



Phage sensitivity and prophage carriage in *Staphylococcus aureus* isolated from foods in Spain and New Zealand



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ABSTRACT

Bacteriophages (phages) are a promising tool for the biocontrol of pathogenic bacteria, including those contaminating food products and causing infectious diseases. However, the success of phage preparations is limited by the host ranges of their constituent phages. The phage resistance/sensitivity profile of eighty seven *Staphylococcus aureus* strains isolated in Spain and New Zealand from dairy, meat and seafood sources was determined for six phages (Φ 11, K, Φ H5, Φ A72, CAPSa1 and CAPSa3). Most of the *S. aureus* strains were sensitive to phage K (*Myoviridae*) and CAPSa1 (*Siphoviridae*) regardless of their origin. There was a higher sensitivity of New Zealand *S. aureus* strains to phages isolated from both Spain (Φ H5 and Φ A72) and New Zealand (CAPSa1 and CAPSa3). Spanish phages had a higher infectivity on *S. aureus* strains of Spanish dairy origin, while Spanish strains isolated from other environments were more sensitive to New Zealand phages. Lysogeny was more prevalent in Spanish *S. aureus* compared to New Zealand strains. A multiplex PCR reaction, which detected Φ H5 and Φ A72 sequences, indicated a high prevalence of these prophages in Spanish *S. aureus* strains, but were infrequently detected in New Zealand strains. Overall, the correlation between phage resistance and lysogeny in *S. aureus* strains was found to be weak.

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1. Introduction

Staphylococcus aureus is a serious threat to human health, due to its ability to cause a multitude of skin and respiratory infections and foodborne illnesses. It is part of the normal microbiota on human skin and in mucous, and is the main cause of *Staphylococcus* infections in hospitals (Figueiredo and Ferreira, 2014) and food contamination during handling (Wattinger et al., 2012). Its ability to form biofilms can lead to persistent contamination of food processing (Gutiérrez et al., 2012; Herrera et al., 2007; Spanu et al., 2013) and hospital environments (Otto, 2013). Recently, the exponential increase in livestock-associated methicillin-resistant *S. aureus* strains (LA-MRSA), such as clone CC398, have become a concern due to their emergence along the whole farm to fork chain (farm animals, meat product and humans) (Fluit, 2012). Moreover, the emergence of vancomycin resistant *S. aureus* strains (VRSA) narrows the antibiotic arsenal available to treat staphylococcal infections (Weigel et al., 2003).

Phages and phage lytic proteins have been proposed as alternative treatments to reduce food contamination and combat infections caused by pathogenic bacteria (García et al., 2010). Some phage-based products are already available in the market to be used in the food industry. These include Listex™ P100 (www.micreosfoodsafety.com) and ListShield™ (www.intralytix.com) that have been recognized as safe by the US Food and Drug Administration (FDA) and approved by the US Department of Agriculture (USDA) as antimicrobial processing aids to combat *Listeria monocytogenes* in foods, and on food processing surfaces.

One of the key factors for the success of phage-based products is likely to be a sufficiently wide host range to ensure efficacy against the majority, if not all, strains of the pathogen. Due to their specificity for certain receptors on the cell wall, phages typically have relatively a narrow host range, and so to overcome this, the use of phage mixtures is usually preferred (Chan et al., 2013; Hagens and Loessner, 2010). Another factor to be considered in the use of phage biocontrol in foods is lysogeny in the target bacterium. Prophages typically impart immunity to super-infection of related phages to the host cell (Bergruber et al., 2010), and so this could be a potential barrier to the successful use of phage biocontrol. Prophages are very often present in the chromosome of pathogenic bacteria, and the majority of *S. aureus* isolates harbor at least one prophage (Goerke et al., 2009).

Previously, we have characterized the temperate phages Φ A72 and Φ H5 isolated from the dairy environment in Spain, and their lytic

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derivatives, vB_SauS-phiIPLA35 and vB_SauS-phiIPLA88, both belonging to the *Siphoviridae* family (García et al., 2007, 2009a). These phages were able to inhibit *S. aureus* growth in milk, curd and cheese manufacturing processes (Bueno et al., 2012; García et al., 2007, 2009b). More recently, biocontrol candidate phages CAPSa1 and CAPSa3 were isolated from milk samples in New Zealand. They are virulent phages that belong to the *Siphoviridae* family (unpublished).

The present work aims to address the efficacy of biocontrol using phages and hosts from distant geographical areas such as Spain and New Zealand. To do this, we have determined phage sensitivity/resistance profiles in a representative *S. aureus* collection containing strains from both countries. In addition, we have characterized the incidence of lysogeny and the carriage of prophages Φ A72 and Φ H5 and its relationship with bacterial resistance.

2. Material and methods

2.1 Bacterial strains, phages, media and growth conditions

Sixty four *S. aureus* strains from three food environments (dairy, meat and seafood) were isolated in Spain, and 23 strains from dairy and one strain from meat were obtained from New Zealand (Table 1). Staphylococcal cells were isolated on Baird Parker Agar (BP) supplemented with egg yolk, and routinely cultured in TSB broth (Triptone Soy Broth, Scharlau) at 37 °C with shaking or in TSB plates containing 2% (w/v) bacteriological agar (TSA).

Phages were routinely propagated as previously described (García et al., 2007). Phage K (O'Flaherty et al., 2005), Φ 11 (Iandolo et al., 2002), Φ A72 and Φ H5 were propagated in *S. aureus* Sa9, while *S. aureus* NZRM2016 was used as host strain for phages CAPSa1 and CAPSa3. Phage enumeration was performed by the double-layer technique (Gutiérrez et al., 2010) using soft TSA medium (0.7% agar plus 10 mM CaCl₂ and 10 mM MgSO₄) in the upper layer.

2.2 Lysogeny determination

The presence of resident prophages in the *S. aureus* strain collection was determined by mitomycin C induction as previously described (Gutiérrez et al., 2010). Briefly, mid-exponential-phase cultures were treated with 0.5 µg/ml of mitomycin C (Sigma-Aldrich, St. Louis, MO) for three hours at 37 °C and shaking. Supernatants were filtered and spotted into agar overlaid lawns of all the *S. aureus* strains.

2.3 Phage host range

The host range of each phage was obtained against a collection of *S. aureus* strains by spotting 5 µl (10⁹ pfu/ml) of the phage suspension

into the lawn of each strain using the double-layer technique. Efficiency of plating (EOP) was calculated using *S. aureus* Sa9 as the reference strain (Gutiérrez et al., 2010).

2.4 Multiplex PCR

The genomic nucleotide sequences of Φ A72 and Φ H5 (García et al., 2009a) were subjected to progressive MAUVE alignment, using the default settings (<http://gel.ahabs.wisc.edu/mauve/>). Regions with no homology were analyzed to design specific primers for each phage, and these primers were submitted to *in silico* PCR amplification (<http://insilico.ehu.es/PCR/>) to verify their specificity. For Φ A72, one pair of primers was designed surrounding the *orf2* (522 bp, from nucleotide 1350 to 1872) and another pair in the region corresponding to a methyl transferase, *orf22* (324 bp, from 11,331 to 11,655). For Φ H5, pairs of oligonucleotides were designed in *orf29* (704 bp, from 151 to 855) and in the integrase region, *orf1* (225 bp, from 13,013 to 13,238). Total DNA was extracted by GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, Madrid, Spain), according to the manufacturer's instructions. PCR reactions were performed with PureTaq Ready-To-Go™ PCR Beads (GE Healthcare, Munich, Germany), 10 ng of DNA and 1 µM of each primer. PCR reactions were based on phage Φ A72 (GenBank NC_011612) using four primers (gp2F: 5'GATAATTACAACCTGGGATACC3'; gp2R: 5'GTATTCAGACAATGTTTTGAAG3'; metrF 5'ATAGAATGCAACATTCACC3'; metrR 5'GATAACAACCATTCTGGTAC3') and the other based on Φ H5 (GenBank NC_011614) (int88F: 5'ATCATTGTGTAATAGATAAGAGC3'; int88R: 5'GTTATTACAGATAAAGCTTATGC3'; gp29F: 5'CATGATGTAAGAGACCATC3'; gp29R: 5'CTACTGCGTCATTTAAATTC3'). As positive control, pure phage DNA from Φ A72 and Φ H5 was used. PCR was performed in a thermocycler (Bio-Rad, Hercules, USA) under the following thermal cycling conditions: one cycle at 95 °C for 5 min; 35 cycles at 95 °C for 1 min, 54 °C for 1 min and 72 °C for 1 min; and a final step of 10 min at 75 °C.

2.5 Statistical analyses

Statistical analyses were performed using R (R Core Team, 2013). The unpaired *t*-test and Chi-square test was conducted to compare the sensitivity/resistance of staphylococcal strains isolated in Spain and New Zealand to six phages. The Chi-square test was used to compare the sensitivity/resistance of Spanish staphylococcal strains isolated from different food environments (dairy, meat, seafood) to the same phages. A significance level of 0.05 was chosen for these purposes. Fisher's exact two tailed test was run using R (R Core Team, 2013), where the null hypothesis was that phage sensitivity and lysogeny are independent. McNemar's Chi-squared test with continuity correction was also run on R (R Core Team, 2013), with the null hypothesis being the proportion

Table 1
Strains used in this study, geographical isolation and origin.

Country	Food Industry	Origin	<i>S. aureus</i> strain	Reference
Spain	Dairy 1	Milk	Sa1, Sa2, Sa3, Sa4, Sa5, Sa6, Sa8, Sa9, Sa10, Sa11, Sa12, Sa13, Sa14, Sa15, Sa16	García et al., 2009b
	Dairy 2	Milk	AAAC9, AAAC10, AAAC11, AFG1, AFG2, GDC3, GDC6, GDC9, GRA16, GRA17, GRA20, JFL2, JFL4, JFL6, JFL8	
	Dairy 3	Milk	IPLA19, IPLA20, IPLA24	Unpublished Gutiérrez et al., 2012
	Dairy 4	Food-contact surfaces	IPLA1, IPLA3	
	Meat 1	Food-contact surfaces	IPLA5, IPLA6, IPLA7, IPLA8, IPLA13, IPLA14, IPLA15, IPLA16, IPLA17, IPLA18	
	Seafood	Food-contact surfaces	IIM201, IIM208, IIM214, IIM222, IIM228, IIM229, IIM233, IIM234, IIM235, IIM237, IIM238, IIM239, IIM240, IIM241, IIM242, IIM245, IIM246, IIM249	
	New Zealand	Dairy 5	Raw milk	PHCFAP1, PHCFAP2, PHCFAP3, S34, S36, S38, S39, S41, S43, S45, S46, S47
Dairy 6		Cheese	S52, S51, FM31, FM34	
Dairy 7		Milk powder	S100, S12	
		Skimmed milk powder	S27, S28	
		Dairy product	S14	
		Cream pie	NZRM3372	
Meat 2		Ham	NZRM3374A	
Bovine	–	NZRM2016		

of hosts sensitive is the same as the proportion of hosts lysogenic, i.e. concordance between the two.

3. Results and discussion

The recent interest in the use of phages as biocontrol agents of pathogenic bacteria in food (García et al., 2010; Sillankorva et al., 2012) raises the question of their efficacy in a globalized market. The use of next generation sequencing technology has profoundly improved our understanding of the dynamic evolutionary processes, transmission and prevalence of staphylococcal clonal lineages in different geographical locations (Castillo-Ramírez et al., 2012; Parkhill and Wren, 2011). However, data on phage susceptibility of this species, and others, is scarce (Argudín et al., 2012).

A collection of *S. aureus* strains composed by isolates from distant locations, Spain (SP) and New Zealand (NZ), was gathered. The Spanish collection encompassed sixty three strains isolated from food-contact surfaces from dairy, meat and seafood factories (Gutiérrez et al., 2012) and also from milk and dairy products (García et al., 2007) (Table 1). These strains had been previously characterized as described in the above references and were chosen on the basis of their distinct phenotypic properties (antibiotic resistance, biofilm as well as genetic diversity based on their RAPD-PCR profiles and presence/absence of endotoxin and biofilm genes. The New Zealand collection was composed by twenty three *S. aureus* strains from dairy samples and one from meat. The genetic relatedness within these strains is not known but they have been gathered from different sources and locations. These collections were used to test the susceptibility to the well characterized lytic phage K, temperate phage Φ 11, phages Φ A72 and Φ H5 isolated in Spain, and phages CAPSa1 and CAPSa3 isolated in New Zealand. The spot-on-the-lawn technique was chosen as a quick and easy screening tool to test all the phages on the same host plates together. Furthermore, to avoid overestimating of the host range due to lysis-from-without, appropriate dilutions were also spotted to confirm the presence of lytic plaques when deemed necessary and the efficiency of plating was calculated using as reference strain *S. aureus* Sa9, susceptible to all tested phages (Supplementary Table 1).

3.1 Infectivity of *S. aureus* with respect to geographic origin of the phage and host

As expected from previous reports, phage K was the phage with the broadest host range showing lysis on all but 4 SP and 1 NZ strains. Phage K is a lytic phage representative of the *Myoviridae* family, infecting staphylococcal strains from human and bovine origin (O'Flaherty et al., 2005), and our results further confirm the polyvalent nature of this phage.

Φ 11 was chosen as a representative of temperate phages belonging to *Siphoviridae* family (Iandolo et al., 2002). This phage displayed a narrow host range and lysed 29% of NZ strains compared to 48% of the SP *S. aureus*, respectively (Fig. 1). Nevertheless, this difference was not statistically significant ($P > 0.05$).

On the contrary, the geographical origin of the *S. aureus* host significantly ($P < 0.01$) determined the infectivity of the Spanish phages Φ A72 and Φ H5. Less than one third of the SP strains were infected, whereas these phages infected >90% of *S. aureus* strains isolated in New Zealand (Fig. 1).

For the phages isolated in New Zealand, CAPSa1 had a broad host range, infecting 62% of SP strains and 71% of NZ strains. By contrast, CAPSa3 preferentially infected NZ over SP strains ($P < 0.01$) (Fig. 1). Overall, NZ *S. aureus* strains were more susceptible to all the phages than SP strains (Fig. 1).

Taken together these results indicate no clear association of infectivity of phages against *S. aureus* strains based on the geographic region of host or phage isolation. These data contrast with those obtained from studies in ecosystems like soil, where phages appear to adapt to become

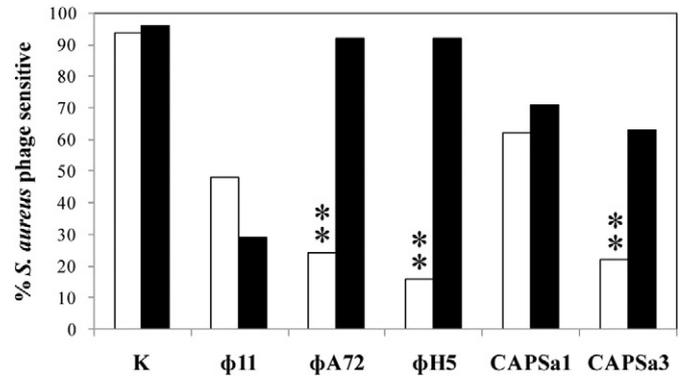


Fig. 1. Phage sensitivity of *S. aureus* isolated from Spain (white bars, n = 63) and New Zealand (black bars, n = 23) and. **, $P < 0.01$.

more infective against bacteria living closely (Vos et al., 2009). A possible explanation for the observed differences may be the globalization of the food industry and the increased frequency of international travel for animals and humans, which are key vectors of *S. aureus*. Therefore, the future design of phage preparations for international markets should consider the potential for both local micro- and global macro-scale interactions of phages and hosts.

3.2 Infectivity of *S. aureus* with respect to environmental origin of the phage and host

Phages Φ A72 and Φ H5 are derivatives of phages isolated from milk, and were previously determined to preferentially infect *S. aureus* strains from dairy compared to clinical origin (García et al., 2007). This observation suggested that the environmental source of hosts, and potentially of phages, may be relevant in the preparation of a phage biocontrol for foods. So we wished to test this further by expanding the panel of strains and phages, including the NZ phages CAPSa1 and CAPSa3 which were also isolated from milk, Φ 11, a temperate phage from a clinical *S. aureus* isolate (Novick, 1963), and phage K, the origin of which is unclear (Burnet and Lush, 1935).

Only SP hosts were tested in this series of experiments as there were only two non-dairy host isolates available from NZ (Table 1). For phage K, Φ 11 and Φ H5, the source of the host was not a significant factor for phage infectivity (Fig. 2). Whereas, for the other three phages Φ A72 ($P < 0.05$), CAPSa1 ($P < 0.01$) and CAPSa3 ($P < 0.05$) there were significant differences in infectivity with hosts from dairy meat and seafood (Fig. 2). Φ A72 infected mostly dairy strains (40%), with no infection of seafood strains. CAPSa1 infected all meat and seafood strains, but fewer (31%) dairy strains. CAPSa3 was most active on strains from meat (60%), than either dairy (17%) or seafood (11%) (Fig. 2). These

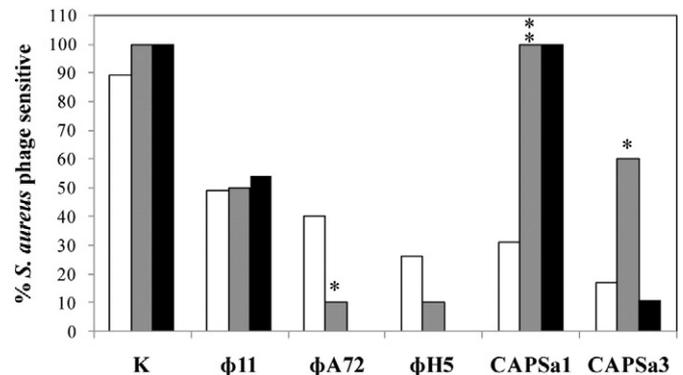


Fig. 2. Phage sensitivity of *S. aureus* isolated from different food environments. Dairy (white columns, n = 35), meat (grey columns, n = 10) and seafood (black columns, n = 18). *, $P < 0.05$; **, $P < 0.01$.

Table 2

Lysogeny and detection of Φ H5- and Φ A72-like sequences by multiplex PCR in *S. aureus* isolated from distantly located regions and environments.

<i>S. aureus</i> (no. strains tested)	Lysogeny ^a	No. strains positive					
		Prophage carriage					
		Φ A72			Φ H5		
		<i>orf2</i>	<i>orf22</i>	both	<i>orf1</i>	<i>orf29</i>	both
Spain							
Dairy 1 (15)	15	0	6	0	1	8	0
Dairy 2 (15)	15	1	6	1	11	0	1
Dairy 3 (3)	3	0	0	2	0	1	1
Dairy 4 (2)	2	0	1	0	0	0	1
Meat 1 (10)	3	0	1	4	0	7	2
Seafood (18)	12	1	1	13	0	11	3
Presence (%)		3	24	32	19	43	11
New Zealand							
Dairy 5 (12)	3	0	1	0	0	0	0
Dairy 6 (4)	1	1	0	0	0	0	0
Dairy 7 (6)	0	1	0	0	0	0	0
Meat 2 (1)	0	0	0	0	0	0	0
Bovine (1)	0	0	0	0	0	0	0
Presence (%)		8	4	0	0	0	0
Total (%)		5	18	23	14	31	9

^a Lysogeny determined by induction with Mitomycin C

results highlight the need of developing specific phage preparations tailored to the food, and food-specific strains, in which they are to be applied.

3.3 Inducible prophages in *S. aureus* strains

The presence of prophages integrated in the bacterial chromosome confers immunity to superinfection and thus resistance to phage attack by closely related phages (Berngruber et al., 2010). Microarray studies have shown that prophages integrated in the bacterial chromosomes are the most widespread mobile genetic elements in *S. aureus* strains, with most of them carrying between one and four prophages (Goerke et al., 2009; Pantucek et al., 2004). However, there is scarce data about the prevalence and diversity of phage populations in specific geographical areas (Rahimi et al., 2012). Some prophages have been determined to be specific for MRSA lineages and are linked to geographical variants, such as the ϕ SP β -like prophage which is characteristic of the ST239 'Asian clade' (Wang et al., 2014).

Mitomycin C induction of our *S. aureus* collection was performed and the presence of phages tested in the culture supernatants. Results showed that only four (16.7%) of the NZ strains were lysogenic for at least one phage, as the supernatants from the induced cultures produced plaques on several strains (Table 2). By contrast, the presence of prophages was detected in 78% of Spanish strains, (note data for some SP strains previously described by Gutiérrez et al. (2012) and García et al. (2007)). It is noteworthy, that lysogeny seems to be more widespread in dairy environment, with 97% of dairy strains positive

for lysogeny, compared to the meat (30% strains) and seafood (67% strains) environments.

Fisher's Tests suggested that the association between *S. aureus* lysogeny and phage sensitivity was not significant, with the exception of CAPSa1 with SP hosts ($P < 0.001$). Similarly, McNemar's Test for dependence between phage sensitivity and lysogeny was rejected for all the phages ($\alpha = 0.05$), with the exception of Φ 11 in both SP and NZ strains, and Φ CAPSa1 in SP strains. Overall, the evidence for an association between inducible prophages in the host and phage resistance is weak.

3.4 Prevalence of prophages Φ H5 and Φ A72 in geographically distant *S. aureus* strains

The prevalence of prophages Φ H5 and Φ A72 in SP and NZ *S. aureus* strains was determined by multiplex PCR. Two primer pairs were designed for each phage as described in the methods and the PCR results for each strain are compiled in Supplementary Table 1. Within all strains, at least one Φ A72-like sequence was detected in 41% of strains, and 40% of the strains harbored a Φ H5-like sequence (Table 2). Both Φ A72-like sequences were detected in 23% of the strains; while only eight strains had both Φ H5-like sequences (Table 2). These results likely reflect the mosaic nature of *S. aureus* phage genomes (Kahankova et al., 2010).

Interestingly, phages Φ A72 and Φ H5 seem to be widely spread among SP strains (32% had both Φ A72-like sequences, and 11% both Φ H5-like sequences), but they were rare in NZ strains (Table 2). No Φ H5-like sequences were detected in the NZ strains, and only three strains were positive for Φ A72 sequences. The absence of Φ A72 and Φ H5 related prophages on NZ strains could explain their higher sensitivity to these phages. Similarly, the prevalence of Φ A72-like prophages among the SP seafood strains might also contribute to the high frequency of strains resistant to this phage (100%).

4. Conclusion

The sensitivity of wild *S. aureus* strains to phage infection was not determined by the geographical origin of the strains. However, there appeared to be an association between phage infectivity and the environment in which the strains were isolated for some phages, but not all. The implications for biocontrol are that it may be more important to isolate and use phages with a naturally broad host range, than to isolate phages from the same region or environment as the target microorganism. While the prevalence of lysogeny appeared to differ between two distant geographic locations, there was no strong correlation between resistance of these strains to phage infection and the presence of prophages.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ijfoodmicro.2016.04.019>.

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References

- Argudín, M.A., Mendoza, M.C., González-Hevia, M.A., Bances, M., Guerra, B., Rodicio, M.R., 2012. Genotypes, exotoxin gene content, and antimicrobial resistance of *Staphylococcus aureus* strains recovered from foods and food handlers. *Appl. Environ. Microbiol.* 78, 2930–2935.

- Berngruber, T.W., Weissing, F.J., Gandon, S., 2010. Inhibition of superinfection and the evolution of viral latency. *J. Virol.* 84, 10200–10208.
- Bueno, E., García, P., Martínez, B., Rodríguez, A., 2012. Phage inactivation of *Staphylococcus aureus* in fresh and hard-type cheeses. *Int. J. Food Microbiol.* 158, 23–27.
- Burnet, F.M., Lush, D., 1935. The staphylococcal bacteriophages. *J. Pathol. Bacteriol.* 40, 455–469.
- Castillo-Ramírez, S., Corander, J., Marttinen, P., Aldeljawi, M., Hanage, W.P., Westh, H., Boye, K., Gulay, Z., Bentley, S.D., Parkhill, J., Holden, M.T., Feil, E.J., 2012. Phylogeographic variation in recombination rates within a global clone of methicillin-resistant *Staphylococcus aureus*. *Genome Biol.* 13, R126.
- Chan, B.K., Abedon, S.T., Loc-Carrillo, C., 2013. Phage cocktails and the future of phage therapy. *Future Microbiol.* 8, 769–783.
- Core Team, R., 2013. R: A Language and Environment for Statistical Computing. Foundation for Statistical Computing, Vienna, Austria.
- Figueiredo, A.M., Ferreira, F.A., 2014. The multifaceted resources and microevolution of the successful human and animal pathogen methicillin-resistant *Staphylococcus aureus*. *Mem. Inst. Oswaldo Cruz* 109, 265–278.
- Fluit, A.C., 2012. Livestock-associated *Staphylococcus aureus*. *Clin. Microbiol. Infect.* 18, 735–744.
- García, P., Madera, C., Martínez, B., Rodríguez, A., 2007. Biocontrol of *Staphylococcus aureus* in curd manufacturing processes using bacteriophages. *Int. Dairy J.* 17, 7.
- García, P., Martínez, B., Obeso, J.M., Lavigne, R., Lurz, R., Rodríguez, A., 2009a. Functional genomic analysis of two *Staphylococcus aureus* phages isolated from the dairy environment. *Appl. Environ. Microbiol.* 75, 7663–7673.
- García, P., Madera, C., Martínez, B., Rodríguez, A., Suarez, J.E., 2009b. Prevalence of bacteriophages infecting *Staphylococcus aureus* in dairy samples and their potential as biocontrol agents. *J. Dairy Sci.* 92, 3019–3026.
- García, P., Rodríguez, L., Rodríguez, A., Martínez, B., 2010. Food biopreservation: promising strategies using bacteriocins, bacteriophages and endolysins. *Trends Food Sci. Technol.* 21, 373–382.
- Goerke, C., Pantucek, R., Holtfreter, S., Schulte, B., Zink, M., Grumann, D., Broker, B.M., Doskar, J., Wolz, C., 2009. Diversity of prophages in dominant *Staphylococcus aureus* clonal lineages. *J. Bacteriol.* 191, 3462–3468.
- Gutiérrez, D., Martínez, B., Rodríguez, A., García, P., 2010. Isolation and characterization of bacteriophages infecting *Staphylococcus epidermidis*. *Curr. Microbiol.* 61, 601–608.
- Gutiérrez, D., Delgado, S., Vázquez-Sánchez, D., Martínez, B., Cabo, M.L., Rodríguez, A., Herrera, J.J., García, P., 2012. Incidence of *Staphylococcus aureus* and analysis of associated bacterial communities on food industry surfaces. *Appl. Environ. Microbiol.* 78, 8547–8554.
- Hagens, S., Loessner, M.J., 2010. Bacteriophage for biocontrol of foodborne pathogens: calculations and considerations. *Curr. Pharm. Biotechnol.* 11, 58–68.
- Herrera, J.J., Cabo, M.L., González, A., Pazos, I., Pastoriza, L., 2007. Adhesion and detachment kinetics of several strains of *Staphylococcus aureus* subsp. *aureus* under three different experimental conditions. *Food Microbiol.* 24, 585–591.
- Iandolo, J.J., Worrell, V., Groicher, K.H., Qian, Y., Tian, R., Kenton, S., Dorman, A., Ji, H., Lin, S., Loh, P., Qi, S., Zhu, H., Roe, B.A., 2002. Comparative analysis of the genomes of the temperate bacteriophages phi 11, phi 12 and phi 13 of *Staphylococcus aureus* 8325. *Gene* 289, 109–118.
- Kahankova, J., Pantucek, R., Goerke, C., Ruzickova, V., Holochova, P., Doskar, J., 2010. Multilocus PCR typing strategy for differentiation of *Staphylococcus aureus* siphoviruses reflecting their modular genome structure. *Environ. Microbiol.* 12, 2527–2538.
- Novick, R.P., 1963. Analysis by transduction of mutations affecting penicillinase formation in *Staphylococcus aureus*. *J. Gen. Microbiol.* 33, 121–136.
- O'Flaherty, S., Ross, R.P., Flynn, J., Meaney, W.J., Fitzgerald, G.F., Coffey, A., 2005. Isolation and characterization of two anti-staphylococcal bacteriophages specific for pathogenic *Staphylococcus aureus* associated with bovine infections. *Lett. Appl. Microbiol.* 41, 482–486.
- Otto, M., 2013. Staphylococcal infections: mechanisms of biofilm maturation and detachment as critical determinants of pathogenicity. *Annu. Rev. Med.* 64, 175–188.
- Pantucek, R., Doskar, J., Ruzickova, V., Kaspárek, P., Oravica, E., Kvardova, V., Rosypal, S., 2004. Identification of bacteriophage types and their carriage in *Staphylococcus aureus*. *Arch. Virol.* 149, 1689–1703.
- Parkhill, J., Wren, B.W., 2011. Bacterial epidemiology and biology—lessons from genome sequencing. *Genome Biol.* 12, 230.
- Rahimi, F., Bouzari, M., Katouli, M., Pourshafie, M.R., 2012. Prophage and antibiotic resistance profiles of methicillin-resistant *Staphylococcus aureus* strains in Iran. *Arch. Virol.* 157, 1807–1811.
- Sillankorva, S.M., Oliveira, H., Azeredo, J., 2012. Bacteriophages and their role in food safety. *Int. J. Microbiol.* 2012, 863945.
- Spanu, V., Scarano, C., Virdis, S., Melito, S., Spanu, C., De Santis, E.P., 2013. Population structure of *Staphylococcus aureus* isolated from bulk tank goat's milk. *Foodborne Pathog. Dis.* 10, 310–315.
- Vos, M., Birkett, P.J., Birch, E., Griffiths, R.I., Buckling, A., 2009. Local adaptation of bacteriophages to their bacterial hosts in soil. *Science* 325, 833.
- Wang, Z., Zhou, H., Wang, H., Chen, H., Leung, K.K., Tsui, S., Ip, M., 2014. Comparative genomics of methicillin-resistant *Staphylococcus aureus* ST239: distinct geographical variants in Beijing and Hong Kong. *BMC Genomics* 15, 529.
- Wattinger, L., Stephan, R., Layer, F., Johler, S., 2012. Comparison of *Staphylococcus aureus* isolates associated with food intoxication with isolates from human nasal carriers and human infections. *Eur. J. Clin. Microbiol. Infect. Dis.* 31, 455–464.
- Weigel, L.M., Clewell, D.B., Gill, S.R., Clark, N.C., McDougal, L.K., Flannagan, S.E., Kolonay, J.F., Shetty, J., Killgore, G.E., Tenover, F.C., 2003. Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. *Science* 302, 1569–1571.