The ISME Journal (2010) 4, 147–150

www.nature.com/ismej

SHORT COMMUNICATION

The opportunistic coral pathogen *Aspergillus sydowii* contains *dddP* and makes dimethyl sulfide from dimethylsulfoniopropionate

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The ascomycete *Aspergillus sydowii* is associated with a serious epizootic of sea fan corals in the Caribbean. Corals are rich in the compatible solute, dimethylsulfoniopropionate (DMSP), produced by their symbionts, the dinoflagellate *Symbiodinium.* As other *Aspergillus* species can catabolize DMSP, liberating dimethyl sulfide (DMS) in the process, we tested *A. sydowii* strains, obtained from diseased corals and other environments, for this Ddd$^+$ phenotype. All the strains, irrespective of their geographical or environmental origins, made DMS from DMSP, and all of them contained homologs (>87% identical) of the *dddP* gene, which encodes an enzyme that releases DMS from DMSP and which occurs in other Ddd$^+$ fungi and in some marine bacteria. The *dddP* gene was likely acquired by the *Aspergillus* fungi by inter-domain horizontal gene transfer from α-proteobacteria.
The ISME Journal (2010) **4,** 147–150; doi:10.1038/ismej.2009.102; published online 24 September 2009
Subject Category: geomicrobiology and microbial contributions to geochemical cycles
Keywords: *A. sydowii*; coral disease; *dddP*; DMS; DMSP; horizontal gene transfer

Corals are sensitive to pollution and thermal stress (Harvell *et al.*, 2007), making them susceptible to infection, exemplified by a severe epizootic of sea fan corals (*Gorgonia ventalina*) in the Caribbean, caused by the opportunistic fungus *Aspergillus sydowii* (Smith *et al.*, 1996; Geiser *et al.*, 1998; Hernández *et al.*, 2008). This pathogen can infect at least eight different species of octocorals (Smith and Weil, 2004), the dominant coral group on many Caribbean reefs, and has caused high rates of mortality throughout the region (Nagelkerken *et al.*, 1997, Kim and Harvell, 2004).

Corals contain photosynthetic dinoflagellates in the genus *Symbiodinium*, which have high intracellular concentrations of dimethylsulfoniopropionate (DMSP), an antistress molecule made by many marine phytoplankton (Hill *et al.*, 1995; Broadbent *et al.*, 2002; Sunda *et al.*, 2002; Jones *et al.*, 2007). When released from such organisms, other marine microbes can use several wholly different ways to catabolize DMSP (Yoch, 2002; Johnston *et al.*, 2007; Howard *et al.*, 2008). Worldwide, these biotransformations annually turn over $\sim 10^9$ tons of DMSP. Some of these pathways liberate dimethyl sulfide (DMS), an environmentally influential gas in its own right, as DMS oxidation products are cloud condensation nuclei, causing cloud cover over the oceans (Sievert *et al.*, 2007). As corals are hot spots for DMSP production, the levels of DMS downwind of the Great Barrier Reef are enhanced (Jones and Trevena, 2005) with possible effects on the abundance of nucleation particles (Modini *et al.*, 2009).

Some ascomycete fungi that occur in the rhizospheres of the salt marsh grass *Spartina*, which is one of the very few angiosperms that make DMSP (Otte *et al.*, 2004), can catabolize this molecule, liberating DMS in the process (Bacic and Yoch, 1998), a phenotype termed Ddd$^+$. This ability was also found in *Aspergillus oryzae*, the fermentative agent for soy sauce, in *Aspergillus flavus* and in the crop pathogens, *Fusarium graminearum* and *F. oxysporum* (Todd *et al.*, 2009). These Ddd$^+$ *Aspergillus* and *Fusarium* strains all contained a gene, termed *dddP*, whose product cleaves DMSP, with the release of DMS. The *dddP* gene likely originated in marine α-proteobacteria such as *Roseovarius*, in which it is important for DMSP

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Received 14 May 2009; revised and accepted 11 August 2009; published online 24 September 2009

Table 1 Dimethylsulfoniopropionate (DMSP)-dependent dimethyl sulfide (DMS) production in strains of *Aspergillus sydowii*

Strain (1)	*Morphology (2)*	*Source (3)*	*Location (4)*	*DMS (5)*
SOMB	G	Infected *Gorgonia ventalina*	Sombrero Reef, Florida	0.83 ± 0.09
SABA	W/P	Infected *G. ventalina*	Saba, Netherland Antilles	2.01 ± 0.48
DumpD	G	Infected *G. ventalina*	San Salvador, Bahamas	0.41 ± 0.03
FK11	G	Infected *G. ventalina*	Key West, Florida	0.5 ± 0.02
15B1	G	Infected *G. ventalina*	Tennessee Reef, Florida	2.89 ± 0.31
NRRL 242	G	Environmental	Austria	2.17 ± 0.16
NRRL 663	G	Environmental	Unknown	4.59 ± 0.20
NRRL 251	G	Environmental	Sri Lanka	0.54 ± 0.06
NRRL 247	W	Environmental	Florida	1.67 ± 0.03
KIR 382A	G	Environmental	Orinoco river sediment	2.31 ± 0.28
SRRC 2540	G	Environmental	Durban, South Africa	2.78 ± 0.44
NRRL 4790	G	Environmental	Japan	0.65 ± 0.28
NRRL 245	W/P	Environmental	Jamaica	0.31 ± 0.02
NRRL 249	W	Environmental	Philadelphia	0.26 ± 0.11
NRRL 1732	G	Environmental	Washington, DC	4.31 ± 0.04
NRRL 5913	G	Environmental	Unknown	3.22 ± 0.53
NRRL 244	G	Environmental	Japan	0.70 ± 0.02
NRRL 253	W/P	Infectious—human	Unknown	0.11 ± 0.02
297072	G	Infectious—human	St Paul, Minnesota	1.20 ± 0.04
SRRC 1112	G	Unknown	Australia	1.10 ± 0.03

Column (1) shows *A. sydowii* strains as in Rypien *et al.* (2008). Column (2) shows mycelial appearance on Potato Dextrose Agar (PDA): G = green, powdery sporulating; W = white, no sporulation; W/P = white, sporulating. Columns (3) and (4) show the source and location of the isolate, respectively. Column (5) shows levels of DMSP-dependent DMS production, in nmol DMS $h^{-1}\,mg^{-1}$ *A. sydowii* mycelial dry weight, with standard errors from two samples. Fungi were grown on solid PDA (Nicholson *et al.*, 1998) at 28 °C for 48 h. A plug of ~25 mm^2 from the growing edge of each mycelium was removed and placed in a sealed vial containing 5 mM DMSP in Vogel's minimal media. Levels of DMS were assayed after 6 h by gas chromatography in a flame photometric detector as in Todd *et al.* (2009).

catabolism and DMS emission (Todd *et al.*, 2009), and was then transferred to fungi by inter-domain horizontal gene transfer (HGT). Other *Aspergillus* species, such as *Aspergillus niger*, do not have a Ddd^+ phenotype and these lacked *dddP* (Todd *et al.*, 2009). As *A. sydowii* associates with DMSP-rich corals, we examined *A. sydowii* isolates, obtained from corals and from other environments for their Ddd phenotypes and for the presence of *dddP*.

All the *A. sydowii* strains examined made DMS from DMSP, with varying levels in different isolates (Table 1). There was no apparent link between DMS production and environmental source or mycelial morphology. Thus, strains from corals had low (strain SOMB) or high (SABA) activities, and 'terrestrial' strains, such as NRRL 242 from Austria, had above-average levels of production, as did 297072, from a human patient.

To test whether these strains, like other Ddd^+ ascomycete fungi, contained *dddP*, their genomic DNAs were used as PCR templates, with primers corresponding to conserved regions near the 5′ and 3′ termini of fungal *dddP*s (Figure 1). In all cases, a single PCR product of the expected size (1.2 kb), corresponding to ~88% of the total *dddP* gene was obtained. These PCR products were sequenced. They all contained a *dddP* homolog, whose DNA and polypeptide products were respectively ~85 and 91% identical to those of *A. oryzae*.

The *dddP* sequences in *A. sydowii* strains more closely resembled each other than *dddP* of *A. oryzae* and some were identical in different strains (Figure 1), so *dddP* was likely present in the last common ancestor of *A. sydowii*. There was no association between the *dddP* sequence and the origin of the *A. sydowii* isolates, consistent with the molecular evidence for a single global population in this species (Rypien *et al.*, 2008).

Aspergillus sydowii (and other fungi) probably acquired *dddP* by inter-domain HGT, either from a bacterium or indirectly from another fungal species (Todd *et al.*, 2009). The bacterial homologs that most closely resemble those in fungi are in a subclass of the DddP sequences in the Global Ocean Sampling metagenomic database of marine bacteria (Rusch *et al.*, 2007), so these are the likeliest sources of the *dddP* gene that was transferred to fungi by inter-domain HGT. Another very different gene, *dddD*, which encodes a class III Coenzyme A transferase that liberates DMS from DMSP, is also subject to HGT among distantly related proteobacteria. DddD homologs occur not only in marine α- and γ-proteobacteria that were already known to have Ddd^+ phenotypes, but also in the terrestrial bacteria, *Burkholderia ambifaria* and *Rhizobium* NGR234, both of which, perhaps significantly, interact with higher plants (Todd *et al.*, 2007).

Raina *et al.* (2009) recently isolated γ- and α-proteobacteria that grew on DMSP as sole carbon source from the mucus or skeletons of the coral *Montipora*, which interacts with DMSP-containing zooxanthellae (Hill *et al.*, 1995). Having shown here that at least some fungi that associate with corals have a Ddd^+ phenotype, it will be of interest to know the relative contributions of bacteria and

	F. oxy	*A. oryzae*	NRRL 263	NRRL 4790	Somb	Dump D	KIR 382A	NRRL 52277	Group A strains	NRRL 663	Group B strains	NRRL 5913	Group C strains
F. graminearum	194	195	210	212	210	212	214	216	214	216	215	216	216
F. oxysporum		160	199	200	201	204	200	201	203	200	199	200	200
A. oryzae			149	148	151	154	153	153	155	153	152	153	153
NRRL 263				26	19	24	28	19	26	23	26	25	27
NRRL 4790					15	18	20	24	22	16	15	16	16
Somb						3	17	17	15	12	13	14	14
Dump D							20	20	18	15	16	17	17
KIR 382A								8	4	13	10	11	11
NRRL 52277									6	16	16	17	17
Group A strains										13	14	15	15
NRRL 663											3	4	4
Group B strains												1	1
NRRL 5913													2

Figure 1 Numbers of nucleotide differences (out of 998) in *A. sydowii dddP* and corresponding regions of *dddP* in *A. oryzae*, *F. graminearum* and *F. culmorum* (Todd *et al.*, 2009) are shown, following comparisons with *Megalign*. Genomic DNA was isolated as in Rypien *et al.* (2008). DNA sequences were generated using primers 5′-GGACCRACTCCGCTGGCGTT-3′ and 5′-TCATARCCCGTCTCCGT CAC-3′ (where R = G or A), which were 238 bp 3′ of the *dddP* ATG start codon and 114 bp 5′ of its TGA stop codon, respectively. Using *PfuUltra* DNA polymerase (Stratagene, La Jolla, CA, USA) to amplify the genomic DNAs, this generated fragments of 1183 bps, which were sequenced. The group A, group B and group C strains each have identical sets of sequences as follows: group A; NRRL 245, NRRL 249; group B; 297072, SRRC 2540, FK11, 15B1, NRRL 251, NRRL 1732; group C; NRRL 242, NRRL 247. The analyses were carried out on 998 bps of unambiguous sequences, which are deposited at GenBank as follows: strain and accession number, respectively; 297072, GQ421799; DumpD, GQ421800; FK11, GQ421801; 15B1, GQ421802; KIR 382A, GQ421803; NRRL242, GQ421804; NRRL245, GQ421805; NRRL247, GQ421806; NRRL249, GQ421807; NRRL251, GQ421808; NRRL263, GQ421809; NRRL663, GQ421810; NRRL1732, GQ421811; NRRL4790, GQ421812; NRRL5913, GQ421813; NRRL52277, GQ421814; SRRC2540, GQ421815; Somb, GQ421816.

eukaryotic microbes in this important process in these critical ecosystems.

The ability to catabolize DMSP may confer selective advantage to those microbes, bacterial and fungal, which live in sites of high DMSP productivity, including corals, as it may give them access to an abundant substrate. Future study, involving the characterization of *A. sydowii* mutants that are defective in their Ddd^+ phenotype, may reveal whether this ability affects pathogenicity and/or colonization of corals or other traits, such as DMSP detoxification, nutrition or chemical signaling.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Alyson Lumley and Nancy Douglas for technical assistance and Andrew Curson and Matthew Sullivan for useful discussions. We thank the UK NERC for financial support.

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