The role of *Scarus ghobban* and *Diadema Savignyi* as potential vectors of coral pathogens on the reefs of SW Madagascar

Sheridan C and Eeckhaut I

* Marine biology lab, Pentagone, 6 Avenue du Champ de Mars, 7000 Mons, Belgium

Introduction

Although there are over 160 species of known corallivorous organisms (Rotjan and Lewis 2008), very little information is available on their role as vectors of potential coral pathogens. It was suggested that both marine vertebrates and invertebrates have the ability to transmit coral pathogens (*e.g.* Sussman et al. 2003, Aeby and Santavy 2006), though in some cases the mode of transmission remains unclear. In this study we investigated the role of two potential vectors of coral bacterial pathogens, the parrotfish *Scarus ghobban* (Fig. 1A) and the sea urchin *Diadema savignyi* (Fig. 1B), on the reefs of SW Madagascar (Toliara). This was achieved by comparing the composition of the bacterial communities associated with the jaws of these organisms to those found on healthy and diseased corals *(Echinopora hirsutissima*), and present in the overlying water column.

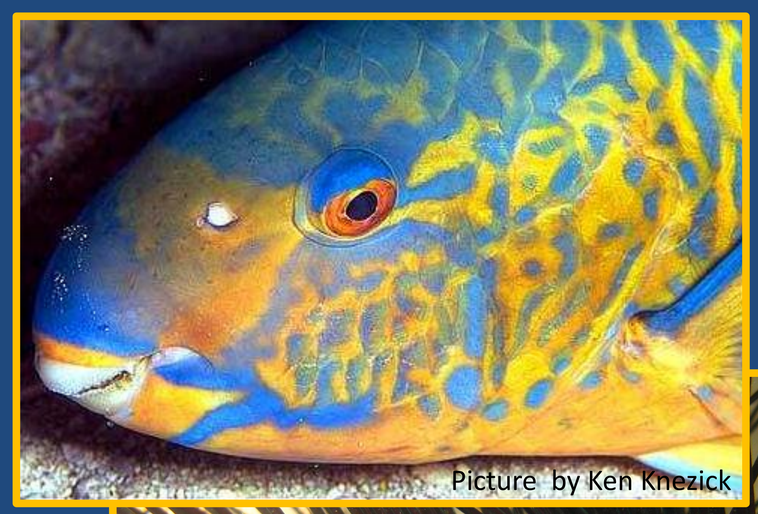

Figure 1. A - Bluebarred parrotfish (*Scarus ghobban*), B - sea urchin *Diadema savignyi*

Material and methods

Sampling (sterile swabs; 5 replicates / sample type)

- Jaws of *S. ghobban*
- Aristotle's lantern of *D. savignyi*
- Tissues of healthy *Echinopora hirsutissima*
- Tissues of diseased *E. hirsutissima*
- Water in vicinity of sampled *E. hirsutissima*

Bacterial cultures → **DNA extraction and amplification of 16S rDNA gene** → **DGGE – band extraction and re-amplification** → **Sequencing**

DGGE gels were analysed using ANOSIM in PRIMER 6.0 The presence of potential bacterial pathogens on the jaws of *S. ghobban* and D. *savignyi* was evaluated through comparison with the bacterial population of healthy and diseased (apparently affected by white syndrome) colonies of the coral *E. hirsutissima*, and through comparison with known bacterial sequences on BLAST (NCBI).

Table 1. Bacterial composition of swab samples analysed through bacterial culture and DGGE followed by sequencing. Numbers represent the amount of BLAST sequences obtained for each sample type and for each OTU.

Phylum	Class	Diseased *E. hirs.*		Diseased *E. hirs.*		Seawater		*D. savignyi*		*S. ghobban*	
		Culture	DGGE	Culture	DGGE	Culture	DGGE	Culture	DGGE	Culture	DGGE
Actinobacteria	Actinobacteridae			1							
Acidobacteria	Unknown										1
	Cytophaga	1									
Bacteroidetes	Unknown									1	
	Bacteroidia									1	
	Sphingobacteria									1	
	Flavobacteria										2
Firmicutes	Unknown			1							
	Bacillales	3	5	8	3		5	4			3
Fusobacteria	Unknown									1	
	Fusobacteriales									1	
Proteobacteria	Unknown			1							
	α-proteobacteria	1		1						5	6
	γ-proteobacteria	7		12		4		6	10		1
Unidentified			2		2			2		4	5

Table 2 Potential pathogens (known coral pathogens and bacteria associated with diseased coral tissues) identified among the various sample types.

	Diseased coral	Healthy coral	Sea water	*Diadema savigny*	*Scarus ghobban*
Uncultured bacteria isolated from black-band diseased coral				X	
Vibrio alginolyticus		X			X
Vibrio coralliilyticus	X				
Vibrio harveyi	X		X		X
Vibrio sp. 3E6 (from YBD lesion)				X	
Vibrio sp. PaD1.23	X				
Vibrio sp. PaD1.27	X				
Vibrio parahaemoliticus					X
Uncultured α-proteobacteria islated from diseased coral					X

Results and discussion

Sequences obtained from bacterial cultures and extracted DGGE bands of all sample types revealed bacteria from a variety of phyla. This double methodology (culture dependent and independent) approach limits the influence of bias associated with bacterial culture and PCR on the results. The bacterial communities of all sample types appeared to be dominated by Bacillales, α- and γ-proteobacteria (Table 1), which supports results obtained by Frias-Lopez et al. (2002; for coral samples only). Though these data provide interesting insight into the bacteria associated with *S. ghobban* and *D. savignyi* jaws (bacteria associated with seawater and hermatypic corals having been described in previous studies (*e.g.* Rohwer et al. 2002, Frias-Lopez et al. 2002)), this is by no means an extensive characterisation of the bacterial communities associated with these sample types.

Interestingly, several potential pathogens (known coral pathogens and bacteria associated with diseased coral tissues) were identified within the bacterial communities of all samples (predominantly in diseased *E. hirsutissima* and *S. ghobban* jaw samples (Table 2)). This suggests that, since *D. savignyi* and *S. ghobban* carry potential pathogens on their oral appendices, they could theoretically transfer these between coral hosts. However, some of these potential pathogens were also found within seawater and healthy *E. hirsutissima* samples (Table 2). This highlights the ubiquitous character of many coral pathogens and suggests that some of them could be present within the coral's normal bacterial community, and upregulated by certain factors (*e.g.* physical/environmental stress). As a result, the bacterial community of the coral host could shift to being dominated by diseased-associated bacteria (Mao-Jones et al. 2010). In theory, bite mark lesions resulting from the action of corallivorous organisms could therefore be sufficient to trigger such shifts, potentially resulting in disease development.

Reference list

Aeby GS, Santavy DL (2006) Factors affecting susceptibility of the coral Montastraea faveolata to black-band disease. Mar Ecol Prog Ser 318:103-110

Sussman M, Loya Y, Fine M, Rosenberg E (2003) The marine fireworm Hermodice carunculata is a winter reservoir and spring-summer vector of the coral – bleaching pathogen Vibrio shiloi. Environ Microbiol 5(4):250-255

Frias-Lopez J, Zerkle AL, Bonheyo GT, Fouke BW (2002) Appl Env Microbiol 68(5):2214-228

Mao-Jones J, Ritchie KB, Jones LE, Ellner SP (2010) How microbial community composition regulates coral disease development. PLoS Biol 8(3):e1000345

Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser 243:1-10

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