

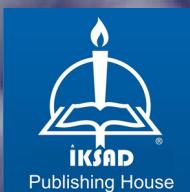
RECENT ADVANCES IN BIOLOGICAL SCIENCES

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Neslihan BAL
Pınar ARSLAN
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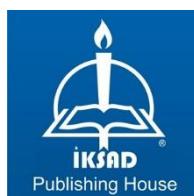
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PREFACE

Biological sciences aim at the study of life and living organisms, their life cycles, adaptations, and the environment. ‘Recent Advances In Biological Sciences’ describes recent progress in various biological sciences, such as microbiology, molecular biology, zoology, and botanic. This book consists of a total of eight chapters, each of which has been contributed by highly qualified professionals in the respective fields of research.

I am proud to present this book, which contains recently updated information on various research areas and techniques in the biological sciences, which will benefit many researchers from different life science institutions around the world.

We would like to thank Iksad Publishing house for their devoted work to bringing together valuable scientists, editing, and publishing the book named **Recent Advances In Biological Sciences**.

With my regards

Res. Assist. Dr. M. Burcu KÜLAHCI

CHAPTER 2

GENETIC INTERPLAY BETWEEN THE DAIRY *STREPTOCOCCUS THERMOPHILUS* AND ITS PHAGES

Res. Assist. Dr. Serap ÇETİNKAYA¹
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INTRODUCTION

Streptococcus thermophilus has long been exploited as a milk clotting agent, starter, especially of yogurt manufacture, and recently, to a much lesser extent, in cheese production. This species has often been encountered in raw milk that constitutes its main natural source. It has generally been accepted that this species came to being very recently, as late as 30,000 years. Its genome has been considered at two levels of organization: the one includes genes indispensable for its survival, and the other, the remainder, may not be essential. Approaches employing computational prediction methods have evidenced that three main driving forces of evolution in prokaryotes, conjugation, competence, and transduction, have all played substantial roles in the shaping of its genome. These studies have also revealed 362 atypical genes of exogenous origin that could have been inserted into the genome of this new species through horizontal gene transfer (HGT) phenomena (Eng et al., 2011).

The production process of industrial yogurts involves the exploitation of two species of the lactic acid bacteria (LAB), *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus*. These starter species protocooperate by reciprocating their specific needs during the fermentation process of milk into yogurt (Fig. 1), and they appear to owe these precious attributes to the mutual events of horizontal gene transfer (Liu et al., 2009).

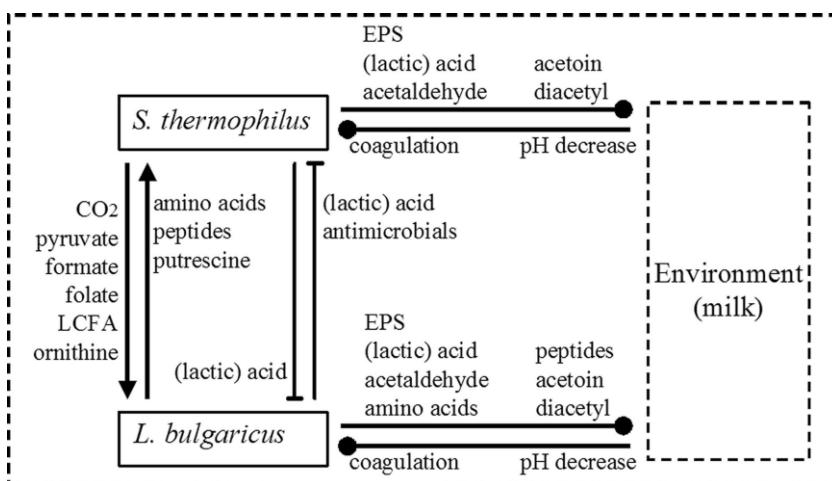


Figure 1. Schematic Illustration Of The Biochemical Interplay Taken Place Between The Two Yogurt Starters (Sieuwerts et al., 2008).

Genomes of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* have been unraveled for some time now, and this information has been used to identify the genetic basis, established through the genomewide horizontal gene transfer (HGT) events, of the biochemical interplay. HGT involves the interchange of DNA segments of chromosomal- and/or episomal origin between interspecies prokaryotes. It serves as a means of cohabitation, which is largely made possible by diverse biochemical activities of distantly related species (Nicolas et al., 2007; Liu et al., 2009).

In silico efforts, comparing genomes of three *S. thermophilus* strains, LMG 18311, CNRZ1066, and LMD9, have provided insightful clues on the HGT events, through which sets of genes, *cbs-cblB(cglB)-cysE*, for example, had been inserted into *S. thermophilus* (Table 1). This gene cluster has been found to be involved in the metabolism of amino acids, cysteine, and methionine. Data produced have also suggested

that this species has evolved together with *Lb. delbrueckii* subsp. *bulgaricus* (Liu et al., 2009).

Cysteine biosynthesis commences with the conversion of L-serine to *O*-acetylserine by the product of *cysE* gene, serine acetyltransferase, and the products of *cglB* and *cbs* convert homocysteine to cysteine (Fig. 2). It has been implied that these genes had originally resided in *Lb. delbrueckii* subsp. *bulgaricus* as a shorter, truncated form and that it had been disabled in the context of cysteine and methionine production in this organism (Liu et al., 2009).

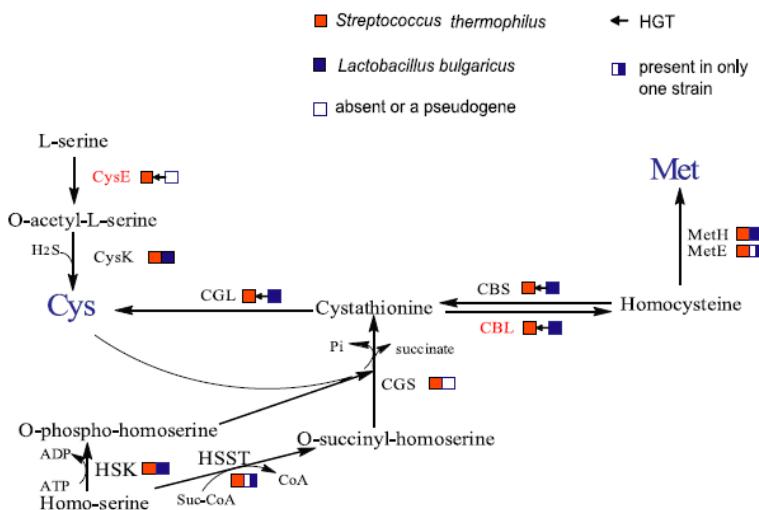


Figure 2: The Role Of HGT In The Synthesis Of Cysteine And Methionine In *S. thermophilus*.

Table 1: The Genes, epsIM and epsIL, Responsible For The Exopolysaccharide Synthesis Genes, On The Other Hand, Have Been Reported To Have Been Transferred From *S. thermophilus* Into *Lb. delbrueckii* subsp. *bulgaricus* And It Has Been Suggested That This Event Might Have Been Mediated Through Physical Contacts During Cocultivation Or In Yoghurt (Liu et al., 2009).

Gene cluster with	Gene ID(s) for strain:			GC content (%)	δ Value (10 ⁻³)	6 Plot position (%)	HGT mechanism-associated feature(s)	Function(s)
	LMG 18311	CNRZ1066	LMD9					
Low GC content								
S1	0098, 0099, 0100, 0102, 0103, 0108	0098, 0099, 0100, 0102, 0103	0131, 0133, 0134, 0135	30	64	75	Transposase, phage integrase	Lantibiotic/bacteriocin biosynthesis protein or exporter, phage integrase, and hypothetical proteins
S2			0141, 0142, 0143, 0144, 0145	36	90	98	Transposase	ABC-type peptide transport system
S3			0146, 0148, 0149, 0150	36	63	75	Transposase	Bacteriocin exporter, EPS-related protein
S4	0182, 0183	0182, 0183		30	102	97		Transcriptional regulator, putative protein kinase
S5	0324, 0325, 0328	0324, 0325, 0328	1694	28	56	49	Transposase	ABC-type transporter, hypothetical protein
S6		0683, 0684, 0685, 0686, 0687, 0688, 0689, 0690		27	73	89	Transposase	Hypothetical proteins
S7	0706, 0707, 0709	0706, 0707, 0709		29	107	98	Phage	Hypothetical proteins
S8			0811, 0812, 0814, 0817	31	103	88	Transposase, phage	Hypothetical proteins
S9		0774, 0782		32	125	85	Phage	Hypothetical protein, phase-associated proteins
S10			1057, 1059, 1060, 1061, 1062, 1066	30	112	99.7	Transposase	EPS biosynthesis
S11	1041, 1042, 1043, 1044	1037, 1040, 1041, 1042, 1044		29	48	57	Transposase	UDP-N-acetylglucosamine endopeptidyl transferase, regulator for MutR family, hypothetical protein, tyrosyl-tRNA synthetase
S12		1077, 1078, 1079, 1080, 1081, 1082		30	67	83	Transposase	EPS biosynthesis
S13	1091, 1092, 1093, 1094, 1095, 1096, 1097, 1098, 1099, 1100, 1102			30	84	99	Transposase	EPS biosynthesis
S14			1296, 1297, 1298, 1299, 1300, 1301	27	94	98		Macrolide efflux protein, peptidase F, regulator for MutR family, hydrolase, hypothetical proteins
S15			1328, 1329	29	68	30		UDP-N-acetylglucosamine 2-epimerase, hypothetical protein
S16	1393	1393	1351, 1352, 1355, 1356, 1358	30	71	90	Transposase	Multidrug efflux protein, regulator for MutR family, hypothetical proteins
S17		1479, 1480	1441, 1442, 1443	30	48	46		Glycosyltransferase involved in cell wall biogenesis and transcriptional activator <i>awrA</i>
S18	1481, 1484, 1486		1445	31	69	78		Hypothetical membrane proteins
S19			1474, 1475, 1476, 1477	31	66	89		CRISPR system-related proteins
S20	1512, 1514	1512, 1514		29	112	94		Hypothetical proteins
S21			1693, 1698	30	64	51	Transposase	Regulator for Xre family, abortive infection phage resistance protein
S22		1943, 1944	1915, 1916	27	89	87	Transposase	Bacteriocin-related proteins
S23	1947, 1948, 1949, 1950, 1951	1947, 1948, 1949, 1950, 1951	1919, 1920, 1921, 1922, 1924	28	88	99	Transposase	Regulator for MutR family and ABC transporter, putative protein kinase, hypothetical protein
S24	1976, 1977, 1978, 1983, 1989	1976, 1977, 1978, 1983, 1989	1955, 1955, 1960, 1966	29	70	49	tRNA	Conserved hypothetical proteins
High GC content								
S25	0040, 0041	0040, 0041	0058, 0059	49	75	67	Transposase	Purine metabolism
S26	0846, 0847, 0848	0846, 0847, 0848	0885, 0886, 0887	43	148	99.4	Transposase	Cys/Met metabolism
S27			1200, 1201	40	00	40	Transposase	Histidine synthesis
S28	1680, 1685	1685		48	161	83	Transposase	Putative bacteriocin

1. PHAGE GROUPS OF *STREPTOCOCCUS THERMOPHILUS*

Infection of a bacterium starts with phage adsorption on the surface of the host that necessitates the presence of a specific receptor and a phage ligand, sometimes called “anti-receptor”. As in most of the other topics of molecular biology, insight gained in this area has been derived from studies on the infection of *E. coli* with its specific phages (Goldberg et al., 1994). Infections with T4 phage for example is largely mediated by the protein of *orf37* gene, gp37 (Wood et al., 1994). This protein constitutes the large subunit of the distal part of the T4 tail and performs the act of an anti-receptor via its C-terminus in a wide host range. Three of its known receptors are B lipopolysaccharide, OmpC, and OmpF (Te'tart et al., 1998). The phage λ anti-receptor, protein J, also interacts with its receptor through its carboxy-terminus. These two phage proteins, thus, appear to be the main determinants of the host specificity (Duplessis and Moineau, 2001).

Mostly *Siphoviridae* family of phages have been isolated from *S. thermophilus* cultures and they have been divided according to the ends and the packaging mode of their genome into two groups, cohesive or headful (*cos* and *pac*, respectively), (Le Marrec et al., 1997) (Fig. 3). Later on, a third group, known as 5093-like phages, have been included (Mills et al., 2011; Mahony et al., 2014). As could be seen below, so far, the number of groups increased to five.

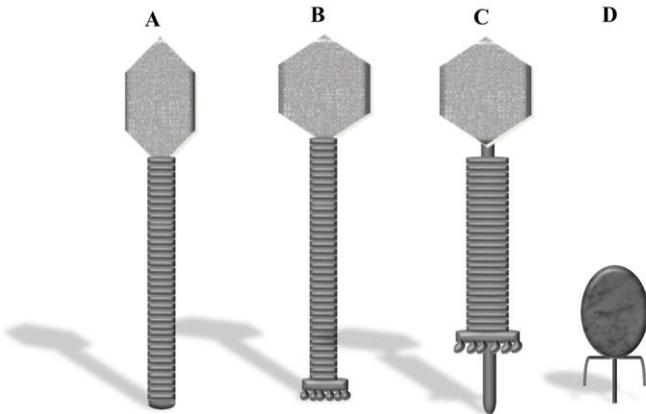


Figure 3: Morphotypes of Common LAB Phages. A: *Siphoviridae* (With Prolate-Head). B: *Siphoviridae* (Isometric-Head). C: *Myoviridae* Phages (With Long Contractile Tail). D: *Podoviridae* (With Short Non-Contractile Tails) (Mahony et al., 2014).

The first group, *cos*, has two major structural proteins (MSPs of 27 and 32 kDa). The latter, packaging, group has been characterized with three MSPs, having molecular weights of 15, 25, and 43 kDa). Computational analyses of the seven phage genomes have suggested that the ordering of the phage genes had a modular structure and that their evolution has followed a modular course (Figs. 4 and 5) (Duplessis and Moineau, 2001).

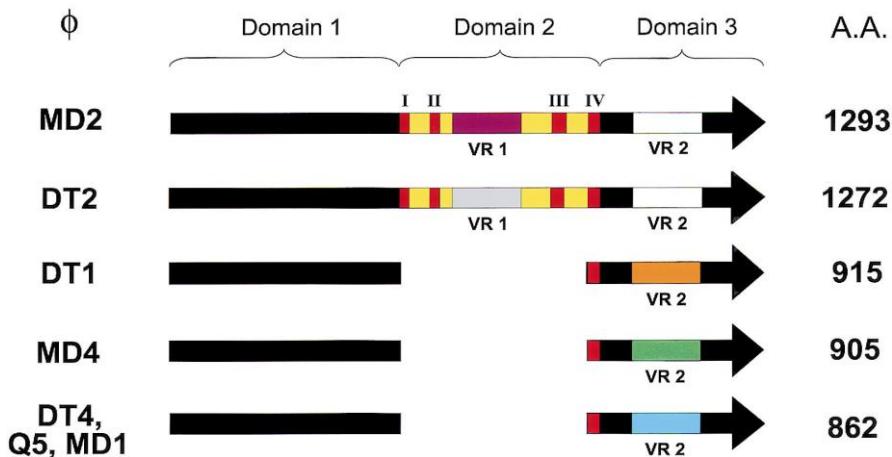


Figure 4: The Organisation Seven ORF18s. VR1, VR2: Variable Regions. Motifs I, II, III and IV: Collagen-Like Repeats.

One of the well-characterized *S. thermophilus* phages, DT1, consists of an isometric head, 60nm in diameter, and a 260nm long, non-contractile tail with 8 nm diameter. Its genome is 34 820 bp linear DNA with cohesive ends. It has two major structural proteins with molecular masses of 26 and 32 kDa. The genome is poor in guanine and cytosine, approximately 39%. So far 46 open reading frames (ORFs) have been mapped, and 20 of these putative functions have been allotted. The protein encoded by *orf18* could be acting as the anti-receptor (Tremblay and Moineau, 1999) because the weight and location of its gene appear to be the same as the gpJ and J gene in Lambda phage. The two proteins also have the same isoelectric (pI) points. Some evolutionary features of *orf18* also has resemblances to those of the other phages in terms of the distribution of conserved regions (Te' tart et al., 1996; 1998; Lucchini et al., 2000). Furthermore, ORF18 of DT1 has similar amino acid composition at its N-terminus to those of seven other phages, including DT4, MD1,

MD2, and Q5, in which coding sequences for collagen-like repeats has been mapped. The carboxy-terminal region of ORF18 also included collagen-like repeats and a variable region (VR2, 145 amino acids) (Duplessis and Moineau, 2001).

Genomic sequencing studies and following computational analyses have shown that the genome of an *S. thermophilus* phage is organised into four large modules: (1) containing genes responsible for the packaging of DNA and tail genes, with *cos* configuration (Sfi21 type) or with *pac* configuration (Sfi11 type) (Lucchini et al., 1999); (2) spanning structural tail genes, and the genes of lysis and lysogeny; (3) often a Sfi21-type replication module, divided into two different gene clusters (Desiere et al., 1997), and (4) a 5kb long module, constituting the 3'-end of the genome (Bru"ssow and Desiere, 2001).

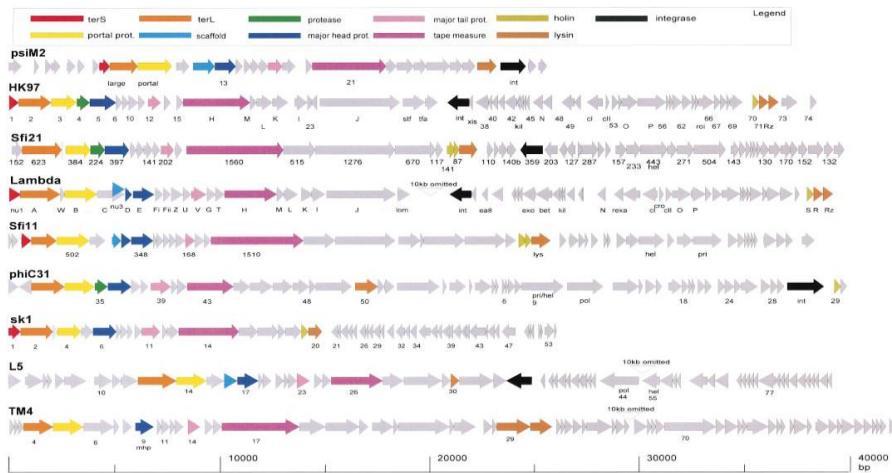


Figure 5: Alignment of The Siphoviridae Genomes (Bru"ssow and Desiere, 2001).

In 2016, a third phage type, forming the group 987 with four members, has been reported. The members appeared to have been the products of hybrid genomes of those phages (Fig. 6.) that have been shown to be able to adsorb onto dairy strains of *S. thermophilus* as well as *Lactococcus lactis*. Thus this discovery has added another dimension to the vulnerability of dairy industries regarding the capability of infectious agents and to the possibilities of countermeasures that should be taken to challenge these devious entities (McDonnell et al., 2016).

Hybrid genomes of the 987 group seemed to have been dependent, within the confines of the dairy industry, on horizontal gene transfer cases, involving the replication- and structural modules of *S. thermophilus* and *L. lactis*, respectively. This group has been morphologically related to those of the *L. lactis* phages. This could be expected but one other striking feature of the new group has been that the genomes of 987 group harbored significant divergence from within, as opposed to the previous data obtained from other groups of the *S. thermophilus* phages. The highly mosaic and diverse nature of the novel hybrid genomes calls to mind both the production of bacteriophage-insensitive mutant (BIM) strains of *S. thermophilus* and the implementation of related rotation programs to alleviate the problem of inadvertent hybrid phage damage in fermentation plants (McDonnell et al., 2016).

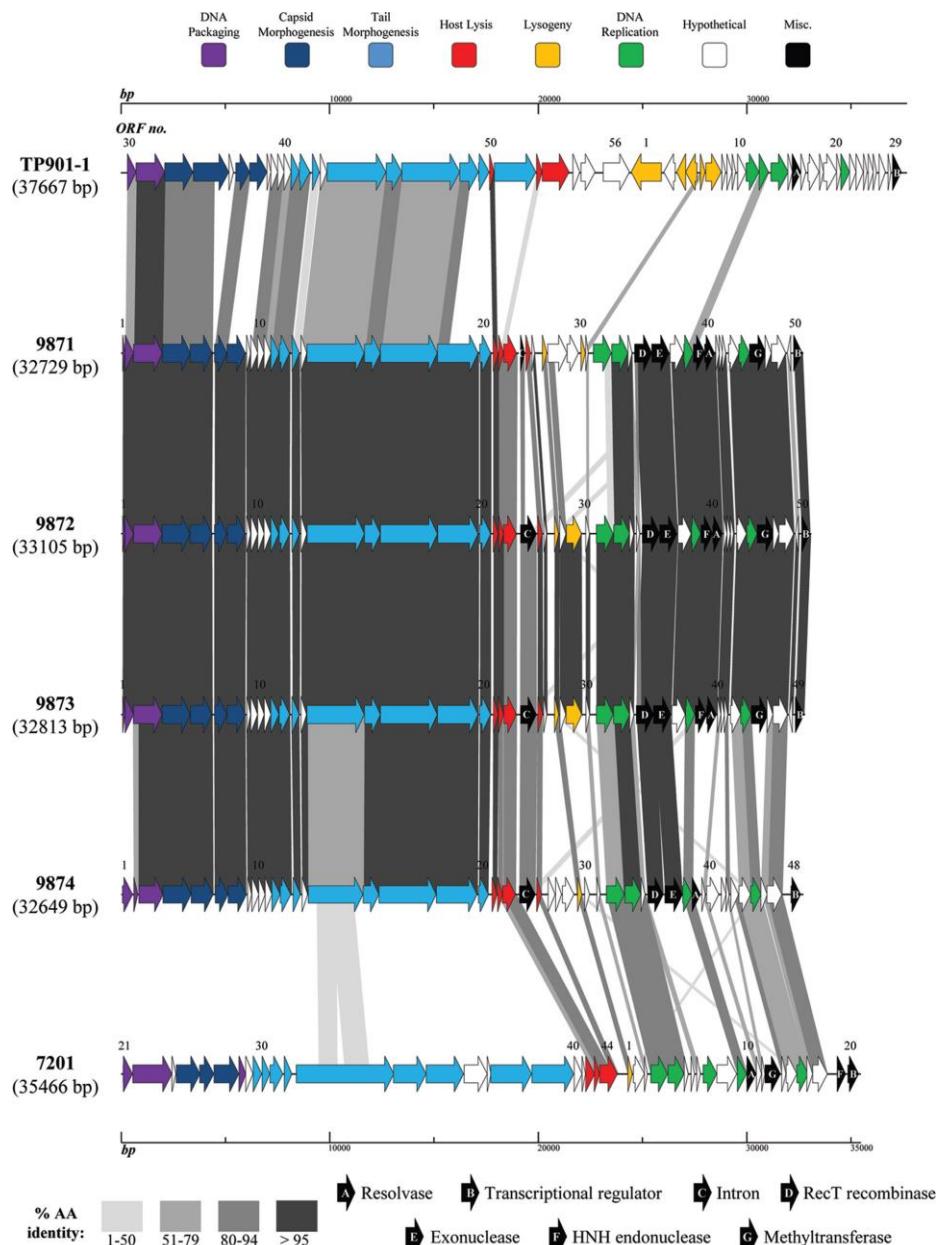


Figure 6: Genomic Organization of Four 987 Phages With Respect To P335 And P7201 (McDonnell et al., 2016).

Arrows: predicted ORFs (their assignments have been indicated with the colored boxes, above). Gene products have been indicated with grey and dark shades (McDonnell et al., 2016).

A fourth type phage assigned for *S. thermophilus* has been 5093, found in a cheese starter, CSK939. Its genome contained 37,184 bp DNA, with a rather dissimilar sequence composition to those of either *cos* or *pac* group. Surprisingly, however, it has shown a significant similarity to the genomes of other streptococci, suggesting that this phage could have a rather large streptococcal host range and that it has experienced substantial interspecies DNA exchange phenomena by HGT or recombination mechanisms (Mills et al., 2011).

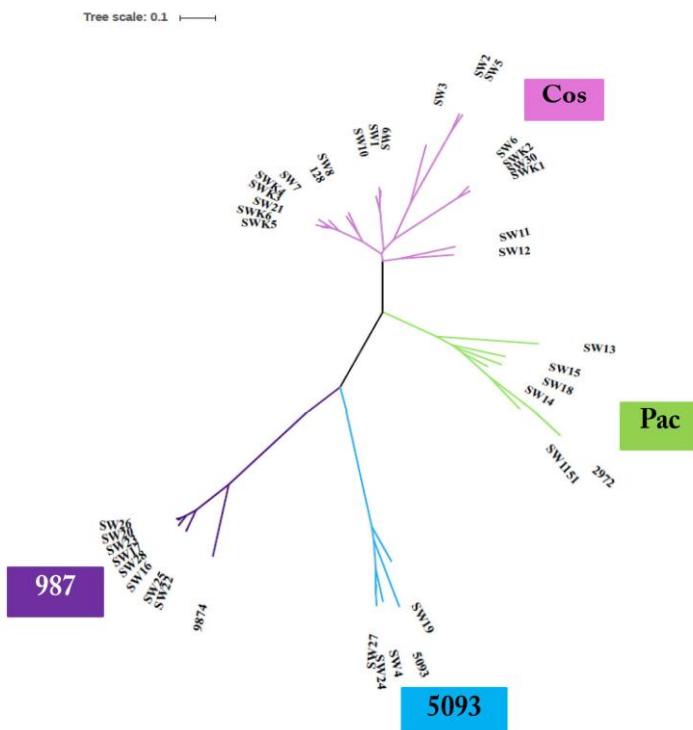
2. PHAGE GENOMIC DIVERSITY

Cos group contains the most comprehensively investigated infectious entities and it has so far been the most recurrent and most diverse group in the dairy industry (Fig. 7). These concordant findings could imply that they hold the longest evolutionary history among the *S. thermophilus* phages. It appears, on the other hand, that such a high diversity in nucleotide sequence has not changed the ordering of the genes (Lavelle et al., 2018).

Pac group also allows a highly significant sequence diversity among its isolated members (Fig. 7). One extremely important feature of this group was that the nucleotide sequence encoding the “the anti-receptor” device displayed very high percentages of dissimilarity. Similarly, gene clusters forming both the modules of lysogeny and

replication seem to have been highly diverged. These findings have also been in concordance with the rather wide host range of the *pac* phages (Lavelle et al., 2018).

Hybrid phage genomes, of 5093 and 987, appear to display lesser diversity from within than those of either *cos* or *pac* phages (Fig. 7). This could be accounted for by the fact that the hybrid phages are much newer entities with respect to the other groups. Nucleotide comparison studies have also revealed that hybrid phages divergence had been generated by the small insertion and deletions (indels) rather than shuffling of the large DNA fragments (Lavelle et al., 2018).



Proteomic data, built in a phylogenetic tree (Fig. 8) at first glance seems to differentiate *cos* group into a single phylogenetic entity and that the three of the remaining four groups were evolutionarily closer to the *cos* proteome than that of the *pac* group (Philippe et al., 2020).

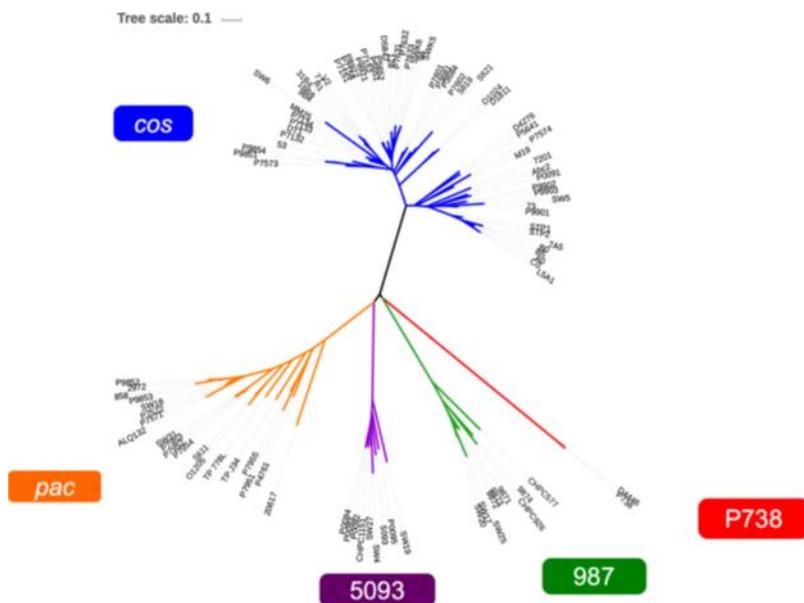


Figure 8: Phylogenetic tree reflecting the proteomic data.

Amino acid comparison studies on the carbohydrate-binding domain of receptor-binding proteins (RBPs) have suggested that these host-specifying devices have evolved independently from the remaining genomic modules in a highly diverse range of phages (Fig. 9). This recalls the possibility to mind that phage modules are not necessarily to have co-evolved. The “independent” evolution could be driven by the gene shuffling mechanism. Hence, phages seem to obtain carbohydrate-binding modules in order to be able to diversify their

host range. Here, the members of *Brussowvirus* and *Moineauvirus* (BVs and MVs, Fig. 10) appear to have served as good donors for the genes of RBP device (Hanemaaijer et al., 2021).

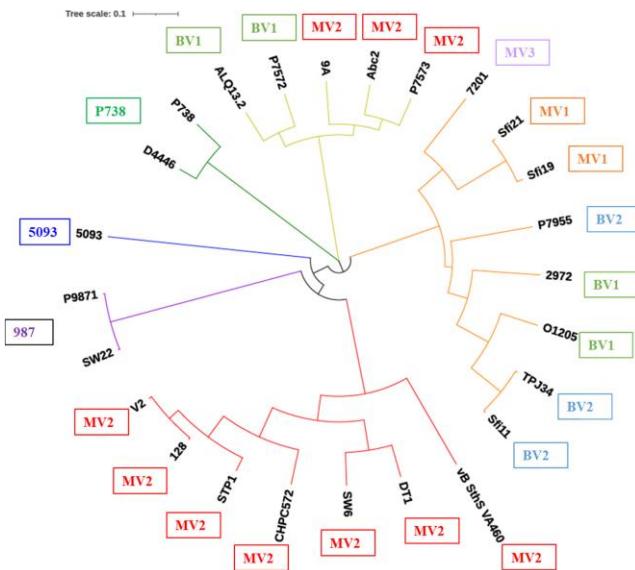
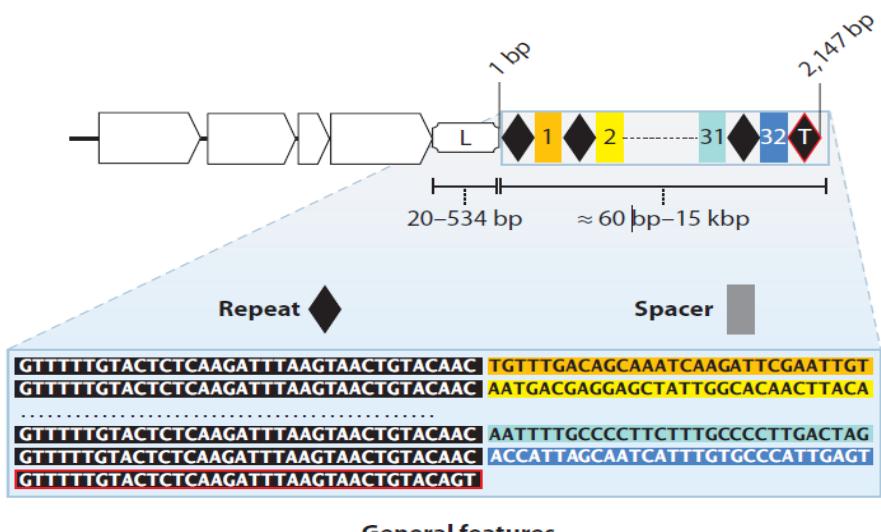


Figure 9: Phylogenetic Tree of Partial RBP Amino Acid Sequence Involved In Carbohydrate Binding.

3. CLUSTERED REGULARLY INTERSPACED SHORT PALINDROMIC REPEATS, CRISPR, OF *S. THERMOPHILUS*

Genome projects have mainly aimed at deciphering the genes and their annotations. Later on, it has become clear that spacer DNA residing between the genes loci has been coding functional molecules, other than proteins, named noncoding RNA (ncRNA). One of these chromosomal elements, having the widest distribution and a very diverse loci organization, has been identified in prokaryotes in 1987 and called clustered regularly interspaced short palindromic repeat CRISPR (Ishino et al., 1987; Deveau et al., 2010).

A CRISPR locus is devoid of open reading frames and includes a fixed range, between 2 and 375, of direct repeats with lengths between 21 and 48 bp. The repeats are interrupted by non-repeating spacers, ranging from 26 to 72 bp (Fig. 10). Approximately one-third of the repeats appear to have been shortened. The final repeat at the rightmost end has often been found to be less preserved with respect to the others. The locus is flanked by an A+T rich leader sequence and a promoter at its upstream end. The CRISPR locus is further specified with some functionally related, 4 to 20 genes within its neighborhood, located up- or downstream, that are named *cas* (CRISPR-associated genes). The genes encode for a series of Cas proteins which form Cas endonucleases. These two entities build together a CRISPR/Cas system. As many as 20 and diverse CRISPRs, making up approximately 1% of the genome and can exist as different loci along the prokaryote chromosome. They can also be mapped in plasmid DNA (Deveau et al., 2010).



General features

Repeats

- 2 to 375 per locus
- Mostly invariable sequence
- 21 to 48 bp

Spacers

- 1 to 374 per locus
- Variable sequence
- 26 to 72 bp

Figure 10: Physical Constitution Of A CRISPR Unit.

CRISPR/Cas loci may constitute a substantial part of the prokaryote immune system against horizontal DNA transfer affected by phage infections and plasmid transformations. This protective system seems to operate via RNA-interference of protein translation (Fig. 11). Palindromic nature of CRISPR enables its transcripts to assume highly firm stem-loop structures, as evidenced for other functional RNA species (Deveau et al., 2010).

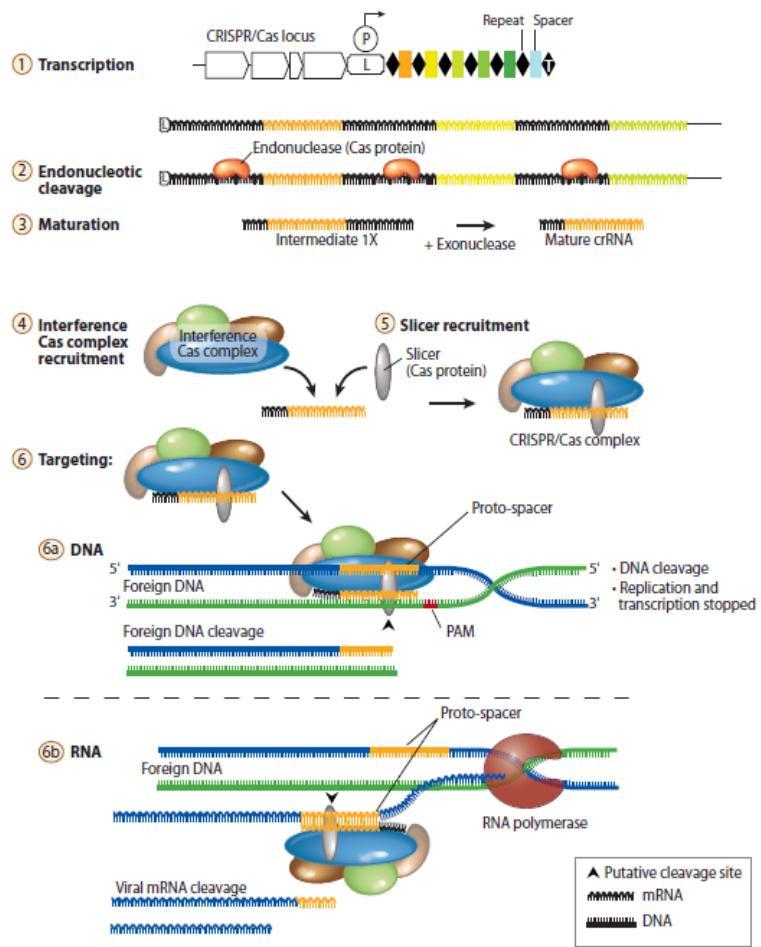


Figure 11: Interference of Translation Through CRISPR RNA.

Unraveling of CRISPR/Cas nucleotide structure and function has prompted some intensive research on *S. thermophilus* to identify or generate bacteriophage-insensitive strains or mutants (BIMs). It has been expected that a “natural”, adaptive, immune strain should have experienced phage infections before its isolation. And it has been evidenced that phage resistance could be made possible by the addition of one single repeat 5' to the CRISPR loci in *S. thermophilus*.

It turns out that a resistant bacterial strain can be infected by a mutant phage. These two findings set a scenario where an interplay might be taking place between the host CRISPRs and phage genome (Fig. 12) (Deveau et al., 2010).

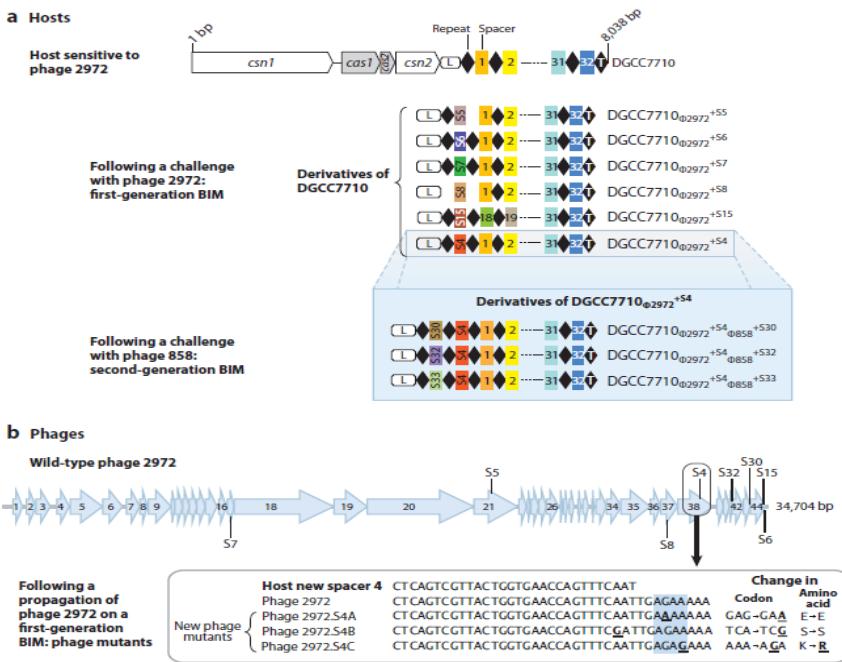


Figure 12: CRISPR Can Mediate A Genetic Interplay Between Dairy *S. Thermophilus* and Its Phages.

Cas1 and Cas2 are expressed in cells harboring CRISPR loci and their abundance appears to be an indicator for the resistance activity of *S. thermophilus* LMD-9 (He et al. 2013; Goh et al. 2011). On the basis of the types of Cas proteins produced, the CRISPR/Cas systems are organized into three subtypes. The presence of Cas3, Cas9, and Cas10, specify the three subtypes I, II, and III, respectively, and they are these are the most frequently found subtypes in *S. thermophilus*. Of the 27

isolates of *S. thermophilus*, more than 96% contained CRISPR/Cas loci (Hu et al., 2020).

Genomic sequencing studies have implied that CRISPR1 (Fig. 13) could be the oldest CRISPR as it has been mapped in 78% of *S. thermophilus* studied and it harboured the highest number of direct repeats (DRs) (Hu et al., 2020).

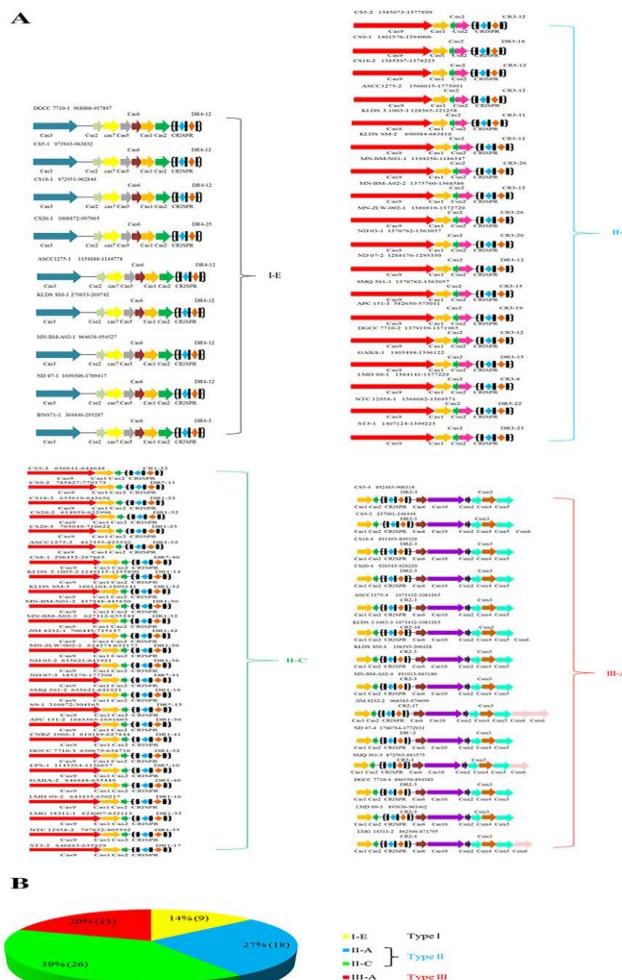


Figure 13: CRISPR Diversity In *S. Thermophilus*.

Seven direct repeats have been mapped in *S. thermophilus* and four of them were most frequently encountered (DR1, DR2, DR3, and DR4), and the remaining three with much fewer frequencies (Fig. 14) (Hu et al., 2020).

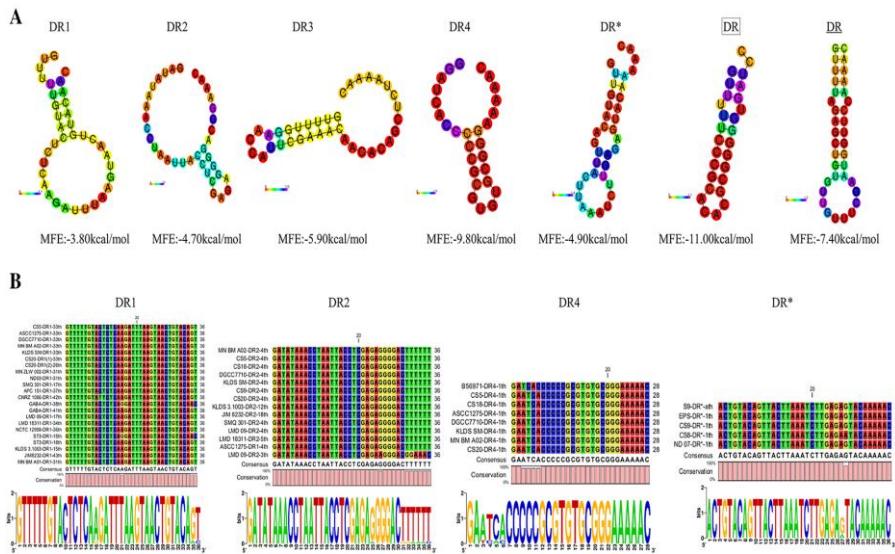


Figure 14: Minimum Free Energy, MFE, Based Secondary Structures of *S. thermophilus* CRISPR RNA.

In the strains of *S. thermophilus*, CNRZ1066, JIM8232, and KLDS 3.1003, the number of spacers differed but their length remained rather similar, between-33 and 35 bp. Again CRISPR1 also appeared to possess the highest number of spacers, while their number was similar in CRISPR3 and CRISPR4 and much lesser in CRISPR2.

Similarity search between CRISPR spacers and available database sequences has revealed that a spacer DNA could be homologous to that of the several *S. thermophilus* phages. The CRISPR-Cas system interferes with phage infections through cutting the phage DNA: upon

invasion by a phage, small pieces of phage DNA are inserted into the host genome as CRISPR spacers. These new spacers could be detected in CRISPR1 and CRISPR3. Then follows transcription of the CRISPR locus and the single-stranded crRNA is produced. This RNA molecule complexes with Cas through its secondary structure, and forms CRISPR nucleoprotein complex (crRNP). The crRNP assumes the role of an endonuclease and is guided by its crRNA to specifically cleave invading phage DNA (Hao et al., 2018).

CONCLUSION

The chapter was prepared with the intention to provide a concise information on the genetics of dairy *S. thermophilus* in relation to its phages. The text was extricated from key references with relevant Figures. In essence, it was tried to make a collection of the key findings on the subject and to reemphasize them together.

It appears that the starter performance of dairy *S. thermophilus* is indispensably dependent on the presence of *Lactobacillus delbrueckii* subsp. *bulgaricus* in the making of yogurt. Evolutionary studies have indicated that the basis of such genetic acquirements is the horizontal gene transfer. Five groups of *S. thermophilus* phages have been established. A *cos* phage, DT1, poses to be the oldest and widest infective agent for dairy *S. thermophilus*. An anti-receptor phage protein, ORF18, has been suggested on the basis of comparative genomic sequence analyses. Such studies have also shed a substantial amount of light as to the physical structures of the phages of dairy *S. thermophilus*. The phage genomes have been found to show

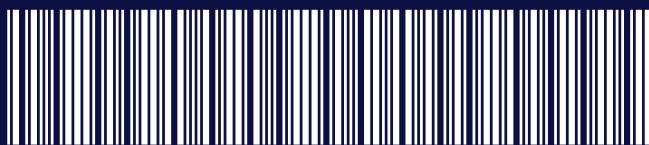
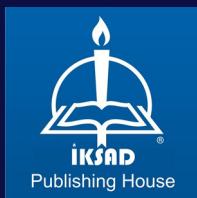
modularity in functional as well as evolutionary senses. Later phage groups seem to possess hybrid genomes that have been established within the confines of dairy industry and involved the infection of both *S. thermophilus* and *Lactococcus lactis*. *Cos* phages form the most diverse and widest group of infectious entities. Nucleotide data have indicated that *cos* and *pac* groups are closer to one another than the other three groups. In the proteomic comparison, on the other hand, *cos* forms one distinct entity, and the remaining four seem to be clustered together in the opposite direction. An even more striking finding has been that the receptor-binding module appeared to have been independent of the remaining three genomic modules in its evolution. Details of the physical features of *S. thermophilus* CRISPR were also reemphasized and text was reinforced with relevant Figures. Here it would be worth reinstating that immune dairy *S. thermophilus* strains could be reinfected with a mutant phage. Three CRISPR subtypes have been suggested for dairy *S. thermophilus* and CRISPR1 appeared to be the oldest. Some secondary structures for CRISPR RNA presented have been generated on the basis of minimum free energy. A schematic representation was also included to show how the CRISPR RNA interfere, through its secondary structure, with the function of translational machinery.

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2	978-605-9885-07-2	2016	مؤسسات العمل الخيري الواقع والمسؤول دراسة تقييمية للمؤسسات في المملكة العربية السعودية	Maha Hamdan Alanazi
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4	978-605-68528-7-9	2018	Экология Зергитеулері: Қазақстан	Медеуова Галия, Садыкова Дамежан, Жайлыбай К.Н., Vd
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	KITAP ADI	YAZAR-EDİTOR	KURUM	KATALOG NUMARASI
1	Tıbbi ve Aromatik Bitkiler Uçucu Yağlı Bitkiler	Komisyon	Tatarstan Academy of Sciences	193479
2	Discussions Between Economic Agents: Individuals, Firms And Countries	Komisyon	Tatarstan Academy of Sciences	193480
3	Klasik Türk Edebiyatı Yazızları (Mecmû'a Tasnîfi, Gazel Şerhi, Küçürek Hikâye, Mesnevî Şerhi Sözlüğü, Münse'ât Mecmû'aları	Prof. Dr. Atabey Kılıç, Öğr. Gör. Mustafa Alkan, vd.	Alisher Navoi Tashkent State University of Uzbek Language and Literature	39836 3836-1
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