

1 **Specific detection and quantification of the marine flavobacterial genus *Zobellia* on**
2 **macroalgae using novel qPCR and CARD-FISH assays**

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7 **SUPPLEMENTARY MATERIAL**

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9 This document contains four supplementary tables, two supplementary figures and references to
10 three separate supplementary files.

11 **Supplementary Tables**

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13 **Supplementary Table 1.** Survey of genes homologous to *Z. galactanivorans* Dsij^T in marine
 14 metagenomes available on the IMG/MER platform. Only metagenome samples showing at least 50
 15 homolog genes with >90% sequence identity to genes in *Z. galactanivorans* Dsij^T are shown.

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Metagenome ID	Origin	Location	Number of 90% hits
3300009421	red alga <i>Porphyra</i>	Sidmouth (UK)	1262
3300027498	red alga <i>Porphyra</i>	Sidmouth (UK)	917
3300009410	red alga <i>Porphyra</i>	Porto (Portugal)	536
3300009192	red alga <i>Porphyra</i>	Porto (Portugal)	480
3300009415	red alga <i>Porphyra</i>	Sidmouth (UK)	393
3300032272	coastal sediment	Maine (USA)	334
3300027262	red alga <i>Porphyra</i>	Sidmouth (UK)	289
3300026840	red alga <i>Porphyra</i>	Porto (Portugal)	283
3300027028	red alga <i>Porphyra</i>	Porto (Portugal)	260
3300009508	epipelagic water	Helgoland (Germany)	231
3300009073	red alga <i>Porphyra</i>	Bantry Bay (Ireland)	162
3300032373	microbial mat	Maine (USA)	129
3300009446	red alga <i>Porphyra</i>	Maine (USA)	114
3300028600	subtidal sediment	Helgoland (Germany)	114
3300025881	epipelagic water	Helgoland (Germany)	113
3300028599	subtidal sediment	Helgoland (Germany)	105
3300027509	red alga <i>Porphyra</i>	Bantry Bay (Ireland)	93
3300032136	coastal sediment	Delaware Bay (USA)	93
2236876004	estuarine water	Columbia River (USA)	83
2236876010	estuarine water	Columbia River (USA)	83
3300010392	coastal sediment	Rhode Island (USA)	82
3300032258	coastal sediment	Maine (USA)	73
3300018420	coastal salt marsh	Skidaway Island (USA)	66
3300010430	coastal sediment	Atlantic coast	66
3300009505	epipelagic water	Helgoland (Germany)	64
3300018048	coastal salt marsh	Skidaway Island (USA)	63
3300018417	coastal salt marsh	Skidaway Island (USA)	61
3300032231	coastal sediment	Maine (USA)	61
3300018876	coastal salt marsh	Skidaway Island (USA)	60
3300027623	red alga <i>Porphyra</i>	Maine (USA)	58
3300018416	coastal salt marsh	Skidaway Island (USA)	58
3300028598	subtidal sediment	Helgoland (Germany)	57
3300025793	wetland	San Francisco Bay (USA)	57
3300009439	red alga <i>Porphyra</i>	Maine (USA)	54
3300018041	coastal salt marsh	Skidaway Island (USA)	51
3300017950	coastal salt marsh	Skidaway Island (USA)	51
3300019459	coastal salt marsh	Skidaway Island (USA)	51

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Supplementary Table 2. List of bacterial strains used in this study.

Strain	Use	Reference
<i>Zobellia galactanivorans</i> Dsij ^T	positive control for qPCR / FISH	(Barbeyron <i>et al.</i> , 2001)
<i>Zobellia russellii</i> KMM 3677 ^T	positive control for qPCR / FISH	(Nedashkovskaya <i>et al.</i> , 2004)
<i>Zobellia amurskyensis</i> KMM 3526 ^T	positive control for FISH	(Nedashkovskaya <i>et al.</i> , 2004)
<i>Zobellia roscoffensis</i> Asnod1-F08 ^T	positive control for FISH	(Barbeyron <i>et al.</i> , 2021)
<i>Cellulophaga baltica</i> NN015840 ^T	negative control for qPCR	(Johansen <i>et al.</i> , 1999)
<i>Cellulophaga</i> sp. strain Asnod2-G02	negative control for qPCR	(Martin <i>et al.</i> , 2015)
<i>Maribacter forsetii</i> KT02ds18-6 ^T	negative control for qPCR	(Barbeyron <i>et al.</i> , 2008)
<i>Lewinella marina</i> MKG-38 ^T	negative control for FISH	(Khan <i>et al.</i> , 2007)
<i>Marinirhabdus citrea</i> MEBiC09412 ^T	negative control for FISH	(Yang <i>et al.</i> , 2018)

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22 Barbeyron, T., Carpentier, F., L'Haridon, S., Schüler, M., Michel, G., and Amann, R. (2008) Description of
23 *Maribacter forsetii* sp. nov., a marine *Flavobacteriaceae* isolated from North Sea water, and emended
24 description of the genus *Maribacter*. *Int J Syst Evol Microbiol* **58**: 790–797.
25 Barbeyron, T., L'Haridon, S., Corre, E., Kloareg, B., and Potin, P. (2001) *Zobellia galactanovorans* gen. nov., sp.
26 nov., a marine species of *Flavobacteriaceae* isolated from a red alga, and classification of [*Cytophaga*]
27 *uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Zobellia uliginosa* gen. nov., comb. nov. *Int J Syst*
28 *Evol Microbiol* **51**: 985–97.
29 Barbeyron, T., Thiébaud, M., Le Duff, N., Martin, M., Corre, E., Tanguy, G., et al. (2021) *Zobellia roscoffensis* sp.
30 nov. and *Zobellia nedashkovskayae* sp. nov., two flavobacteria from the epiphytic microbiota of the brown
31 alga *Ascophyllum nodosum*, and emended description of the genus *Zobellia*. *Int J Syst Evol Microbiol*.
32 Johansen, J., Nielsen, P., and Sjøholm, C. (1999) Description of *Cellulophaga baltica* gen. nov., sp. nov. and
33 *Cellulophaga fucicola* gen. nov., sp. nov. and reclassification of [*Cytophaga*] *lytica* to *Cellulophaga lytica*
34 gen. nov., comb. nov. *Int J Syst Bacteriol* **49**: 1231–1240.
35 Khan, S.T., Fukunaga, Y., Nakagawa, Y., and Harayama, S. (2007) Emended descriptions of the genus *Lewinella*
36 and of *Lewinella cohaerens*, *Lewinella nigricans* and *Lewinella persica*, and description of *Lewinella lutea* sp.
37 nov. and *Lewinella marina* sp. nov. *Int J Syst Evol Microbiol* **57**: 2946–2951.
38 Martin, M., Barbeyron, T., Martin, R., Portetelle, D., Michel, G., and Vandenberg, M. (2015) The Cultivable Surface
39 Microbiota of the Brown Alga *Ascophyllum nodosum* is Enriched in Macroalgal-Polysaccharide-Degrading
40 Bacteria. *Front Microbiol* **6**: 1–14.
41 Nedashkovskaya, O., Suzuki, M., Vancanneyt, M., Cleenwerck, I., Lysenko, A., Mikhailov, V., and Swings, J.
42 (2004) *Zobellia amurskyensis* sp nov., *Zobellia laminariae* sp nov and *Zobellia russellii* sp nov., novel marine
43 bacteria of the family *Flavobacteriaceae*. *International J Syst Evol Microbiol* **54**: 1643–1648.
44 Yang, S.H., Oh, J.H., Seo, H.S., Lee, J.H., and Kwon, K.K. (2018) *Marinirhabdus citrea* sp. nov., a marine
45 bacterium isolated from a seaweed. *Int J Syst Evol Microbiol* **68**: 547–551.

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48 **Supplementary Table 3.** Number of copies of 16S rRNA genes in available genomes from the genus
49 *Zobellia*.
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Strain	Number of 16S rRNA genes
<i>Z. amurskyensis</i> KMM 3526 ^T	4
<i>Z. amurskyensis</i> MAR_2009_138	3
<i>Z. russellii</i> KMM 3677 ^T	2
<i>Z. laminariae</i> KMM 3676 ^T	2
<i>Z. uliginosa</i> DSM 2061 ^T	2
<i>Z. galactanivorans</i> DsiJ ^T	2
<i>Z. galactanivorans</i> OII3	1
<i>Z. roscoffensis</i> Asnod1-F08 ^T	1
<i>Z. roscoffensis</i> Asnod2-B02-B	1
<i>Z. nedashkovskayae</i> Asnod2-B07-B ^T	1
<i>Z. nedashkovskayae</i> Asnod3-E08-A	1

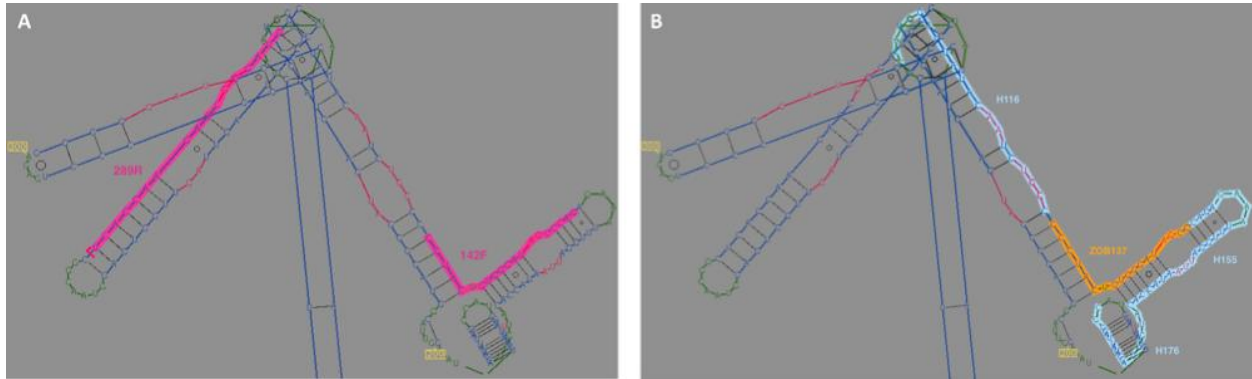
51 **Supplementary Table 4.** Blastn results on the NCBI 16S rRNA gene database for sequences of qPCR
52 products
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Seq. ID	Best blastn hit		First blastn hit outside <i>Zobellia</i>	
	strain	% id	strain	% id
LdigO-1	<i>Zobellia laminariae</i> KMM 3676 ^T	99.32	<i>Euzebyella marina</i> CY01 ^T	87.59
LdigO-2	<i>Zobellia russellii</i> KMM 3677 ^T	99.32	<i>Jejudonia soesokkakensis</i> SSK1-1 ^T	89.39
LdigO-3	<i>Zobellia laminariae</i> KMM 3676 ^T	98.65	<i>Euzebyella marina</i> CY01 ^T	86.86
LdigO-4	<i>Zobellia russellii</i> KMM 3677 ^T	97.97	<i>Jejudonia soesokkakensis</i> SSK1-1 ^T	87.88
LdigO-5	<i>Zobellia russellii</i> KMM 3677 ^T	100.00	<i>Jejudonia soesokkakensis</i> SSK1-1 ^T	90.15
LdigO-6	<i>Zobellia laminariae</i> KMM 3676 ^T	99.32	<i>Euzebyella marina</i> CY01 ^T	87.59
LdigO-7	<i>Zobellia laminariae</i> KMM 3676 ^T	99.32	<i>Euzebyella marina</i> CY01 ^T	87.59
LdigO-8	<i>Zobellia laminariae</i> KMM 3676 ^T	99.32	<i>Euzebyella marina</i> CY01 ^T	87.59
LdigO-9	<i>Zobellia laminariae</i> KMM 3676 ^T	99.32	<i>Euzebyella marina</i> CY01 ^T	89.05
LdigO-10	<i>Zobellia laminariae</i> KMM 3676 ^T	99.32	<i>Euzebyella marina</i> CY01 ^T	87.59
PpalS-1	<i>Zobellia laminariae</i> KMM 3676 ^T	99.32	<i>Euzebyella marina</i> CY01 ^T	87.59
PpalS-2	<i>Zobellia laminariae</i> KMM 3676 ^T	99.32	<i>Euzebyella marina</i> CY01 ^T	87.59
PpalS-3	<i>Zobellia laminariae</i> KMM 3676 ^T	97.97	<i>Euzebyella marina</i> CY01 ^T	86.13
PpalS-4	<i>Zobellia laminariae</i> KMM 3676 ^T	99.32	<i>Euzebyella marina</i> CY01 ^T	87.59
PpalS-5	<i>Zobellia laminariae</i> KMM 3676 ^T	98.66	<i>Euzebyella marina</i> CY01 ^T	85.51
PpalS-6	<i>Zobellia russellii</i> KMM 3677 ^T	100.00	<i>Jejudonia soesokkakensis</i> SSK1-1 ^T	90.15
PpalS-7	<i>Zobellia laminariae</i> KMM 3676 ^T	99.32	<i>Euzebyella marina</i> CY01 ^T	87.59
PpalS-8	<i>Zobellia laminariae</i> KMM 3676 ^T	99.32	<i>Euzebyella marina</i> CY01 ^T	87.59
PpalS-9	<i>Zobellia russellii</i> KMM 3677 ^T	99.32	<i>Euzebyella marina</i> CY01 ^T	90.51
PpalS-10	<i>Zobellia russellii</i> KMM 3677 ^T	98.65	<i>Euzebyella marina</i> CY01 ^T	89.78

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56 **Supplementary Figures**

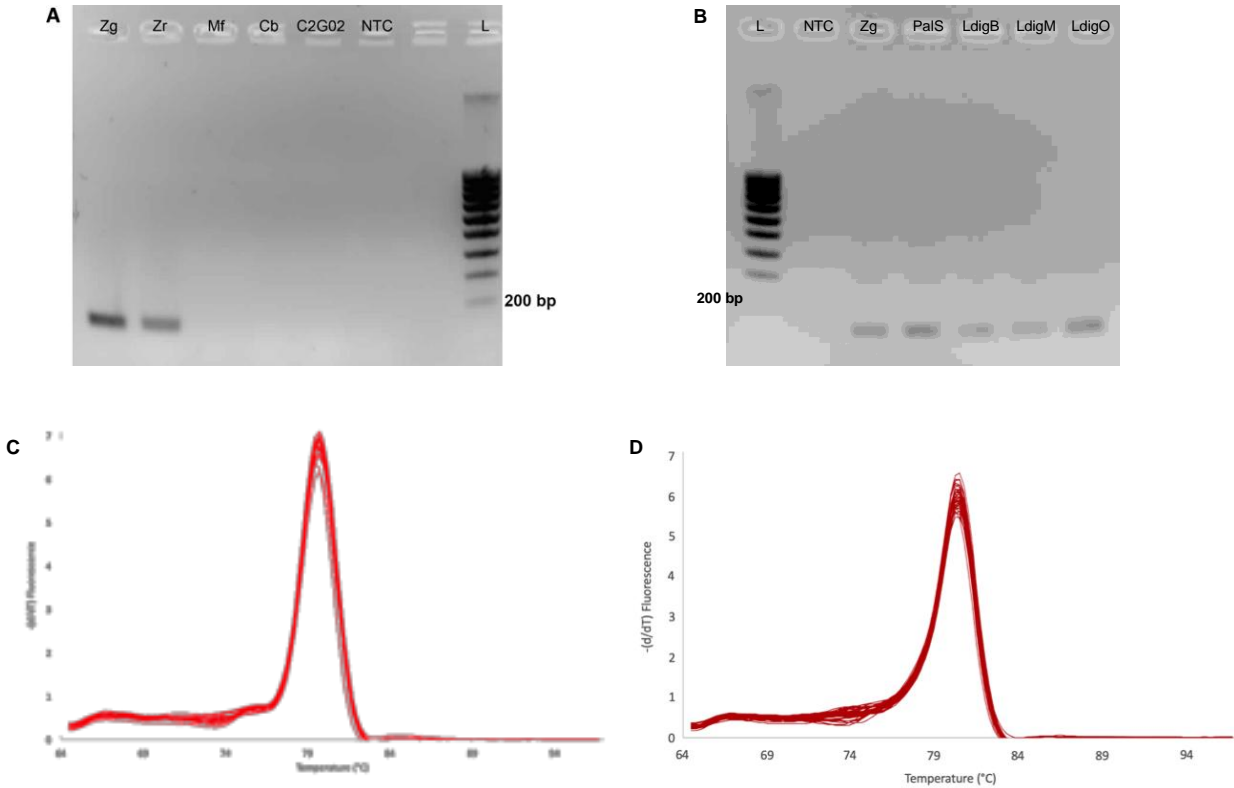
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60 **Supplementary Figure 1. Secondary structure of the *Z. galactanivorans* Dsij^T 16S rRNA V2 region.**
61 qPCR primers 142F/289R (A) and ZOB137 FISH probe and helpers (B) designed for the study are
62 highlighted. This graphical representation was prepared using the ARB secondary structure editor.
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Supplementary Figure 2. (A) Agarose gel electrophoresis of PCR products obtained on *Zobellia galactanivorans* Dsj1^T (Zg), *Z. russellii* KMM 3677^T (Zr), *Maribacter forsetii* KT02ds18-6^T (Mf), *Cellulophaga baltica* NN015840^T (Cb) and *Cellulophaga* sp. strain Asnod2-G02 (C2G02). NTC, no-template control; L, molecular weight ladder. (B) Agarose gel electrophoresis of PCR products obtained on DNA from *Zobellia galactanivorans* Dsj1^T pure culture (Zg) and four algae-associated microbiota (PpalS, LdigB, LdigM and LdigO; see details in Figure 4 legend). NTC, no-template control; L, molecular weight ladder. (C, D) Melting curves obtained from the qPCR assays on pure *Z. galactanivorans* Dsj1^T gDNA (C) and all environmental samples used in this study (D).

76 **Supplementary Files**

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79 **Supplementary File 1 (Fasta file).** List of the 139 aligned 16S rRNA gene sequences from flavobacteria,
80 including 52 *Zobellia* strains and 87 sequences representing 23 close genera, used to design *Zobellia*-
81 specific primers with DECIPHER.

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83 **Supplementary File 2 (Excel file).** Details of the qPCR assay on the environmental samples, following
84 the Minimum Information for publication of Quantitative real-time PCR Experiments (MIQE) guidelines.

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86 **Supplementary File 3 (Fasta file).** Sequences of qPCR products obtained from environmental samples
87 LdigO and PpalS. Ten clones were randomly picked for each environmental sample.

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