The following supplement accompanies the article

Screening bacterial metabolites for inhibitory effects against *Batrachochytrium dendrobatidis* using a spectrophotometric assay

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Supplement. The taxonomic affiliation of bacterial isolates that were totally inhibitory to *Batrachochytrium dendrobatidis* and details of the molecular methods that were used to generate these data

MATERIALS AND METHODS

DNA extraction

Axenic isolates in 400 µl molecular grade water were subjected to 3 freeze–thaw cycles (+70/–80°C; 10 min each) and then centrifuged at 7500 × *g* (5 min) to pellet the cells. The supernatant was used directly as a template in the DNA amplification reaction. If this was unsuccessful, isolates were extracted using a Qiagen DNeasy Blood and Tissue Kit as per the manufacturer's protocol, with pretreatment for Gram-negative bacteria.

Amplification of 16s rRNA gene

DNA from pure bacterial isolates was amplified by polymerase chain reaction (PCR) on Bio-Rad C1000/S1000 thermal cyclers with the bacteria-specific primer 8F (5'-AGA GTT TGA TCC TGG CTC AG-3') and the universal primer 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') (Lane 1991). The PCR reaction mix contained 0.2 µM of each primer, 0.2 mM dNTPs, 3 mM $MgCl_2$, 0.2 mg ml^{-1} bovine serum albumin, 1.25 U HotStar *Taq* polymerase (Qiagen) with 1× buffer and <1 µg template DNA. The thermocycling parameters were: 95°C for 15 min followed by 35 cycles of 94°C for 1 min, 48°C for 1 min, 72°C for 1.5 min and a final elongation for 10 min at 72°C. Presence of PCR products was examined by electrophoresis in 1.5% agarose gels stained with GelGreen™ (Biotium).

DNA sequencing

PCR product purification and DNA sequencing were conducted by Macrogen Inc. (South Korea). Forward and reverse nucleotide sequences were aligned in Geneious Pro (Biomatters; www.geneious.com) to create a consensus sequence of approximately 1400 bp. All consensus sequence editing and alignments were performed using the ClustalW algorithm (Larkin et al. 2007) within Geneious Pro. Nucleotide sequences were subject to a National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) search against those in the GenBank database (Benson et al. 2011), and identification was assigned based on the highest sequence similarity.

RESULTS

Table S1. Taxonomy of bacterial isolates that exhibited total inhibition against *Batrachochytrium dendrobatidis*

Taxonomy/GenBank closest match	GenBank accession no.	% sequence similarity	Number of frogs with taxon
Actinobacteria			
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Microbacteriaceae			
Curtobacterium flaccumfaciens	EU977762	99.5	1
Proteobacteria			
Gammaproteobacteria			
Pseudomonadaceae			
Pseudomonas fluorescens	GU726880	99.9	3
Pseudomonas sp.	HM996802	99.4	1
Pseudomonas fluorescens	FJ608707	99.7	1
Enterobacteriaceae			
Averyella dalhousiensis	DQ158204	99.6	1
Averyella dalhousiensis	DQ158205	99.2	1
Yokenella regensburgei	AB519796	99.6	1
Xanthomonadaceae			
Stenotrophomonas maltophilia	AY445079	99.7	1
Stenotrophomonas maltophilia	CP001111	100	1
Stenotrophomonas sp.	FJ193149	99.8	1
Luteibacter rhizovicinus	EU022023	99.7	1

LITERATURE CITED

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