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Genomic and phenotypic analyses of *Carnobacterium jeotgali* strain MS3^T, a lactate-producing candidate biopreservative bacterium isolated from salt-fermented shrimp

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One sentence summary: Genomic and phenotypic analyses of *Carnobacterium jeotgali* strain MS3^T revealed promising features that make this bacterium a desirable candidate for exploitation by the fermented food industry.
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ABSTRACT

Carnobacterium jeotgali strain MS3^T was isolated from traditionally fermented Korean shrimp produced with bay salt. The bacterium belongs to the family *Carnobacteriaceae*, produces lactic acid and contains gene clusters involved in the production of lactate, butyrate, aromatic compounds and exopolysaccharides. *Carnobacterium jeotgali* strain MS3^T was characterized through extensive comparison of the virulence potential, genomic relatedness and sequence similarities of its genome with the genomes of other Carnobacteria and lactic acid bacteria. In addition, links between predicted functions of genes and phenotypic characteristics, such as antibiotic resistance and lactate and butyrate production, were extensively evaluated. Genomic and phenotypic analyses of strain MS3^T revealed promising features, including minimal virulence genes and lactate production, which make this bacterium a desirable candidate for exploitation by the fermented food industry.

Keywords: *Carnobacterium jeotgali*; lactic acid bacteria; salt-fermented shrimp; jeotgal; genome sequence

The traditionally fermented Korean food *jeotgal* is made by adding large amounts of bay salt (salt of marine origin) to a diverse range of marine animals, such as shrimp, shellfish and fish, and incubating to allow fermentation, whereupon the *jeotgal* acquires a high nutritional content and unique flavor profile (Cha and Lee 1985; Roh *et al.* 2010). Studies carried out to isolate and characterize the roles of microorganisms during *jeotgal* fermentation resulted in the isolation of a novel lactic acid

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bacterium (LAB), *Carnobacterium jeotgali* strain MS3^T (Kim *et al.* 2009). The genus *Carnobacterium* contains Gram-positive, facultatively anaerobic, psychrotolerant and heterofermentative LABs that produce predominantly L-(+)-lactic acid from glucose (Collins *et al.* 1987).

The use of biological food preservation to minimize the loss of nutritional and organoleptic qualities of perishable food products has attracted attention in recent years (Cortesi *et al.* 2009). LABs are promising candidates for this biopreservation as they are naturally present in a wide range of food products and play pivotal roles in food preservation by producing antimicrobial metabolites such as organic acids, hydrogen peroxide and/or bacteriocins (De Vuyst and Leroy 2007; Galvez *et al.* 2007). In addition to their presence in fish from fresh and saltwater environments, Carnobacteria have been found in preserved seafood products prepared using diverse treatments including vacuum packaging, cold smoking, high-pressure processing, modified-atmosphere packaging (MAP) and chilled MAP. This is in contrast to other organisms, which are rarely found in foods preserved by these methods (Ghanbari and Jami 2013). Accordingly, some species belonging to the *Carnobacterium* genus are of great interest for the biopreservation of meat and fish products (Leisner *et al.* 2007). However, the virulence potential of any target species must be considered prior to its use as a biopreservative culture. For example, *Carnobacterium maltaromaticum* ATCC 35586 was isolated from diseased salmon alongside seafood-associated pathogenic bacteria, but comparative genomic analysis showed that it presented no risk to human health (Leisner *et al.* 2012). Several differences, e.g. chromosomal genome size, were observed between the genomes of *C. maltaromaticum* and other species belonging to the *Carnobacterium* genus (Cailliez-Grimal *et al.* 2013). Here, we report the genome sequence of *C. jeotgali* MS3^T, which was isolated from salt-fermented shrimp prepared with additional marine salt.

Genomic DNA sequencing was performed using an Ion Torrent Personal Genome Machine with a 318 sequencing chip in accordance with the manufacturer's instructions (Rothberg *et al.* 2011). The sequence data consisted of 4 858 745 reads with an average length of 275 bp, which covered a total of 1.3 Gb (representing ~568.8-fold coverage of the genome). Assembly with the CLC Genomics Workbench 6.5 program (CLC Bio, Aarhus, Denmark) yielded 107 large contigs (>1 kb in size; longest contig = 153 kb; mean contig size = 22 kb). Preliminary predictions of 2347 and 2376 coding sequences within the genome were respectively determined with Rapid Annotations using Subsystems Technology (RAST) server 4.0 (Aziz *et al.* 2008) and the Glimmer 3.02 modeling software package (Delcher *et al.* 2007). The unclosed draft genome of *C. jeotgali* MS3^T comprised 2 349 614 bp with a G + C content of 35.2%. The FindtRNA program (http://www.bioinformatics.org/findtrna/) and RNAmmer 1.2 (Lagesen *et al.* 2007) analyses of the genome predicted 53 tRNA genes (11 on the forward strand and 42 on the reverse strand), three 5S rRNA genes, one 23S rRNA gene and one 16S rRNA gene. Data generated by the whole-genome shotgun project were deposited in DDBJ/EMBL/GenBank under accession number JEMH00000000. The genome described in this paper is version JEMH01000000. The 107 large contigs contained in the genome were deposited under accession numbers JEMH01000001–JEMH01000107.

The virulence potential of the *C. jeotgali* MS3^T isolate was assessed by examination of virulence-associated genes within the RAST-annotated genome (Table 1). Among 38 genes categorized as virulence, disease and defense, 1, 24 and 13 genes were predicted to be involved in adhesion, resistance to

Table 1. Summary of subsystems of homologous genes in related other Carnobacteria and lactic acid bacteria to predicted *Carnobacterium jeotgali* MS3^T virulence genes.

Species	Genome size (Mb)	Number of predicted coding sequences	Source	Subsystem feature counts[a] (subcategory)[b]						ANI (%)
				A	B	C	D	E		
								E1	E2	
C. jeotgali MS3^T	2.35	2347	Salt-fermented shrimp	38	97		1	10	5	
Carnobacterium sp. AT7	2.44	2308	Deep seawater from the Aleutian Trench	43	104	7	7	12	5	98.46
C. gilichinskyi WN1359^T	2.35	2154	Siberian permafrost	41	95	5	8		5	83.18
Carnobacterium sp. 17-4	2.64	2446	Permanently cold seawater	52	109	5	8	11	7	83.05
C. maltaromaticum LMA 28	3.65	3473	Ripened soft cheese	93	162	30	8		8	70.93
Lactobacillus brevis KB290	2.4	2344	Traditional Japanese fermented vegetable	58	122	8	6	6	11	64.55
Lactobacillus helveticus DPC 4571	2.08	2,247	Swiss cheese	43	81		5		13	64.10
Lactobacillus oryzae SG293^T	1.86	1885	Fermented rice grains	49	117		1		14	64.62
Lactobacillus paracasei N1115	2.94	2919	Traditional Chinese fermented milk	64	93	6	5	3	12	64.28
Lactobacillus sakei ssp. *sakei* LS25	2.04	1973	Commercial starter for fermented sausage	56	108	1	5		10	66.06
Lactococcus lactis ssp. *lactis* IL1403	2.37	2440	Cheese starter culture	59	107		1		11	65.73
Lactococcus lactis ssp. *cremoris* HP^T	2.27	2403	Cheddar cheese starter culture	57	97		1		8	65.40
Leuconostoc carnosum JB16	1.65	1564	Traditional Korean fermented vegetable	21	71		1		12	64.54
Leuconostoc gelidum JB7	1.89	1818	Traditional Korean fermented vegetable	23	83		1		13	64.39
Weissella koreensis KACC 15510	1.42	1307	Traditional Korean fermented vegetable	36	79		1		7	64.61
Weissella oryzae SG25^T	2.13	2107	Fermented rice grains	31	84	1	1	5	6	64.08
Listeria monocytogenes N53-1	2.78	3491	Fish processing plant	76	151	25	10	6	8	66.22

[a]A, virulence, disease and defense; B, cell wall and capsule; C, iron acquisition and metabolism; D, dormancy and sporulation; E, fermentation.
[b]E1, acetyl-CoA fermentation to butyrate; E2, fermentation: lactate.

antibiotics and toxic compounds, and invasion and intracellular resistance, respectively. The predicted genes involved in resistance to antibiotics contained several genes that putatively encode β-lactamases and other enzymes involved in resistance to fluoroquinolones (e.g. DNA gyrase subunits A and B and topoisomerase IV subunits A and B) or streptothricin (e.g. streptothricin acetyltransferase). We additionally found that the genome of *C. jeotgali* MS3^T contains genes associated with the cell wall and capsule ($n = 97$) and dormancy and sporulation ($n = 1$). To evaluate the links between predicted functions of genes and phenotypic characteristics, we assessed the resistance of *C. jeotgali* MS3^T to several antibiotics including β-lactams (penicillin and ampicillin) and non-β-lactams (chloramphenicol, tetracycline and erythromycin). Disk diffusion tests based on Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI 2013) indicated that *C. jeotgali* MS3^T is susceptible to penicillin (10 units), ampicillin (10 μg) and chloramphenicol (30 μg), but resistant to tetracycline (30 μg) and erythromycin (15 μg). Next, we compared the virulence potential of the *C. jeotgali* MS3^T genome with that of genomes of other Carnobacteria and with publically available LAB genomes, with a focus on LABs involved in food fermentation. The number of putative *C. jeotgali* MS3^T virulence genes ($n = 38$) was comparable with *Carnobacterium* sp. AT7 ($n = 43$), *Carnobacterium gilichinskyi* WN1359^T ($n = 41$) and LABs such as *Lactobacillus helveticus* DPC 4571 ($n = 43$) and *Weissella koreensis* KACC 15510 ($n = 36$), whereas the number of genes was smaller when compared with *C. maltaromaticum* LMA 28 ($n = 93$) or the known food pathogen *Listeria monocytogenes* ($n = 76$). Genes categorized as iron acquisition and metabolism were absent in the genome of *C. jeotgali* MS3^T, but frequently found in other strains belonging to the genus *Carnobacterium*. These results imply that *C. jeotgali* MS3^T may have minimal virulence potential.

The chromosomal genome size varies between Carnobacteria. The *C. jeotgali* MS3^T genome (2.35 Mb) was similar in size to those of *Carnobacterium* sp. AT7, *C. gilichinskyi* WN1359^T and *Carnobacterium* sp. 17-4; however, these four genomes were ~1 Mb smaller than the two sequenced *C. maltaromaticum* genomes (draft genome of strain ATCC 35586 and complete genome of strain LMA 28). Next, we used average nucleotide identity (ANI) analysis, which provides a robust measurement of genomic relatedness between strains (Konstantinidis, Ramette and Tiedje 2006; Kim *et al.* 2014), to evaluate the relatedness between the *C. jeotgali* MS3^T genome and other Carnobacteria genomes. Sequence similarities between the *C. jeotgali* MS3^T genome and other Carnobacteria genomes were variable: the draft genome of *Carnobacterium* sp. AT7 showed 98.46% similarity; the complete genome of *C. gilichinskyi* WN1359^T showed 83.18% similarity; the complete genome of *Carnobacterium* sp. 17-4 showed 83.05% similarity; the draft genome of *C. maltaromaticum* ATCC 35586 showed 70.95% similarity; and the complete genome of *C. maltaromaticum* LMA 28 showed 70.93% similarity. We next compared genomic similarity using whole-genome multiple alignment with consideration of gene gain, loss and rearrangement (progressiveMauve; Darling, Mau and Perna 2010). Alignment results concurred with the ANI analysis and showed that *C. jeotgali* MS3^T was highly similar to *Carnobacterium* sp. AT7, *C. gilichinskyi* WN1359^T and *Carnobacterium* sp. 17-4, but was more distantly related to *C. maltaromaticum* LMA 28 (Fig. 1). These results suggest that *C. jeotgali* MS3^T, along with *Carnobacterium* sp. AT7, *C. gilichinskyi* WN1359^T and *Carnobacterium* sp. 17-4, may adapt to diverse habitats (primarily seawater, Table 1) by loss of genes not necessary for survival.

Further genomic analysis of *C. jeotgali* MS3^T revealed five, seven and five genes predicted to be involved in lactate production, biosynthesis of aromatic compounds and biosynthesis of exopolysaccharides, respectively. As shown in Table 1, all strains belonging to the genus *Carnobacterium* and/or LABs possess 5–14 genes involved in lactate production. The products of these genes may play key roles in food fermentation by accelerating and steering the fermentation process (Leroy and De Vuyst 2004). In this regard, we evaluated the lactate production of *C. jeotgali* MS3^T. The strain was cultured anaerobically in YPG medium and cell-free supernatant was analyzed by gas

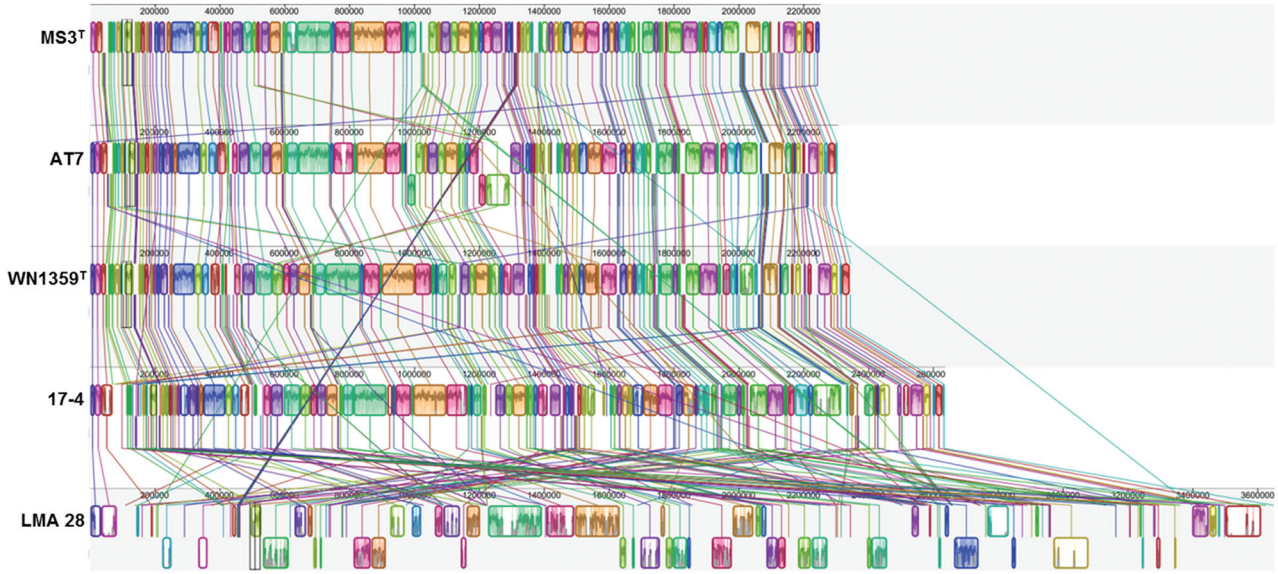

Figure 1. Whole-genome multiple alignments of the *C. jeotgali* MS3^T genome and various Carnobacteria genomes. Contig mapping for gap closure of the draft genome was achieved using Projector 2 based on reference genome sequences (van Hijum *et al.* 2005). After *in silico* scaffolding, alignment of the five genomes was conducted using the Mauve algorithm. Blocks displayed in the same color indicate locally collinear blocks that can be aligned between genomes without rearrangement. MS3^T, *C. jeotgali* MS3^T; AT7, *Carnobacterium* sp. AT7; WN1359^T, *C. gilichinskyi* WN1359^T; 17-4, *Carnobacterium* sp. 17-4; LMA 28, *C. maltaromaticum* LMA 28.

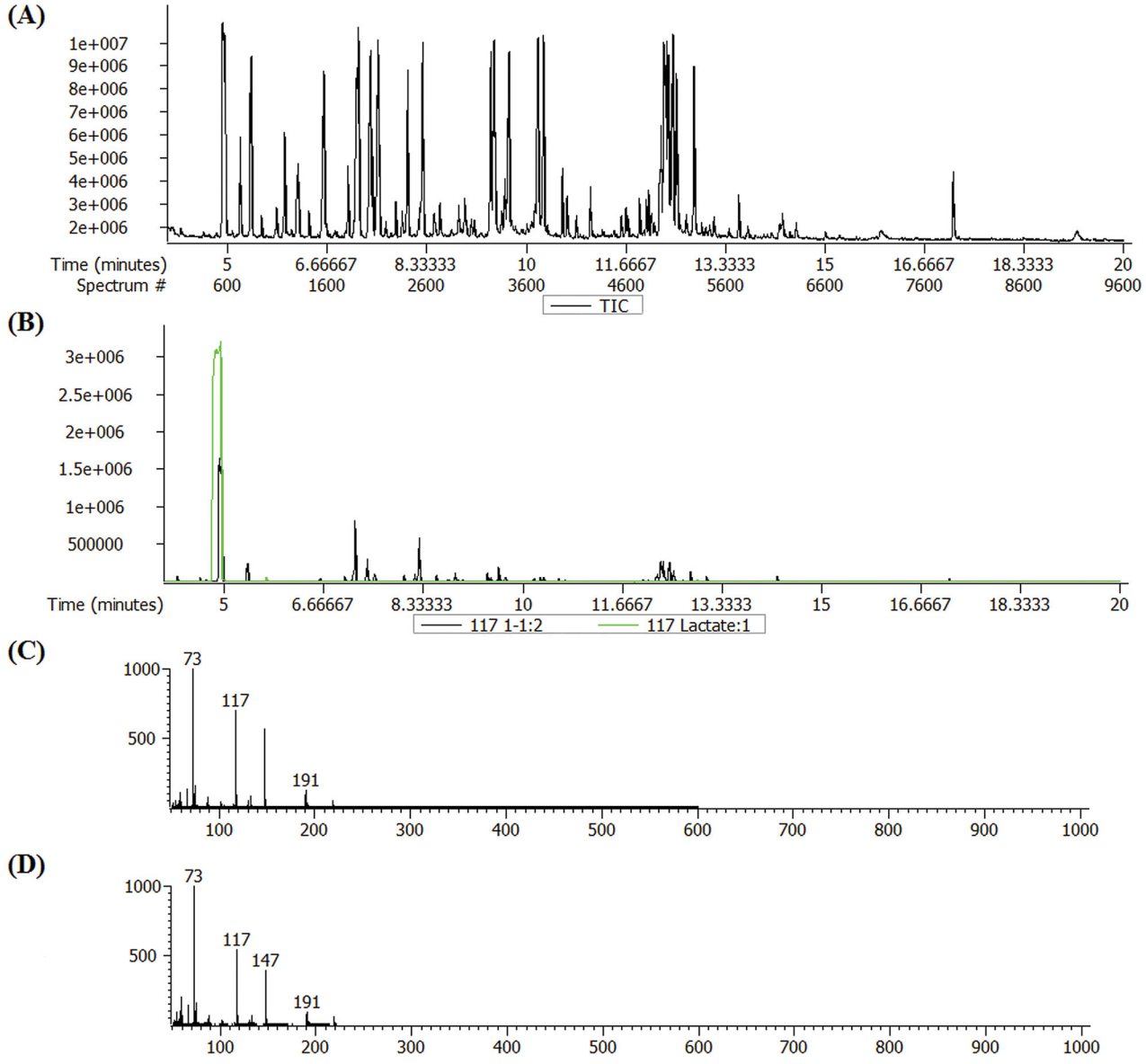

Figure 2. Measurement for lactate production in *C. jeotgali* MS3^T based on gas chromatography time-of-flight mass spectrometry analysis. (**A**) Chromatogram of derivatized sample from cell-free supernatant of the anaerobically cultured strain *C. jeotgali* MS3^T. (**B**) Chromatogram of the derivatized sample overlapped with the lactic acid standard compound in single ion mode (*m/z* 117). (**C**) Spectrum of lactic acid in the derivatized sample. (**D**) Spectrum of lactic acid standard compound.

chromatography time-of-flight mass spectrometry (GC-TOF-MS) for detecting lactic acid (see Supplementary text for detailed procedures of the culture condition and GC-TOF-MS analysis). Both the chromatogram and spectrum of the derivatized bacterial culture medium overlapped with the lactic acid standard compound, indicating that *C. jeotgali* MS3^T is capable of producing lactate as a result of the fermentation process (Fig. 2). In addition, we identified 10 genes predicted to be involved in the fermentation of acetyl-CoA to butyrate within the genome of *C. jeotgali* MS3^T. Butyrate is a product of microbial fermentation and as such is the major energy source for the colonic mucosa. Butyrate is also involved in the regulation of various host cell signaling pathways that act to maintain human gut homeostasis (Hamer *et al.* 2008; Louis and Flint 2009; Furusawa *et al.* 2013). Interestingly, not all strains belonging to the genus *Carnobacterium* carry genes involved in butyrate production, as evidenced by the absence of these genes in the genomes of *C. gilichinskyi* WN1359^T and *C. maltaromaticum* LMA 28. Among the reference LABs used in this study, only a few members, such as *Lactobacillus brevis* KB290, *Lactobacillus paracasei* N1115 and *Weissella oryzae* SG25^T, possess genes capable of producing butyrate. Accordingly, we used GC-TOF-MS analysis as described above to measure the butyrate production of *C. jeotgali* MS3^T. Under our culture conditions, however, we found no evidence of butyrate production by MS3^T, since neither the chromatogram nor the spectrum of the cell-free supernatant of the anaerobically cultured strain overlapped with the butyric acid standard compound (Supplementary Fig. 1).

In summary, the genomic and phenotypic features of *C. jeotgali* MS3^T revealed promising characteristics that would allow

its exploitation by the fermented food industry in product biopreservation and functional food science.

SUPPLEMENTARY DATA

Supplementary data is available at FEMSLE online.

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Conflict of interest. None declared.

REFERENCES

Aziz RK, Bartels D, Best AA, *et al*. The RAST Server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 2008;**9**:75.

Cailliez-Grimal C, Chaillou S, Anba-Mondoloni J, *et al*. Complete chromosome sequence of *Carnobacterium maltaromaticum* LMA 28. *Genome Announc* 2013;**1**:e00115-12.

Cha YJ, Lee EH. Studies on the processing of low salt fermented sea foods. *Bull Korean Fish Soc* 1985;**18**:206–13.

CLSI. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-third Informational Supplement. CLSI document M100-S23*. Wayne, PA: Clinical and Laboratory Standards Institute, 2013.

Collins MD, Farrow JAE, Phillips BA, *et al*. Classification of *Lactobacillus divergens*, *Lactobacillus piscicola*, and some catalase-negative, asporogenous, rod-shaped bacteria from poultry in a new genus, *Carnobacterium*. *Int J Syst Bacteriol* 1987;**37**:310–16.

Cortesi ML, Panebianco A, Giuffrida A, *et al*. Innovations in seafood preservation and storage. *Vet Res Commun* 2009;**33 Suppl 1**:15–23.

Darling AE, Mau B, Perna NT. ProgressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 2010;**5**:e11147.

De Vuyst L, Leroy F. Bacteriocins from lactic acid bacteria: production, purification, and food applications. *J Mol Microbiol Biotechnol* 2007;**13**:194–9.

Delcher AL, Bratke KA, Powers EC, *et al*. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 2007;**23**:673–9.

Furusawa Y, Obata Y, Fukuda S, *et al*. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013;**504**:446–50.

Galvez A, Abriouel H, Lopez RL, *et al*. Bacteriocin-based strategies for food biopreservation. *Int J Food Microbiol* 2007;**120**:51–70.

Ghanbari M, Jami M. Lactic acid their bacteriocins: a promising approach to seafood biopreservation. In: Kongo M (ed). *Lactic Acid Bacteria-R & D for Food, Health and Livestock Purposes* 2013;381–404.

Hamer HM, Jonkers D, Venema K, *et al*. Review article: the role of butyrate on colonic function. *Aliment Pharmacol Ther* 2008;**27**:104–19.

Kim M, Oh HS, Park SC, *et al*. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 2014;**64**:346–51.

Kim MS, Roh SW, Nam YD, *et al*. *Carnobacterium jeotgali* sp. nov., isolated from a Korean traditional fermented food. *Int J Syst Evol Microbiol* 2009;**59**:3168–71.

Konstantinidis KT, Ramette A, Tiedje JM. The bacterial species definition in the genomic era. *Philos Trans R Soc Lond B Biol Sci* 2006;**361**:1929–40.

Lagesen K, Hallin P, Rodland EA, *et al*. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 2007;**35**:3100–8.

Leisner JJ, Laursen BG, Prevost H, *et al*. *Carnobacterium*: positive and negative effects in the environment and in foods. *FEMS Microbiol Rev* 2007;**31**:592–613.

Leisner JJ, Hansen MA, Larsen MH, *et al*. The genome sequence of the lactic acid bacterium, *Carnobacterium maltaromaticum* ATCC 35586 encodes potential virulence factors. *Int J Food Microbiol* 2012;**152**:107–115.

Leroy F, De Vuyst L. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci Technol* 2004;**15**:67–78.

Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett* 2009;**294**:1–8.

Roh SW, Kim KH, Nam YD, *et al*. Investigation of archaeal and bacterial diversity in fermented seafood using barcoded pyrosequencing. *ISME J* 2010;**4**:1–16.

Rothberg JM, Hinz W, Rearick TM, *et al*. An integrated semiconductor device enabling non-optical genome sequencing. *Nature* 2011;**475**:348–52.

van Hijum SA, Zomer AL, Kuipers OP, *et al*. Projector 2: contig mapping for efficient gap-closure of prokaryotic genome sequence assemblies. *Nucleic Acids Res* 2005;**33**:W560–6.