

Title	A novel erythromycin resistance plasmid from Bacillus sp strain HS24, isolated from the marine sponge Haliclona simulans
Authors	Barbosa, Teresa M.;Phelan, Robert W.;Leong, Dara;Morrissey, John P.;Adams, Claire;Dobson, Alan D. W.;O'Gara, Fergal
Publication date	2014
Original Citation	Barbosa TM, Phelan RW, Leong D, Morrissey JP, Adams C, Dobson ADW, et al. (2014) A Novel Erythromycin Resistance Plasmid from Bacillus Sp. Strain HS24, Isolated from the Marine Sponge Haliclona Simulans. PLoS ONE 9(12): e115583. doi:10.1371/journal.pone.0115583
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1371/journal.pone.0115583
Rights	© 2015 Barbosa et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited - <a href="http://creativecommons.org/licenses/by/4.0/">http://creativecommons.org/licenses/by/4.0/</a>
Download date	2024-11-22 11:47:48
Item downloaded from	<a href="https://hdl.handle.net/10468/2318">https://hdl.handle.net/10468/2318</a>



# UCC

**University College Cork, Ireland**  
 Coláiste na hOllscoile Corcaigh



CrossMark  
click for updates

## OPEN ACCESS

**Citation:** Barbosa TM, Phelan RW, Leong D, Morrissey JP, Adams C, et al. (2014) A Novel Erythromycin Resistance Plasmid from *Bacillus* Sp. Strain HS24, Isolated from the Marine Sponge *Haliclona Simulans*. PLoS ONE 9(12): e115583. doi:10.1371/journal.pone.0115583

**Editor:** Jose Luis Balcazar, Catalan Institute for Water Research (ICRA), Spain

**Received:** September 16, 2014

**Accepted:** December 1, 2014

**Published:** December 30, 2014

**Copyright:** © 2014 Barbosa et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability:** The authors confirm that all data underlying the findings are fully available without restriction. The 16S rRNA gene sequence of *Bacillus* sp. HS24 and the complete nucleotide sequence of plasmid pBHS24B have been deposited in the GenBank database with the accession numbers JF803858 and KC991136, respectively.

**Funding:** This research was supported in parts by grants awarded to FOG by the Science Foundation of Ireland (SSPC2 12/RC/2275, 13-TIDA-B2625, 07/IN.1/B948, 12/TIDA/B2411, 12/TIDA/B2405,09/RFP/BMT 2350); the Department of Agriculture, Fisheries and Food (DAFF11/F/009 MabS, FIRM/RSF/CoFoRD; FIRM 08/RDC/629); the Environmental Protection Agency (EPA 2008-PhD/S-2), the Irish Research Council for Science, Engineering and Technology (PD/2011/2414; RS/2010/2413), the European Commission (FP7-PEOPLE-2013-ITN, 607786; OCEAN2012, 287589; FP7-KBBE-2012-6, CP-TP 311975; FP7-KBBE-2012-6, CP-TP-312184; Marie Curie 256596); and the Marine Institute (Beaufort award C2CRA 2007/082); Teagasc (Walsh Fellowship 2013) and the Health Research Board (HRA/2009/146). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

## RESEARCH ARTICLE

# A Novel Erythromycin Resistance Plasmid from *Bacillus* Sp. Strain HS24, Isolated from the Marine Sponge *Haliclona Simulans*

Teresa M. Barbosa<sup>1\*</sup>, Robert W. Phelan<sup>2,3\*</sup>, Dara Leong<sup>2</sup>, John P. Morrissey<sup>2,4</sup>, Claire Adams<sup>2,3</sup>, Alan D. W. Dobson<sup>2,4</sup>, Fergal O’Gara<sup>2,3,4,5\*</sup>

1. School of Pharmacy, University College Cork, Cork, Ireland, 2. Department of Microbiology, University College Cork, Cork, Ireland, 3. Biomerit Research Centre, University College Cork, Cork, Ireland, 4. Marine Biotechnology Centre, Environmental Research Institute, University College Cork, Cork, Ireland, 5. Curtin University, School of Biomedical Sciences, Perth WA 6845, Australia

\*[t.barbosa@ucc.ie](mailto:t.barbosa@ucc.ie) (TMB); [f.ogara@ucc.ie](mailto:f.ogara@ucc.ie) (FO’G)

These authors contributed equally to this work.

## Abstract

A better understanding of the origin and natural reservoirs of resistance determinants is fundamental to efficiently tackle antibiotic resistance. This paper reports the identification of a novel 5.8 kb erythromycin resistance plasmid, from *Bacillus* sp. HS24 isolated from the marine sponge *Haliclona simulans*. pBHS24B has a mosaic structure and carries the erythromycin resistance gene *erm*(T). This is the first report of an erythromycin resistance plasmid from a sponge associated bacteria and of the *Erm*(T) determinant in the genus *Bacillus*.

## Introduction

Antibiotic resistance is recognised as a major public health problem and resistance determinants have been identified in a wide variety of different clinical and environmental settings [1, 2, 3, 4, 5]. However, despite many years of research, the origin of these resistance determinants remains elusive [6, 7]. Resistance genes are frequently associated with promiscuous mobile genetic elements which drive their evolution and facilitate their horizontal spread [8]. Knowledge on the prevalence and nature of these in natural habitats is therefore fundamental to increasing our understanding of the development of antibiotic resistance [9]. Additionally, these plasmids can provide a backbone for the creation of new cloning vectors for use in

the genetic manipulation of natural isolates, which are frequently refractory to the uptake and integration of exogenous DNA [10].

While the marine sponge microbiota is attracting increasing interest, research to date has primarily focused on the overall microbial diversity and biotechnological potential of this unique microbial ecosystem [11, 12, 13, 14]. However the antimicrobial susceptibility of the sponge microbiota coupled with their ability to act as a possible reservoir for antibiotic resistance determinants; potentially transmissible to the food chain and clinical relevant bacteria [5], has not to date been adequately examined [15, 16].

We have recently isolated a *Bacillus* sp. isolate, HS24, from the marine sponge *Haliclona simulans* [17, 18]. *Bacillus* sp. HS24 displays resistance towards erythromycin and tetracycline and was shown to contain two small plasmids, of which, pBHS24 carries the tetracycline resistance determinant Tet(L) [17]. pBHS24 was shown to be almost identical to three other mobilisable tetracycline resistance plasmids identified in the honey bee pathogen *Paenibacillus larvae* (pMA67), in the anaerobe *Lactobacillus sakei* Rits 9, isolated from an Italian Sola cheese (pLS55) and in the spore-former *Sporosarcina ureae* (pSU1), isolated from the subsurface beneath a broiler chicken farm [17].

In this background, the aim of the present study was to characterise the nature of erythromycin resistance of a halophilic *Bacillus* strain isolated from the marine sponge *Haliclona simulans*.

## Materials and Methods

### Growth and antibiotic susceptibility testing

Sponge-associated *Bacillus* sp. HS24 was routinely grown and maintained aerobically, on Difco marine agar/broth (MA/MB) (Difco 2216), at 30°C, unless otherwise stated. Luria-Bertani medium was routinely used for growth and maintenance of *E. coli* and *B. subtilis* 168.

Susceptibility to erythromycin was determined by spotting MB cultures onto Muller–Hinton (MH, Merck, Darmstadt, Germany) plates supplemented with different concentrations of erythromycin (Sigma-Aldrich, Munich, Germany) and incubated aerobically at 30°C. Initial tests were performed with plates supplemented with 0 to 0.5 mg ml<sup>-1</sup> erythromycin. The concentration range of erythromycin was subsequently expanded, with plates supplemented with 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mg ml<sup>-1</sup>. MIC values are defined as the minimal concentration of antibiotic able to inhibit the growth. As there are no specific established antibiotic breakpoints values for marine sponge *Bacillus* isolates, the breakpoint values used for categorizing strain HS24 as resistant were those recommended by EFSA [19].

## DNA extraction, PCR amplification and transformation

Total genomic DNA of *Bacillus* sp. HS24 was extracted from 24 h MB cultures as previously described [20]. Total plasmid DNA was extracted from overnight MB cultures using the QIAprep Spin miniprep kit optimized for *Bacillus* (Qiagen GmbH, Hilden, Germany).

Plasmid DNA isolated from isolate HS24 was used to transform *B. subtilis* 168 competent cells as previously described [21, 22].

The universal eubacterial primers 27f (5'-AGA GTT TGA TCM TGG CTC AG-3', M=C or A) and 1492r (5'-GGT TAC CTT GTT ACG ACT T-3') [23] were used to amplify the small-subunit rRNA (16S rRNA) gene sequence of *Bacillus* sp. HS24. PCR mixtures (50  $\mu$ l) contained 50 ng of genomic DNA as template, 1  $\times$  BioTaq PCR Buffer (Bioline, London, UK), 1.5 mmol l<sup>-1</sup> of MgCl<sub>2</sub>, 0.2 mmol l<sup>-1</sup> of dNTPs, 0.5  $\mu$ mol l<sup>-1</sup> of each primer and 2.5 U of BioTaq DNA polymerase (Bioline). PCR was carried out under the following cycling conditions: initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 45 s, with a final extension at 72°C for 10 min.

## DNA sequencing

The near complete 16S rRNA gene sequence of *Bacillus* sp. HS24 (1441 nt) (GenBank JF803858) obtained with the primers 27f and HS24F2 (GTGAAATGCGTAGATATGTGG) (GATC Biotech AG, Germany) was compared with sequences in the Genbank nucleotide sequence database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) using BLASTn [24, 25]. A Neighbour-joining phylogenetic tree was generated by analysing near complete 16S rRNA gene sequences of *Bacillus* sp. HS24 and strains of closely related *Bacillus* species. The tree was constructed using maximum composite likelihood and pairwise deletion. Percentage bootstrap values (>50% only) from 1000 re-samplings are indicated at each node. Bar, 5% estimated sequence divergence.

The pBHS24B plasmid was sequenced as follows: pBHS24B DNA restricted with *Hind*III and *Eco*RI was cloned into the vector pUC18 and initial nucleotide sequences obtained with the M13 primers (GATC Biotech AG, Germany), as previously described for plasmid pBHS24 [17]. The complete nucleotide sequence of the plasmid (GenBank KC991136) was subsequently determined by primer walking, using pBHS24B as a template. The sequencing data was manually assembled using Bioedit [26]. Open reading frames (ORFs) were determined and annotated using ORF Finder (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>), and Basic Local Alignment Search Tool (BLAST) at NCBI [27].

## Nucleotide sequence accession numbers

The 16S rRNA gene sequence of *Bacillus* sp. HS24 and the complete nucleotide sequence of plasmid pBHS24B have been deposited in the GenBank database with the accession numbers JF803858 and KC991136, respectively.

## Results and Discussion

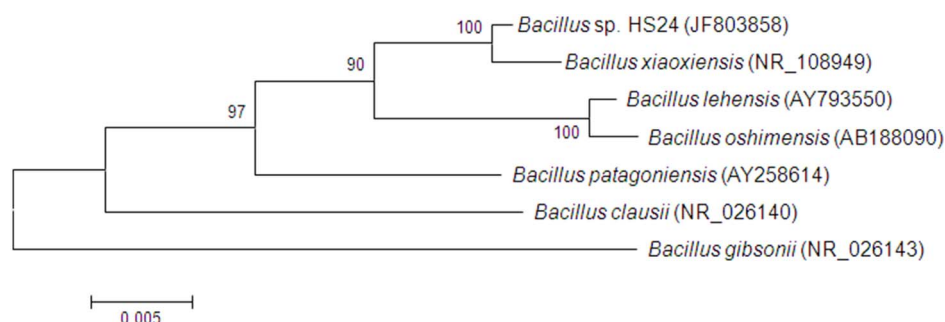
Phylogenetic analysis of the 16S rRNA gene sequence of *Bacillus* sp. HS24 indicates a 99% sequence identity with the 16S rRNA gene of its closest relative, the slightly halophilic *Bacillus xiaoxiensis* strain JSM081004 [28] (Fig. 1).

Although *Bacillus* sp. HS24 displays high levels of resistance to erythromycin (MIC of 3 mg ml<sup>-1</sup>), transformation of the erythromycin susceptible strain *Bacillus subtilis* 168, with total plasmid DNA purified from isolate HS24, yielded no colonies on LB medium supplemented with 5 µg ml<sup>-1</sup> erythromycin [17]. However, in the current study a large number of erythromycin resistant transformants were obtained when selection was performed at a lower concentration (1 µg ml<sup>-1</sup>). As expected no colonies were observed on antibiotic control plates when plasmid DNA was not added to cells. A single plasmid of approximately 5.8 kb in size, here named pBHS24B, was purified from the erythromycin resistance *B. subtilis* transformants (Fig. 2). While the level of tetracycline resistance conferred by pBHS24 (>100 µg ml<sup>-1</sup>) in the *B. subtilis* background was significantly higher than that in strain HS24 (75 µg ml<sup>-1</sup>) [17], there was no difference in the level of erythromycin resistance conferred by pBHS24B in the native and cloning hosts. Attempts to transform pBHS24B into chemically competent *E. coli* DH5α or K12 MG1655 cells proved unsuccessful.

The pBHS24B sequencing data was manually assembled using Bioedit [26], generating a circular element of 5837 nt (Fig. 3). A total of six putative open reading frames (ORFs) were determined and annotated (Fig. 3, Table 1). Results from BLASTx searches revealed that pBHS24B has a mosaic structure, which is more than likely to have evolved through the occurrence of multiple recombination events in one or more hosts. Different sections of the plasmid appear to have assorted origins as indicated by the level of sequence homology to different extra chromosomal elements from host strains isolated from a wide range of environments and the different G/C content of the respective open reading frames (Table 1, Fig. 3).

pBHS24B encodes a truncated copy of the recombinase/mobilisation gene, *pre/mob*, whose deduced amino acid sequence shows the highest homology (47% amino acid sequence identity) with the N-terminal 186 aa of the Pre/Mob protein from plasmid pBM02 of *Lactococcus lactis* subsp. *cremoris* [29] (Table 1). The shorter size of the pBHS24B Mob protein (196 amino acid) contrasts with the usually larger Pre/Mob proteins of the pMV158 family (350–500 amino acid) [30]. Although this region spans the three conserved motifs of the pMV158 family of Pre/Mob proteins (Fig. 4) it is not clear as yet if the truncated protein is functional. Sequence analysis suggest that a 894 bp segment of unknown origin, which appears to encode a 297 amino acids hypothetical protein (ORF1), might have integrated at this point in the plasmid resulting in the truncation of the original *pre/mob* gene (Fig. 3, Table 1).

The putative replication region of pBHS24B is highly homologous to that of the *erm*(B) encoding rolling circle-replication (RCR) plasmid pLFE1, from the raw milk cheese isolate *Lactobacillus plantarium* M345 [31]. This includes the copy

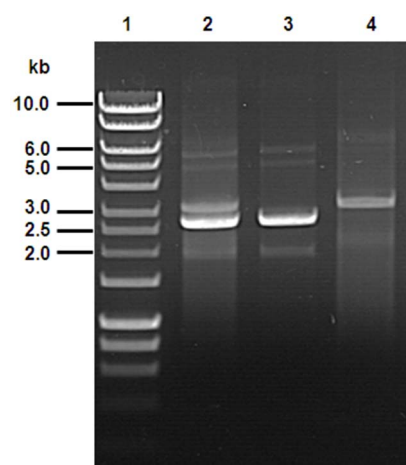


**Fig. 1. Neighbour-joining phylogenetic tree generated by analysing near complete 16S rRNA gene sequences of *Bacillus* sp. HS24 and strains of closely related *Bacillus* species.** Accession numbers are in parentheses. The tree was constructed using maximum composite likelihood and pairwise deletion. Percentage bootstrap values (>50% only) from 1000 re-samplings are indicated at each node. Bar, 5% estimated sequence divergence.

doi:10.1371/journal.pone.0115583.g001

number control protein, CopG and the replication initiation protein, RepB (with only one and two nucleotide differences between the *copG* and *repB* genes, respectively, in the two plasmids) (Table 1). This homology also extends to a 580 bp region upstream of *copG*, which includes a putative replication initiation site with a single-strand origin (*sso*)-like region and a characteristic pMV158 family double-strand origin (*dso*) (100% nt sequence identity) [31]. This again suggests that pBHS24B belongs to the pMV158 family of plasmids [30], and therefore is likely to replicate by a RCR mechanism, like many of the plasmids derived from Gram positive hosts [32].

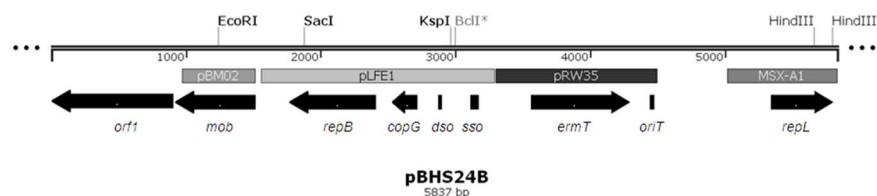
A second putative replication initiation protein with 83% amino acid sequence identity to the putative RepL protein from *Bacillus cereus* MSX-A1 (Genbank



**Fig. 2. Comparison of plasmid DNA extracted from *Bacillus* sp. strain HS24 and *B. subtilis* 168 transformed with the tetracycline resistance plasmid pBHS24 and the erythromycin resistance plasmid pBHS24B.** Lane 1, DNA marker; Lane 2, *Bacillus* sp. HS24; Lane 3, *B. subtilis* 168 - pBHS24; Lane 4, *B. subtilis* 168 - pBHS24B; Multiple faint bands on lanes 2 to 4 correspond to the different conformational forms of plasmid DNA.

doi:10.1371/journal.pone.0115583.g002





**Fig. 3. Graphical representation of the genomic structure of pBHS24B from *Bacillus* sp. HS24.** Restriction sites and regions with homology to previously reported sequences are indicated. Arrow heads indicate the direction of transcription of the different open reading frames. The 525 bp region immediately upstream from *repL* does not share homology to any other sequence in the database. MSX-A1, *B. cereus* whole genome shotgun (WGS) entry; preliminary data, plasmid content unknown. Figure created using Snapgene viewer.

doi:10.1371/journal.pone.0115583.g003

accession number EJQ95744) is also present in pBHS24B (Fig. 3, Table 1). Replication proteins of the RepL family are frequently found in small cryptic or erythromycin resistance encoding RCR plasmids previously identified in *Staphylococcus* and *Bacillus* species [33]. The presence of more than one replication protein has previously been reported for other plasmids, such as the *Streptococcus faecalis* plasmid pAM $\alpha$ 1 [34] and the *Bacillus* plasmid pTB19 [35, 36].

Erythromycin resistance in pBHS24B is conferred by a macrolide-lincosamide-streptogramin B (MLS<sub>B</sub>) resistance methylase Erm(T), which has been previously reported only in the genera *Enterococcus*, *Lactobacillus*, *Streptococcus* and *Staphylococcus* (<http://faculty.washington.edu/marilynr/>). The pBHS24B Erm(T) protein shares 100% amino acid sequence homology with the Erm(T) of pUR2940, pUR2941, pKKS25, pRW35, pGA2000, pGB2001 and pGB2002 isolated

**Table 1. Sequence homology of the proteins encoded by pBHS24B\*.**

ORF	% G/C content	No. aa <sup>&amp;</sup>	Closest protein homologue	Strain/Origin	% aa Identity	E value <sup>#</sup>	Accessio no.
1	44.7	297	— <sup>*</sup>	—	—	—	—
2	47	196	Mob like protein, pBM02	<i>Lactococcus lactis</i> subsp. <i>Cremoris</i> P8-2-47; component of a German industrial starter culture	47	2E-47	NC_004930
3	33.8	212	RepB, pLFE1	<i>Lactobacillus plantarum</i> M345; raw-milk cheese	99	5E-157	NC_012628
4	30	59	CopG, pLFE1	<i>Lactobacillus plantarum</i> M345; raw-milk cheese	97	8E-22	NC_012628
5	25	244	Erm(T), pRW35	<i>Streptococcus pyogenes</i> RW35; nosocomial sample <sup>£</sup>	100	1E-174	NC_010423
6	36.6	152	Predicted RepL <sup>**</sup>	<i>Bacillus cereus</i> MSX-A1 <sup>***</sup>	83	2E-85	EJQ95744

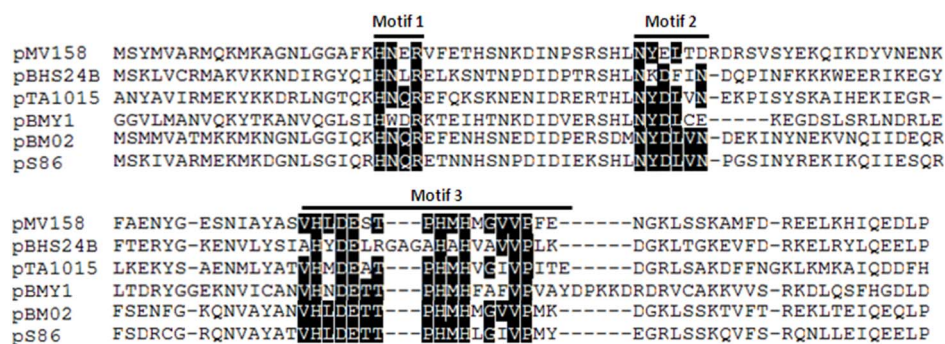
\*Results are from a BLASTx search of the GenBank non-redundant protein database on 13/8/13. <sup>&</sup>aa, amino acids. <sup>#</sup>Expectation value.

<sup>£</sup>100% identity also found to other plasmids as described in the text.

<sup>\*\*</sup>Whole genome shotgun (WGS) entry; preliminary data, plasmid content unknown. <sup>\*\*\*</sup>anthrax-like illness; isolated in Antarctica.

<sup>\*</sup>ORF1 shows a low homology hit (27%; E value 3E-05) with a *Leishmania major* structural maintenance of chromosome (SMC) protein domain (CAJ07774).

doi:10.1371/journal.pone.0115583.t001



**Fig. 4. Alignment of pBHS24B and selected pMV158-superfamily relaxases.** pS86, *Enterococcus faecalis*; pBM02, *Lactococcus lactis*; pBMY1, *Bacillus mycoides*; pTA1015, *Bacillus subtilis*; pMV158, *Streptococcus agalactiae*. The three conserved motif sequences typical of the pMV158 family of Pre/Mob proteins are identified [30]. Conserved amino acids within the motifs are highlighted in black.

doi:10.1371/journal.pone.0115583.g004

from *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus pyogenes* strains [34, 37, 38, 39, 40] (Table 1). The *oriT* sequence is located downstream of *erm*(T) and should have the same origin as the resistance gene (Fig. 3). The sequences encompassing the leader peptide-encoding sequence and the *erm*(T) translational start regions of these plasmids are also identical [38]. Previous comparisons of the *erm*(T) up- and downstream sequences in the streptococcal pGB2002, pGB2001, pGA2000, pRW35 and the staphylococcal pUR2940, pUR2941 plasmids, identified 56 to 58 bp long conserved imperfect direct repeat (IDR) regions [39], which are believed to play a role in the acquisition of the erythromycin resistance determinants. Although the downstream sequence is clearly identifiable and relatively well conserved in pBHS24B (4437–4492 nt), the acquisition of a 1730 bp fragment of DNA from plasmid pLFE1 (Fig. 3), appears to have resulted in the deletion of the IDR region upstream of *erm*(T). *Bacillus* sp. HS24 has not been screened for other previously described erythromycin resistance determinants, nor has it been cured of plasmid pBHS24B, and therefore, the concomitant existence of other erythromycin resistance gene(s) in the genome of this strain cannot be excluded.

To our knowledge this is the first report of the erythromycin resistance Erm(T) determinant in the genus *Bacillus*. Erythromycin resistance through methylation of the 23S rRNA within this genus, has been previously associated with Erm(B), Erm(C), Erm(D), Erm(G) and Erm(34), with evidence for specific species association for some of the determinants (<http://faculty.washington.edu/marilynr/ermweb4.pdf>) [41]. The *erm*(T) gene has previously been identified in bacterial isolates from agricultural and clinical settings [42, 43, 44, 45], where the widespread use of antibiotics is likely to have contributed to the development of resistance within the associated microbiota. While the prevalence of erythromycin resistance among marine sponge bacteria is unknown, *B. licheniformis* HS147, was the only other *Bacillus* isolate from *H. simulans* to display resistance to this antibiotic [18]. Antibiotics used in therapy and agriculture are known to accumulate in the environment and to contaminate aquatic habitats where they



can exert their selective pressure on the native flora [7, 46, 47]. Erythromycin in particular is widely used to control the spread of infection in the aquaculture industry [48]. Interestingly, the *H. simulans* sponge host of isolate HS24, was recovered from Gurraig Sound in Kilkieran Bay, off the coast of Galway in Ireland [49], in an area that is well known for aquaculture (Status of Irish Aquaculture 2007, <http://www.marine.ie/home/Aquaculture.htm>). Given that sponges are known to filter large quantities of seawater, up to 24,000 L Kg<sup>-1</sup> per day; they are thus likely to be susceptible to accumulate environmental contaminants, such as heavy metals and antibiotics, which could ultimately drive the acquisition of resistance by the associated microbiota [15]. Despite fears that intensive aquaculture processes may contribute to the development and dissemination of antibiotic resistance, little is known about this practise in comparison to animal husbandry. The use of antibiotics to treat infection in aquaculture generally focuses on specific fish pathogens and not the complex commensal microbiota of the fish and surrounding marine environments [5].

The mosaic structure of plasmid pBHS24B supports the importance of these elements in the evolution and acquisition of antibiotic resistance through horizontal gene transfer. The question as to whether resistance in this habitat arose as a consequence of environmental contamination or if resistance determinants are a common part of the genome of environmental bacteria where they have alternative functional roles remains highly debatable [9]. Although the overuse and misuse of antibiotics is reported to be responsible for the spread of antibiotic resistant bacteria, a large number of environmental strains produce antibiotics and so potentially carry genes encoding resistance to these compounds. As a result, antibiotics produced in the environment may exert a selective pressure on neighbouring microorganisms [7].

In conclusion *Bacillus* sp. HS24 contains two antibiotic resistance plasmids, one of which is nearly identical to plasmids from commensal and pathogenic bacterial species from four different genera, isolated from quite distinct ecological habitats [17]. The second plasmid shows a mosaic structure, which is likely to have been derived as a result of multiple recombination events between different plasmids within multiple hosts, the order of which remains unknown. Our results further illustrate the promiscuity of the nature of antibiotic resistance and suggest that sponge associated bacteria, as with other environmental bacteria; may represent a reservoir of resistance genes with the potential to transfer resistance to the food chain or indeed clinically relevant organism.

## Acknowledgments

We would like to thank Pat Higgins for his technical assistance.

## Author Contributions

Conceived and designed the experiments: TMB RWP ADWD JPM FO'G.

Performed the experiments: RWP DL TMB. Analyzed the data: TMB RWP.

Contributed reagents/materials/analysis tools: TMB JPM ADWD FO'G.  
Contributed to the writing of the manuscript: TMB RWP CA FO'G.

## References

1. **Barbosa TM, Levy SB** (2000) The impact of antibiotic use on resistance development and persistence. *Drug Resist Updat* 3: 303–311.
2. **Bhullar K, Wagglechner N, Pawlowski A, Koteva K, Banks ED, et al.** (2012) Antibiotic resistance is prevalent in an isolated cave microbiome. *PLoS One* 7: e34953.
3. **Cabello FC** (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environ Microbiol* 8: 1137–1144.
4. **Salyers AA, Gupta A, Wang Y** (2004) Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends Microbiol* 12: 412–416.
5. **Marshall BM, Levy SB** (2011) Food animals and antimicrobials: impacts on human health. *Clin Microbiol Rev* 24: 718–733.
6. **Rolain JM, Canton R, Cornaglia G** (2012) Emergence of antibiotic resistance: need for a new paradigm. *Clin Microbiol Infect* 18: 615–616.
7. **Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, et al.** (2010) Call of the wild: antibiotic resistance genes in natural environments. *Nat Rev Microbiol* 8: 251–259.
8. **Frost LS, Leplae R, Summers AO, Toussaint A** (2005) Mobile genetic elements: the agents of open source evolution. *Nat Rev Microbiol* 3: 722–732.
9. **D'Costa VM, King CE, Kalan L, Morar M, Sung WW, et al.** (2011) Antibiotic resistance is ancient. *Nature* 477: 457–461.
10. **Duitman EH, Wyczawski D, Boven LG, Venema G, Kuipers OP, et al.** (2007) Novel methods for genetic transformation of natural *Bacillus subtilis* isolates used to study the regulation of the mycosubtilin and surfactin synthetases. *Appl Environ Microbiol* 73: 3490–3496.
11. **Phelan RW, Barret M, Cotter PD, O'Connor PM, Chen R, et al.** (2013) Subtilomycin: a new lantibiotic from *Bacillus subtilis* strain MMA7 isolated from the marine sponge *Haliclona simulans*. *Mar Drugs* 11: 1878–1898.
12. **Taylor MW, Radax R, Steger D, Wagner M** (2007) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiol Mol Biol Rev* 71: 295–347.
13. **Kennedy J, Flemer B, Jackson SA, Lejon DP, Morrissey JP, et al.** (2010) Marine metagenomics: new tools for the study and exploitation of marine microbial metabolism. *Mar Drugs* 8: 608–628.
14. **Jackson SA, Flemer B, McCann A, Kennedy J, Morrissey JP, et al.** (2013) Archaea Appear to Dominate the Microbiome of *Inflatella pellicula* Deep Sea Sponges. *PLoS One* 8: e84438.
15. **Selvin J, Shanmugha Priya S, Seghal Kiran G, Thangavelu T, Sapna Bai N** (2009) Sponge-associated marine bacteria as indicators of heavy metal pollution. *Microbiol Res* 164: 352–363.
16. **Cabello FC, Godfrey HP, Tomova A, Ivanova L, Dolz H, et al.** (2013) Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. *Environ Microbiol* 15: 1917–1942.
17. **Phelan RW, Clarke C, Morrissey JP, Dobson AD, O'Gara F, et al.** (2011) Tetracycline resistance-encoding plasmid from *Bacillus* sp. strain #24, isolated from the marine sponge *Haliclona simulans*. *Appl Environ Microbiol* 77: 327–329.
18. **Phelan RW, O'Halloran JA, Kennedy J, Morrissey JP, Dobson AD, et al.** (2012) Diversity and bioactive potential of endospore-forming bacteria cultured from the marine sponge *Haliclona simulans*. *J Appl Microbiol* 112: 65–78.
19. **EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)** (2008) Technical guidance - Update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance *EFSA Journal* 732: 1–15.

20. **Barbosa TM, Serra CR, La Ragione RM, Woodward MJ, Henriques AO** (2005) Screening for *Bacillus* isolates in the broiler gastrointestinal tract. *Appl Environ Microbiol* 71: 968–978.
21. **Bott KF, Wilson GA** (1967) Development of competence in the *Bacillus subtilis* transformation system. *J Bacteriol* 94: 562–570.
22. **Wilson GA, Bott KF** (1968) Nutritional factors influencing the development of competence in the *Bacillus subtilis* transformation system. *J Bacteriol* 95: 1439–1449.
23. **Lane DJ** (1991) 16S/23S rRNA sequencing. In: Stackebrandt EaG, M, editor. *Nucleic Acid Techniques in Bacterial Systematics*. New York: John Wiley and Sons. 115–175.
24. **Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, et al.** (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25: 3389–3402.
25. **Zhang Z, Schwartz S, Wagner L, Miller W** (2000) A greedy algorithm for aligning DNA sequences. *J Comput Biol* 7: 203–214.
26. **Hall T** (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41: 95–98.
27. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ** (1990) Basic local alignment search tool. *J Mol Biol* 215: 403–410.
28. **Chen YG, Zhang YQ, Chen QH, Klenk HP, He JW, et al.** (2011) *Bacillus xiaoxiensis* sp. nov., a slightly halophilic bacterium isolated from non-saline forest soil. *Int J Syst Evol Microbiol* 61: 2095–2100.
29. **Sanchez C, Mayo B** (2003) Sequence and analysis of pBM02, a novel RCR cryptic plasmid from *Lactococcus lactis* subsp *cremoris* P8-2-47. *Plasmid* 49: 118–129.
30. **Francia MV, Varsaki A, Garcillan-Barcia MP, Latorre A, Drainas C, et al.** (2004) A classification scheme for mobilization regions of bacterial plasmids. *FEMS Microbiol Rev* 28: 79–100.
31. **Feld L, Bielak E, Hammer K, Wilcks A** (2009) Characterization of a small erythromycin resistance plasmid pLFE1 from the food-isolate *Lactobacillus plantarum* M345. *Plasmid* 61: 159–170.
32. **del Solar G, Giraldo R, Ruiz-Echevarria MJ, Espinosa M, Diaz-Orejas R** (1998) Replication and control of circular bacterial plasmids. *Microbiol Mol Biol Rev* 62: 434–464.
33. **Sprincova A, Javorsky P, Pristas P** (2005) pSRD191, a new member of RepL replicating plasmid family from *Selenomonas ruminantium*. *Plasmid* 54: 39–47.
34. **Perkins JB, Youngman P** (1983) *Streptococcus* plasmid pAM alpha 1 is a composite of two separable replicons, one of which is closely related to *Bacillus* plasmid pBC16. *J Bacteriol* 155: 607–615.
35. **Imanaka T, Ano T, Fujii M, Aiba S** (1984) Two replication determinants of an antibiotic-resistance plasmid, pTB19, from a thermophilic bacillus. *J Gen Microbiol* 130: 1399–1408.
36. **Osborn AM, da Silva Tatley FM, Steyn LM, Pickup RW, Saunders JR** (2000) Mosaic plasmids and mosaic replicons: evolutionary lessons from the analysis of genetic diversity in IncFII-related replicons. *Microbiology* 146 (Pt 9): 2267–2275.
37. **Kadlec K, Schwarz S** (2010) Identification of a plasmid-borne resistance gene cluster comprising the resistance genes *erm(T)*, *dfxK*, and *tet(L)* in a porcine methicillin-resistant *Staphylococcus aureus* ST398 strain. *Antimicrob Agents Chemother* 54: 915–918.
38. **Woodbury RL, Klammer KA, Xiong Y, Bailiff T, Glennen A, et al.** (2008) Plasmid-Borne *erm(T)* from invasive, macrolide-resistant *Streptococcus pyogenes* strains. *Antimicrob Agents Chemother* 52: 1140–1143.
39. **Gomez-Sanz E, Kadlec K, Feßler AT, Zarazaga M, Torres C, et al.** (2013) Novel *erm(T)*-carrying multiresistance plasmids from porcine and human isolates of methicillin-resistant *Staphylococcus aureus* ST398 that also harbor cadmium and copper resistance determinants. *Antimicrob Agents Chemother* 57: 3275–3282.
40. **DiPersio LP, DiPersio JR, Beach JA, Loudon AM, Fuchs AM** (2011) Identification and characterization of plasmid-borne *erm(T)* macrolide resistance in group B and group A *Streptococcus*. *Diagn Microbiol Infect Dis* 71: 217–223.
41. **Adimpong DB, Sorensen KI, Thorsen L, Stuer-Lauridsen B, Abdelgadir WS, et al.** (2012) Antimicrobial susceptibility of *Bacillus* strains isolated from primary starters for African traditional bread

- p>production and characterization of the bacitracin operon and bacitracin biosynthesis. Appl Environ Microbiol 78: 7903–7914.
42. **Whitehead TR, Cotta MA** (2001) Sequence analyses of a broad host-range plasmid containing *ermT* from a tylosin-resistant *Lactobacillus* sp. Isolated from swine feces. Curr Microbiol 43: 17–20.
43. **Tannock GW, Luchansky JB, Miller L, Connell H, Thode-Andersen S, et al.** (1994) Molecular characterization of a plasmid-borne (pGT633) erythromycin resistance determinant (*ermGT*) from *Lactobacillus reuteri* 100-63. Plasmid 31: 60–71.
44. **Chen J, Yu Z, Michel FC Jr, Wittum T, Morrison M** (2007) Development and application of real-time PCR assays for quantification of *erm* genes conferring resistance to macrolides-lincosamides-streptogramin B in livestock manure and manure management systems. Appl Environ Microbiol 73: 4407–4416.
45. **Teng LJ, Hsueh PR, Ho SW, Luh KT** (2001) High prevalence of inducible erythromycin resistance among *Streptococcus bovis* isolates in Taiwan. Antimicrob Agents Chemother 45: 3362–3365.
46. **Baquero F, Martinez JL, Canton R** (2008) Antibiotics and antibiotic resistance in water environments. Curr Opin Biotechnol 19: 260–265.
47. **Aminov RI** (2009) The role of antibiotics and antibiotic resistance in nature. Environ Microbiol 11: 2970–2988.
48. **Esposito A, Fabrizi L, Lucchetti D, Marvasi L, Coni E, et al.** (2007) Orally administered erythromycin in rainbow trout (*Oncorhynchus mykiss*): residues in edible tissues and withdrawal time. Antimicrob Agents Chemother 51: 1043–1047.
49. **Kennedy J, Codling CE, Jones BV, Dobson AD, Marchesi JR** (2008) Diversity of microbes associated with the marine sponge, *Haliclona simulans*, isolated from Irish waters and identification of polyketide synthase genes from the sponge metagenome. Environ Microbiol 10: 1888–1902.