Microbiol. Biotechnol. Lett. (2020), 48(3), 322–327
http://dx.doi.org/10.4014/mbl.2002.02017
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

Identification of a Prophage-encoded Abortive Infection System in *Levilactobacillus brevis*

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Received: February 25, 2020 / Revised: April 10, 2020 / Accepted: April 24, 2020

Abortive infection systems (Abi) are phage resistance systems that can be prophage-encoded. Here, two genes encoding an Abi system were identified on a prophage sequence contained by the chromosome of the *Levilactobacillus brevis* strain UCCLBBS124. This Abi system is similar to the two-component AbiL system encoded by *Lactococcus lactis* biovar. *diacetylactis* LD10-1. The UCCLBBS124 prophage-derived Abi system (designated here as AbiL$_{124}$) was shown to exhibit specific activity against phages infecting *L. brevis* and *L. lactis* strains. Expression of the AbiL$_{124}$ system was shown to cause reduction in the efficiency of plaquing and cell lysis delay for phages of both species.

Keywords: **Abi system, bacteriophage, lactic acid bacteria, beer spoilage**

Lactobacillus brevis, recently reclassified as *Levilactobacillus brevis* REFERENCE: PMID: 32293557, belongs to the lactic acid bacteria (LAB), food and fermentation industry [1]; however, it is also associated with the spoilage of beer. There is an ever-increasing consumer demand for natural food preservation methods, and in this context, bacteriophages possess the potential to control such spoilage microorganisms [2, 3]. Virulent phages active against *L. brevis* have been isolated and characterized, revealing a narrow host range [4, 5] and suggesting the presence of resistance mechanisms against these bacteriophages. Various naturally occurring, phage-derived defence systems against LAB phages have been identified, including abortive infection (Abi) systems [6–9]. Abi systems block phage multiplication leading to the release of few (if any) infective virions and cause death of infected cells, thereby protecting the overall bacterial population [10]. More specifically, Abi systems interfere with phage development following phage adsorption and DNA injection into the host, resulting in an absence of plaques or a reduction in plaque size coupled with significant cell death [11–14]. Many Abi systems are encoded by a single gene, though two-component Abi systems have also been identified [8]. A notable feature among Abi systems is the high A+T content (usually ~70%) of the genes encoding these systems [15]. Studies focused on phage-resistance systems in lactobacilli are very limited and just a single, plasmid-encoded phage-resistance system has been described for Lactobacillus plantarum NGRI0101 plasmid pLKS [16]. In the present study, analysis of *L. brevis* prophages revealed the presence of a potential prophage-encoded Abi system located within the chromosome of a beer-spoiling *L. brevis* strain. This Abi system was shown to be functional and is encoded by two genes, with sequence similarity to the previously characterized lactococcal AbiL system [8].

Predicted prophage regions were identified on the chromosome of nineteen *L. brevis* strains using PHASTER. Among these predicted prophage regions a

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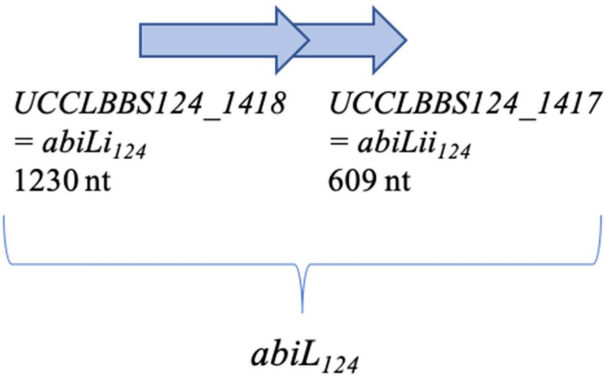

Fig. 1. Genetic organization of the AbiL$_{124}$ system.

BlastP analysis revealed the presence of an identical putative *abi* system carried by three prophage regions located on the chromosome of *L. brevis* strains UCCLBBS124, NPS-QW-145 and SRCM101174. This putative *abi* system is composed of two genes with a A+T content of 68% similar to that observed for other *abi* genes [15]. The proteins encoded by this putative *abi* system display similarity (approximately 35% at amino acid level; data not shown) to the two proteins of the AbiL system previously identified in *L. lactis* [8]. Of these three *L. brevis* strains, only *L. brevis* strain UCCLBBS124 was available in our collection for further characterization of this putative Abi system encoded by its temperate phage TPMB124. The putative *abi* system carried by prophage TPMB124, designated here as *abiL$_{124}$*, is composed of two genes which appear to be organized as an operon due to their apparent translational coupling as had previously been observed for the lactococcal AbiL system (Fig. 1) [8].

To assess the effect of AbiL$_{124}$ on the phage sensitivity profile of different host bacteria, the genes encoding AbiL$_{124}$ were cloned into pNZ44 to generate plasmid pNZ44:*abiL$_{124}$*, which was then introduced into *L. brevis* strains UCCLB521 and SA-C12. In the case of UCCLB521 pNZ44:*abiL$_{124}$* significant phage resistance was observed against phages 3-521, 521B and SAC12B with an EOP (Efficiency of plaquing) that was lower than 10^{-8} (Table 1), demonstrating the functionality and efficacy of this prophage-encoded phage resistance system. Conversely, no such resistance was observed for SA-C12 and its derivative expressing the putative Abi system, SA-C12 pNZ44:*abiL$_{124}$* against phages 3-SAC12 and SAC12B (Table 1). The role of the individual genes constituting *abiL$_{124}$* in conferring phage resistance to UCCLB521 was also investigated. After introduction of the individual genes separately in UCCLB521, no resistance to phages was observed (data not shown) confirming the requirement for both genes for an active AbiL$_{124}$ phage resistance system. Lysis-in-broth and cell death experiments were conducted to study the effect of the AbiL$_{124}$ system on actively growing cells. Expression of AbiL$_{124}$ in *L. brevis* UCCLB521 allowed significantly improved survival after phage infection relative to the wild-type and the strain carrying an empty plasmid pNZ44 as shown by lysis-in-broth experiments (Fig. 2). The strain expressing the AbiL$_{124}$ system exhibits growth in the presence of phages 3-521 or 521B, yet the level of growth is significantly lower than that observed for the strain in the absence of either of these phages (Fig. 2). Cell death was calculated following phage infection of the *L. brevis* strain in broth for 30 h, which indicated that approximately 64 and 60% of the cells of UCCLB521 expressing the AbiL$_{124}$ system lost viability following infection with phages 3-521 and 521B, respectively (Table 2). Adsorption assays revealed a significant lower adsorption efficiency of phages 3-521 and 521B on *L. brevis* strain UCCLB521 expressing the AbiL$_{124}$ sys-

Table 1. Efficiency of plaquing of tested bacteriophages against the putative *L. brevis* AbiL$_{124}$ system.

		Lb. brevis strains					
		UCCLB521			SA-C12		
		WT	pNZ44	pNZ44:*abiL$_{124}$*	WT	pNZ44	pNZ44:*abiL$_{124}$*
Phage	3-521 (Group I)	1	0.58	< 10^{-8}	N/A[a]	N/A	N/A
	521B (Group I)	1	1.52	< 10^{-8}	N/A	N/A	N/A
	SAC12B (Group I)	1	0.98	< 10^{-8}	1	0.97	0.90
	3-SAC12 (Group II)	N/A	N/A	N/A	1	0.96	0.94

[a]N/A: not applicable

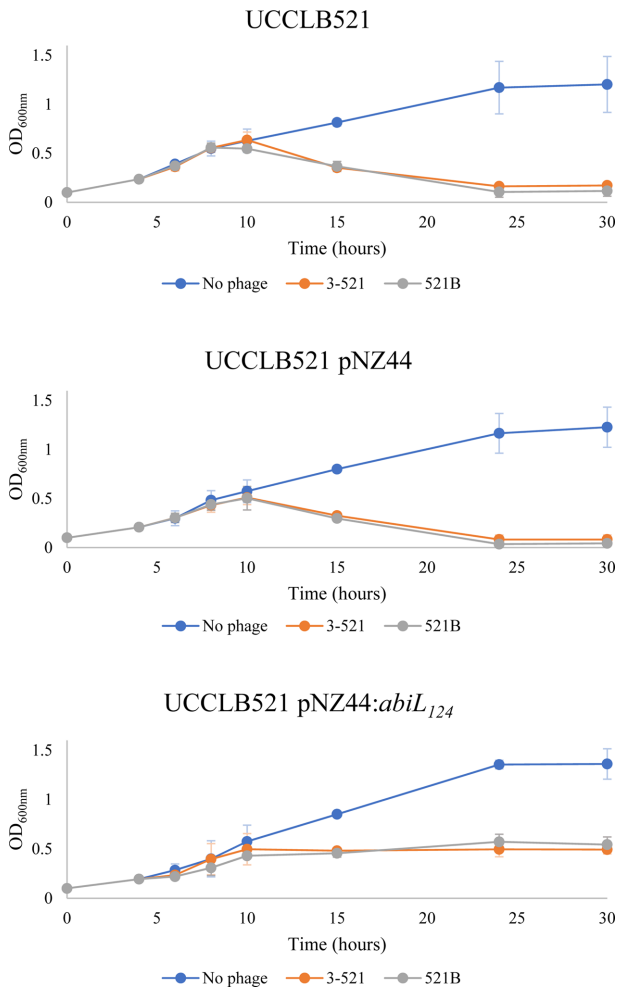

Fig. 2. Lysis-in-broth experiments comparing the lysis profiles of UCCLB521 WT, harboring the empty vector pNZ44 or expressing the AbiL$_{124}$ system when infected with phages 3-521 (orange) or 521B (grey) (MOI = 1). Absence of phage in the culture was used as a negative control (blue).

tem with an adsorption efficiency 2.5 (for phage 521B) to 4 (for phage 3-521) times lower than that observed for the WT sensitive strain (Table 2). These observations are unusual for an Abi system as Abi systems predominantly act intracellularly by interfering with phage development following phage adsorption and DNA injection [8]. To determine if phage-infected strains released viable phages, ECOI (Efficiency of the Center Of Infection) experiments were performed demonstrating the significant inability of the strain expressing the AbiL$_{124}$ system (UCCLB521 pNZ44:*abiL$_{124}$*) to produce viable phages (Table 2). AbiL$_{124}$ was shown to provide complete phage resistance for one strain of *L. brevis* and its activity was also tested on *L. lactis* strains. The *abiL$_{124}$* genes were expressed in *L. lactis* NZ9000 and 3107 and tested for their effectiveness against a range of phages (Table 3). AbiL$_{124}$ was shown to confer almost complete resistance to *L. lactis* 3107 pNZ44:*abiL$_{124}$* against all tested phages with EOPs lower than 10^{-9}. The AbiL$_{124}$ system was shown to be active against phages belonging to different phage groups (936, 949, P335 and P087 groups), thus showing a broad activity-range against lactococcal phages. Phage resistance was also observed in *L. lactis* NZ9000 carrying the *abiL$_{124}$* genes but not to the same extent (Table 3) as in 3107. Significant ($p < 0.05$) resistance against phages jj50, p2 and sk1 were observed for NZ9000 expressing the AbiL$_{124}$ system, however no significant difference was observed against phage 712 which remained active in infection of the derivative strain (Table 3).

In this study, a novel Abi system was identified on prophage regions of three *L. brevis* strains. This Abi system comprises two translationally coupled ORFs, both required for conferring phage resistance to the host. Consequences of this AbiL$_{124}$ system on targeted phages are elimination of their efficiency of plaquing, a reduction in their adsorption efficiency and a significant

Table 2. Phenotypic characteristics of the potential AbiL$_{124}$ system.

L. brevis strains	Phage	Cell death (%)	Adsorption (%)	ECOI[a]
UCCLB521 WT	3-521	100	95.9 ± 2.6	1
	521B	100	96.1 ± 2.5	1
UCCLB521 pNZ44	3-521	99.2 ± 2.3	86.7 ± 1.7	0.92 ± 0.27
	521B	98.7 ± 1.3	94.4 ± 1.2	0.97 ± 0.03
UCCLB521 pNZ44:*abiL$_{124}$*	3-521	64.1 ± 0.9	22.7 ± 4.7	0
	521B	60.2 ± 0.7	37.1 ± 5.6	0.04 ± 0.03

[a]ECOI: Efficiency of the center of infection

http://dx.doi.org/10.4014/mbl.2002.02017

Table 3. Efficiency of plaquing of tested lactococcal bacteriophages against the putative *L. brevis* AbiL$_{124}$ system (results are average of triplicate assays).

		L. lactis strains					
		3107			NZ9000		
		WT	pNZ44	pNZ44:*abiL*$_{124}$	WT	pNZ44	pNZ44:*abiL*$_{124}$
Phage (phage group)	TP901-1 (P335)	1	0.66	$< 10^{-11}$	N/A[a]	N/A	N/A
	LC3 (P335)	1	0.75	$< 10^{-11}$	N/A	N/A	N/A
	Dub35A (P335)	1	1	$< 10^{-12}$	N/A	N/A	N/A
	62601 (936)	1	1	$< 10^{-12}$	N/A	N/A	N/A
	66901 (936)	1	1.57	$< 10^{-10}$	N/A	N/A	N/A
	949 (949)	1	0.96	$< 10^{-10}$	N/A	N/A	N/A
	WRP3 (949)	1	0.83	6.1×10^{-7}	N/A	N/A	N/A
	P087 (P087)	1	0.86	$< 10^{-9}$	N/A	N/A	N/A
	jj50 (936)	N/A	N/A	N/A	1	1	0.70
	p2 (936)	N/A	N/A	N/A	1	1.09	0.71
	sk1 (936)	N/A	N/A	N/A	1	0.96	0.71
	712 (936)	N/A	N/A	N/A	1	1.12	1.02

[a]N/A: not applicable

decline in the number of progeny phage released. Interestingly, the Abi system identified in this study on *Lb. brevis* prophages was shown to be active against phages infecting *L. lactis* strains revealing a broad activity range. This Abi system only shows sequence similarity to the already described AbiL system [8] even though the AbiL$_{124}$ system showed to be active against lactococcal phages of the P335 group unlike the AbiL system. The phage resistance system described here shows similarities and impacts on phage/host interaction that are consistent with other Abi systems. However, further experiments are needed to elucidate if this phage resistance mechanism is due to the inability of the phage to penetrate the host (low adsorption efficiency) or to exit the host once inside (e.g. due to DNA replication inhibition as observed for AbiA [12] or due to phage protein synthesis inhibition as described for AbiL [8]). The findings presented here reveal new insights into phage resistance mechanisms in *L. brevis* strains and the benefits conferred by their resident prophages. Considering the negative impact of *L. brevis* strains on beer spoilage and the increased demand in bioremediation process during the fermentation process, it is important to understand natural phage defence systems in order to develop effective phage-based treatments to eliminate bacterial beer spoilage.

Acknowledgments

Marine Feyereisen is the recipient of an Irish Research Council Enterprise Partnership Scheme postgraduate scholarship (Ref. No. EPSPG/2015/7). Douwe van Sinderen is supported by a Principal Investigator award (Ref. No. 450 13/IA/1953) through Science Foundation Ireland (SFI). Jennifer Mahony is in receipt of Starting Investigator Research Grant (SIRG) (Ref. No. 15/SIRG/3430) funded by Science Foundation Ireland (SFI).

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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