues lying in the mechanism acquiring the resistance in *streptococcus thermophilus* since the HGT of these

genes has never been studied in this species. This work is also important to create the dairy bacterium without antibiotic-resistance.

## MATERIALS AND METHODS

### Strains and antibiotic sensitivity test

*Streptococcus thermophilus* was isolated from a new yogurt starter (NO. M20080903, China Mengniu Dairy Company Limited,) and grown on MRS agar plates. An ideal single colony on MRS plate was picked to 5 ml MRS liquid medium and inoculated overnight at 37°C. The strains was analyzed using 16s rRNA and found to have 100% homology with that of the strain LMD-9. Subsequently, 55 ul culture coming from one colony was aliquoted into 11 new tubes and added with 1.5 ml fresh MRS medium containing 50 ug/ml of each ampicillin, tetracycline, kanamycin, streptomycin, cephamycin, erythromycin, zeocin, chloramphenicol, neomycin, G418, or vancomycin respectively.

### Candidate genes relatd to antibiotics-resistance and phylogenetic trees

All the genes related to antibiotics-resistance were found by blasting the NCBI archives according to the previous reports (7-14) (Table 1). The distances between all the genes were depicted as phylogenetic trees with maximum sequence difference (http://www.ncbi.nlm. nih.gov/blast/Blast.cgi?). In order to understand how consistently the data support taxon bipartitions, bootstrapping measures were set up. When a node generally appeared in more than 50% of the total number of analyses, it was considered "accurate" while lower percentage was "less confidence"[15].

### Quantification of HGT events.

Usually, the HGT emerges when a gene sequence from a particular organism shows the strong similarity with a homolog in a distant taxon. To make the case convincing, phylogenetic analysis is required[16]. From more than four sets of data, HGT from/to *streptococcus thermophilus* should be positive if more than 75% of the data sets support *Streptococcus thermophilus* as a coherent group, while negative if less than 25% of the data sets from other taxa, or if less than 25% of the data sets to *Streptococcus thermophilus* grouping somewhere else[17].

## RESULTS

### Strains and antibiotic sensitivity test

The isolated *Streptococcus thermophilus* (Supposed LMD-9 according to 16s rRNA analysis) was tested to be resistant to the following antibiotics: ampicillin, cephamycin, vancomycin,

**Table 1**. The blast search for the antibiotic-resistance-related genes of *Streptococcus thermophilus*

| The name of gene | Gene numbers |
|---|---|
| ABC-type antimicrobial peptide transport system | 5 |
| ABC-type cobalt transport system, ATPase component | 3 |
| ABC-type Fe3+-hydroxamate transport system | 3 |
| ABC-type multidrug transport system | 16 |
| ABC-type oligopeptide transport system | 30 |
| ABC-type phosphate transport system | 5 |
| ABC-type uncharacterized transport system | 8 |
| Glycopeptide antibiotics resistance protein | 2 |
| Glycosyltransferase | 02 |
| Glycosyltransferase involved in cell wall biogenesis | 07 |
| Heat shock protein | 01 |
| L-lactate dehydrogenase | 01 |
| Membrane carboxypeptidase (penicillin-binding protein) | 01 |
| Na+-driven multidrug efflux pump | 03 |
| Predicted ABC-type exoprotein transport system | 01 |
| Transposase | 058 |
| Type II secretory pathway/competence component | 03 |
| Plasmid 1 and 2 | |

chloramphenicol, kanamycin, erythromycin, G418, tetracycline, streptomycin, zeocin and neomycin. *Streptococcus thermophilus* in this study exhibited a wide range of resistance, which is different from that compared with the earlier reports[2].

**Blast and quantification of HGT events related to antibiotic-resistance**

Tansposon, an mobile genetic element, translocated by transposase, plays an important role in HGT. The antibiotics resistance in *streptococcus thermophilus* might be traced to the contsribution of transposase. The 16 sequences from the genes in *streptococcus thermophilus* with near nodes of that coding transposase in enterococcus (Genbank Accession No: NC_008532) suggest that the genes were transferred from enterococcus to *streptococcus thermophilus* LMD-9 (Fig. 1).

During gene blasting, we found that the sequence of transposase gene was more similar with that of EPS operon gene, which was one of main components for antibiotics resistance and found to be transferred from *streptococcus thermophilus* to *streptococcus salivarius* (Fig. 2). Multidrug transport system is also involved with the mechanism for causing antibiotics resistance[10]. Since our phylogenetic analysis indicated that the gene coding multidrug transport system was also transferred from *streptococcus thermophilus* to *Streptococcus salivarius* (data not shown), it is possible for *streptococcus salivarius* acquireing some antibiotics resistance gene from *streptococcus thermophilus*. Similarly, the macrolide efflux genetic assembly of *streptococcus pneumoniae* was reported to be present in erythromycin-resistant *streptococcus salivarius*[18].

Na-driven multidrug efflux pump gene[19] is also closely related with antibiotic resistance. Through the blast, the gene was found only in *streptococcus thermophilus* and *streptococcus mutans*, but not in the third genome-sequenced strains. Obviously, the gene might be transferred from other unnamed or unsequenced strains. As the result, the HGT of antibiotic resistance could occur between probiotic bacterial strains. The HGT of glycopeptide antibiotics resistance protein gene occurred between *streptococcus thermophilus* and *lactibacillus lactis* (Fig. 3). The four sequences in *streptococcus thermophilus* with high bootstrap values (96%) suggested the gene coding glycopeptide antibiotics resistance protein was also transferred from *streptococcus thermophilus* to *streptococcus salivarius*.

Plasmid2 was also suggested to be involved in HGT events. The sequences of *streptococcus thermophilus* with high bootstrap values (99%) suggested that the partial sequence of plasmid2 was transferred from *streptococcus*

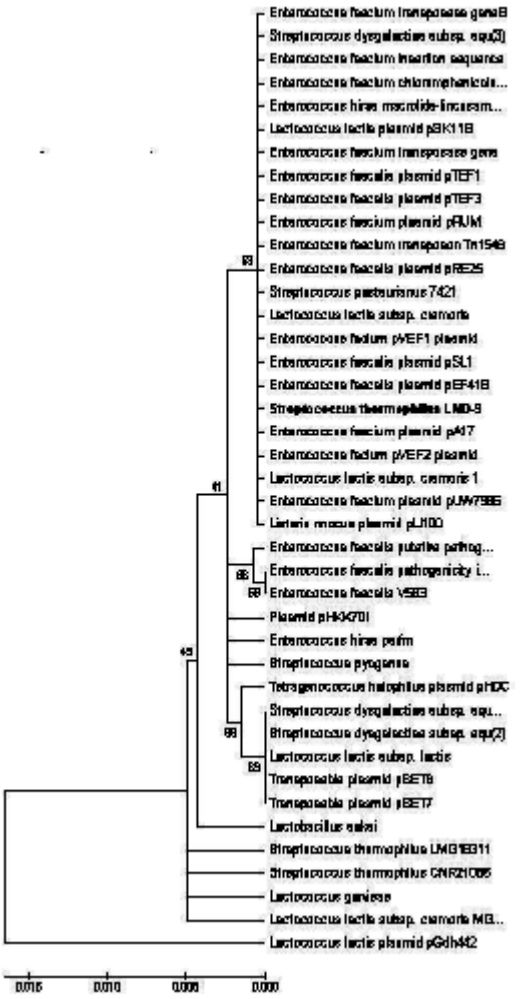


**Fig. 1.** Phylogeny of transposase gene (Genbank Accession No: NC_008532) from *Streptococcus thermophilus*. Numbers of the branch show bootstrap values for maximum likelihood and distance analyses, respectively. In this tree, *Streptococcus thermophilus* (in boldface) had no a detectable homolog in its annotation, while the sequences of 16 enterococcus with high bootstrap (69%) support suggested the gene coding transposae was transferred from enterococcus to *Streptococcus thermophilus* LMD-9.

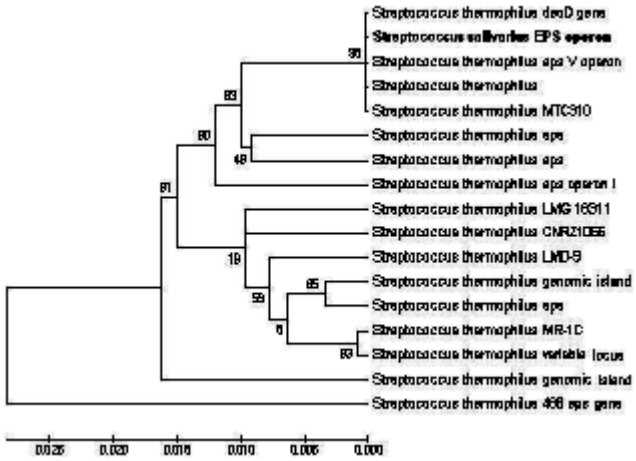


**Fig. 2.** Phylogeny of exopolysaccharide (EPS) operon gene (Genbank Accession No: NC_008532) from *Streptococcus thermophilus*. Numbers of the branch show bootstrap values for maximum likelihood and distance analyses, respectively. In this tree, *Streptococcus salivarius* (in boldface) had no a detectable homolog in its annotation, while the sequences of four *Streptococcus thermophilus* with high bootstrap (95%) support suggested the gene coding EPS operon was transferred from *Streptococcus thermophilus* to *Streptococcus salivarius*

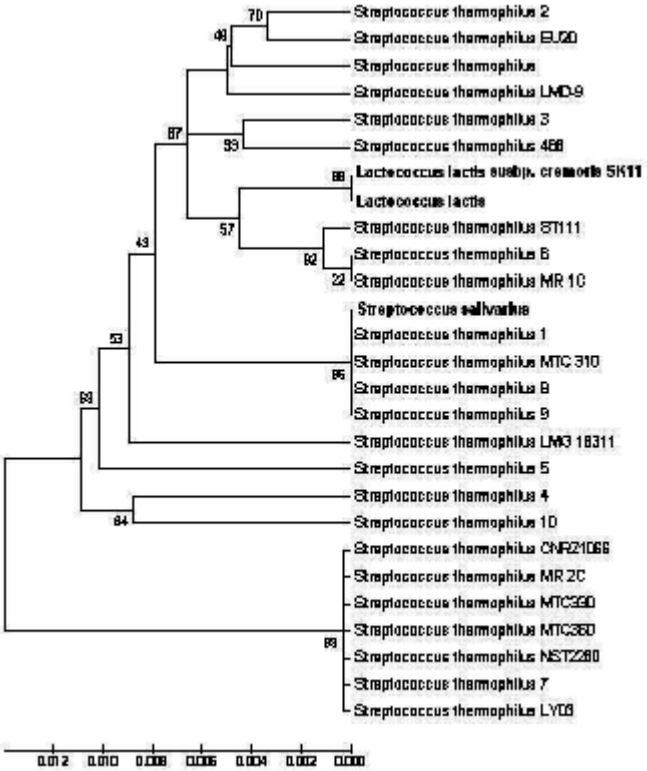


**Fig. 3.** Phylogeny of glycopeptide antibiotics resistance protein gene (Genbank Accession No: NC_008532) from *Streptococcus thermophilus*. Numbers of the branch showed bootstrap values for maximum likelihood and distance analyses, respectively. In this tree, *Streptococcus salivarius* and *Lactobacillus lactis* (in bold letter size) had no a detectable homolog in its annotation, while the sequences of *Streptococcus thermophilus* with high bootstrap support suggested the gene coding glycopeptide antibiotics resistance protein was transferred from *Streptococcus thermophilus* to *Streptococcus salivarius* and *Lactobacillus lactis.*

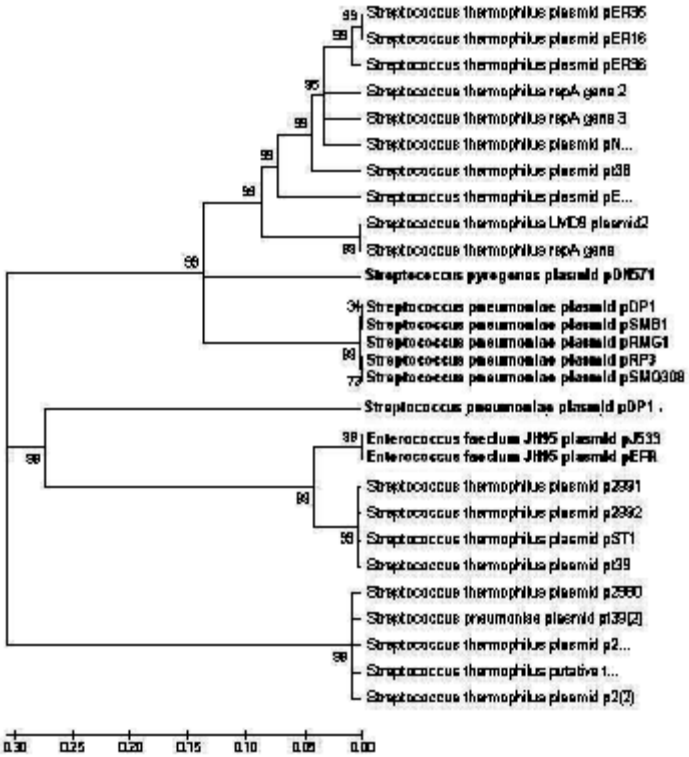


**Fig. 4.** Phylogeny of plasmid2 (Genbank Accession No: NC_008501) from *Streptococcus thermophilus*. Numbers of the branch showed bootstrap values for maximum likelihood and distance analyses, respectively. In this tree, *Streptococcus pyrogenes* and *Enterococcus faecium* (in boldface) had no a detectable homolog in its annotation, while the sequences of *Streptococcus thermophilus* with high bootstrap support suggested the partial sequence of plasmid2 was transferred from *Streptococcus thermophilus* to *Streptococcus pyrogenes* and *Enterococcus faecium*. For *Streptococcus pneumoniae* had a little homology in its annotation, so the HGT between *Streptococcus thermophilus* and *Streptococcus pneumoniae* seemed less confident.

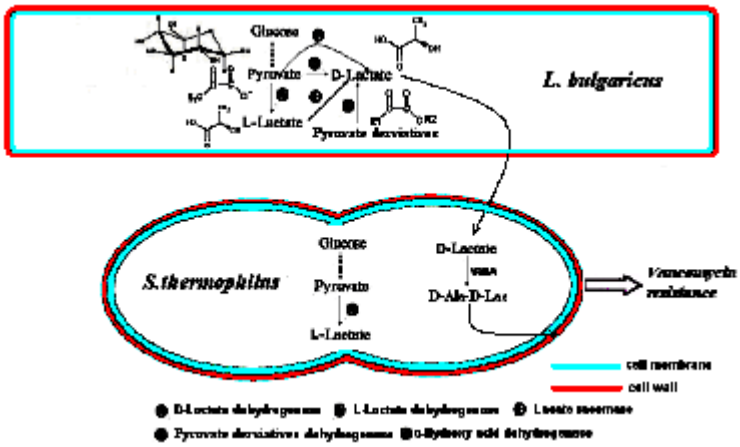


**Fig. 5.** The possible pathways of D-lactate production in dairy bacteria (*Lactobacillus delbrueckii bulgaricus* and *Streptococcus thermophilus*). For *Streptococcus thermophilus*, the acquired resistance to vancomycin was developed when the D-Ala-D-Ala terminating peptidoglycan precursors was replaced by the altered D-Ala-D-Lac terminating ones. In the yogurt culture, it was quite possible that *Streptococcus thermophilus* acquires the resistance to vancomycin using the D-lactate produced by *Lactobacillus delbrueckii bulgaricus*

*thermophilus* to *streptococcus pyrogenes*, *enterococcus faecium* and *etreptococcus pneumoniae* (Fig. 4).

## DISCUSSION

According to our knowledge, *streptococcus thermophilus* is getting wide resistance to available antibiotics, including penicillin, tetracycline, erythromycin and vancomycin. However, the mechanism of resistance in *streptococcus thermophilus* has not yet been clarified. Cui et al. showed that cell wall thickening process is a common feature of vancomycin resistance[20]. In another experiment, they demonstrated that the thickened cell wall of Staphylococcus aureus resistant to vancomycin could protect peptidoglycan biosynthesis ongoing in the cytoplasmic membrane from the inhibition by vancomycin, allowing the cells to continue producing nascent cell wall peptidoglycan and making the cells much more resistant to vancomycin.

From our results, according to the OD value of growth, *streptococcus thermophilus* was high resistant to streptomycin, zeocin, neomycin (high molecular weight antibiotics), middle resistant to kanamycin, erythromycin, G418 , tetracycline (middle molecular weight antibiotics) and low resistant to ampicillin, cephamycin, chloramphenicol (low molecular weight antibiotics) and vancomycin (high molecular weight antibiotics but limiting cell wall synthesis). Based upon above results, we concluded that cell wall thickening was a common feature for the broad antibiotics resistance of *streptococcus thermophilus*.

D-lactate is an important substance for cell wall thickening[20], but there is no D-lactate in *streptococcus thermophilus*, which might come from D-lactate production of *lactobacillus delbrueckii bulgaricus* during coculturing (Fig. 5).

D-Lactate is produced by a number of species including Lactobacillus[21-23]. D-Lactate can be formed by D-lactate dehydrogenase in *Lactobacillus delbrueckii bulgaricus*. It can also be formed by the action of a racemase[24]. It is presently unknown whether α-hydroxy acid dehydrogenase functions in the dairy bacteria although D-lactate can be produced through the enzyme using pyruvate as substrate. The other possible pathway for producing D-lactate is pyruvate derivatives dehydrogenase using pyruvate derivatives as substrate (Fig. 5). However, there had not been such a result reported by for now.

In general, HGT is believed to be the main mechanism for acquiring antibiotics resistance in bacteria. During bacterial evolution, the ability of bacteria adapts to new environments most often results from the acquisition of new genes through HGT. In our investigation, one transposase gene is transferred from *Enterococcus* to *Stremptococcus thermophilus*, and plasmid2 from *Streptococcus thermophilus* to *Streptococcus pyrogenes*, *Enterococcus faecium* and *Streptococcus pneumoniae*. Thus, controlling the dissemination of multidrug-resistant bacteria remains a big challenge. Additionally, the acquired resistance elements, exopolysaccharide operon gene and glycopeptide antibiotics resistance protein gene might be transferred from *streptococcus thermophilus* to *streptococcus salivarius*. The latter gene was also found to be transferred from *streptococcus thermophilus* to *lactococcus lactis*. Meanwhile, the high bootstrap values convinced that the HGT of antibiotics resistance occurred widely between *streptococcus thermophilus* and other taxa.

Summarily, regarding for many respects mentioned above, the safety status of the dairy *streptococcus thermophilus* should be considered carefully and properly.

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