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Catabolite regulation of enzymatic activities in a white pox pathogen and commensal bacteria during growth on mucus polymers from the coral *Acropora palmata*

Cory J. Krediet[1], Kim B. Ritchie[2,3], Max Teplitski[1,3,*]

[1]Interdisciplinary Ecology Graduate Program, School of Natural Resources and Environment, University of Florida, Institute of Food and Agricultural Sciences (IFAS), 103 Black Hall, PO Box 116455, Gainesville, Florida 32611, USA

[2]Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, Florida 34236, USA

[3]Department of Soil and Water Science, University of Florida, IFAS, 106 Newell Hall, PO Box 110510, Gainesville, Florida 32611, USA

ABSTRACT: Colonization of host mucus surfaces is one of the first steps in the establishment of coral-associated microbial communities. Coral mucus contains a sulfated glycoprotein (in which oligosaccharide decorations are connected to the polypeptide backbone by a mannose residue) and molecules that result from its degradation. Mucus is utilized as a growth substrate by commensal and pathogenic organisms. Two representative coral commensals, *Photobacterium mandapamensis* and *Halomonas meridiana*, differed from a white pox pathogen *Serratia marcescens* PDL100 in the pattern with which they utilized mucus polymers of *Acropora palmata*. Incubation with the mucus polymer increased mannopyranosidase activity in *S. marcescens*, suggestive of its ability to cleave off oligosaccharide side chains. With the exception of glucosidase and *N*-acetyl galactosaminidase, glycosidases in *S. marcescens* were subject to catabolite regulation by galactose, glucose, arabinose, mannose and *N*-acetyl-glucosamine. In commensal *P. mandapamensis*, at least 10 glycosidases were modestly induced during incubation on coral mucus. Galactose, arabinose, mannose, but not glucose or *N*-acetyl-glucosamine had a repressive effect on glycosidases in *P. mandapamensis*. Incubation with the mucus polymers upregulated 3 enzymatic activities in *H. meridiana*; glucose and galactose appear to be the preferred carbon source in this bacterium. Although all these bacteria were capable of producing the same glycosidases, the differences in the preferred carbon sources and patterns of enzymatic activities induced during growth on the mucus polymer in the presence of these carbon sources suggest that to establish themselves within the coral mucus surface layer commensals and pathogens rely on different enzymatic activities.

KEY WORDS: Coral microbiology · Mucus surface polysaccharide · Coral pathogen

INTRODUCTION

Attachment, colonization and establishment on host mucus surfaces are the first steps in the interactions between bacteria and their eukaryotic hosts (Kurz et al. 2003, Ritchie & Smith 2004, Rosenberg & Falkovitz 2004, Nehme et al. 2007). Pathogens and commensals differ in the way they colonize host surfaces and degrade components of the host surface-associated mucus (Vine et al. 2004, Fabich et al. 2008). In studies of bacteria associated with fish and corals, pathogens rapidly degraded host surface mucus and reached high numbers (Vine et al. 2004, Sharon & Rosenberg 2008). When grown on host mucus *in vitro,* populations of commensal bacteria stabilized at densities that were at least an order of magnitude lower than those reached

*Corresponding author. Email: maxtep@ufl.edu

by pathogens (Vine et al. 2004). Similarly, *Vibrio* spp., which are related to coral pathogens, dominated microbial communities formed on mucus of the coral *Oculina patagonica* after an extended incubation *in vitro* (Rosenberg & Falkovitz 2004, Sharon & Rosenberg 2008), even though vibrios make up less than 5% of culturable bacteria in the mucus layer of this coral under normal conditions (Koren & Rosenberg 2006). Collectively, these observations support the hypothesis that in the absence of other factors, opportunistic pathogens can overgrow and dominate coral-associated microbial communities, thus contributing to the appearance of disease signs (Ritchie 2006).

This hypothesis also implies that opportunistic pathogens of corals have the ability to outcompete native coral-associated bacteria under the conditions that favor this overgrowth. Consistent with this hypothesis, of all the tested coral and environmental bacteria, *Serratia marcescens* PDL100 reached the highest final population density when grown *in vitro* on mucus of *Acropora palmata* (Krediet et al. 2009). This strain of *S. marcescens* was originally isolated as a cause of white pox, a rapidly progressing coral tissue necrosis of the threatened Caribbean coral *A. palmata* (Patterson et al. 2002). The observations that the white pox strain was more efficient at utilizing mucus of the host coral *in vitro* suggested that this ability to efficiently degrade mucus may be linked to its pathogenicity (Krediet et al. 2009).

The mechanisms by which *Serratia marcescens* PDL100 colonizes, infects, and causes disease in corals are not yet clear. The progression of infections caused by *Serratia* spp. in other invertebrates suggests that colonization of host surfaces is the first in a series of events leading to the appearance of disease signs. For example, to cause disease in nematodes and flies, *S. marcescens* first colonizes the intestines, degrades cells of the alimentary tract, and then spreads to other organs (Kurz et al. 2003, Nehme et al. 2007). Based on studies in other invertebrates, it seems likely that *S. marcescens* PDL100 first needs to attach to and then establish itself within the coral mucus surface layer.

The ability to utilize coral mucus as a growth substrate involves glycosidases, proteases and esterases (Vacelet & Thomassin 1991, Krediet et al. 2009). These enzymatic activities are generally consistent with the composition of coral mucus. The major component of the coral mucus surface layer is a sulfated glycoprotein, and mucus also contains a significant amount of low molecular weight compounds that probably result from microbial degradation of the polymers (Meikle et al. 1988). In mucus of acroporid corals, arabinose, *N*-acetyl-glucosamine, mannose, glucose, galactose, *N*-acetyl-galactosamine and fucose are the major sugars; serine and threonine are the major amino acids; and lipids make up 4.2% of the mucus (Ducklow & Mitchell 1979, Meikle et al. 1988). The chemical composition of the mucus glycoprotein differs among coral species (Ducklow & Mitchell 1979, Molchanova et al. 1985, Meikle et al. 1987, 1988, Klaus et al. 2007). Mucus of *Acropora formosa*, for example, contains 36 to 38% neutral sugars, 18 to 22% amino sugars and 19 to 30% amino acids (Meikle et al. 1988). The oligosaccharide decorations (2 to 4 sugar residues in length) are attached to the polypeptide backbone by an *O*-glycosidic linkage to serine or threonine through the carbon 1 of mannose (Meikle et al. 1987). The glycoproteins from *A. formosa* contain terminal arabinose residues linked by a β 1→3 bond (Meikle et al. 1987). The entire spectrum of enzymatic activities involved in the degradation of coral mucus by pathogenic, commensal and environmental bacteria is not yet characterized.

Preparations of coral mucus contain a significant amount of low molecular weight compounds (Meikle et al. 1987, 1988, Krediet et al. 2009). While the chemical nature of these compounds is not yet known, the low molecular weight fraction of mucus most likely contains simple sugars and oligosaccharides that resulted from enzymatic degradation of the polymer. Even though these compounds appear to be plentiful in mucus, their effect on enzymatic activities in coral mucus-associated bacteria is not yet known. Kuntz et al. (2005) hinted at a potential role of sugars in coral ecology: exposure of coral fragments to sugars at 5 to 25 mg l^{-1} led to the appearance of various disease signs. For example, a 30 d treatment with lactose (at 25 mg l^{-1}), a disaccharide that is not known to occur in the coral reef environment, led to nearly 100% mortality of the *Montastraea annularis* fragments; however, survivorship of *Porites furcata* was only modestly reduced by the treatment (Kuntz et al. 2005). A 30 d incubation with mannose caused significant mortality in *P. furcata* but not in *M. annularis* (Kuntz et al. 2005). It is not yet clear whether these observed pathologies and mortalities were due to (1) the destabilization of the symbiosis between the coral animal and its photosynthetic dinoflagellates, (2) potential growth promotion of opportunistic pathogens by the easily metabolizable carbon sources, or (3) a role of these sugars in affecting virulence gene regulation in the existing microbial communities.

In order to better understand functions of carbon sources in the behavior of coral-associated bacteria, the present study tested the hypothesis that simple sugars, which would be released from the mucus glycoprotein by enzymatic digest, affect behaviors involved in the interactions of bacteria with mucus of the coral host (e.g. enzymatic degradation and bacterial attachment to mucus).

The results of the present study demonstrated significant differences in the sequence with which the white

pox pathogen and 2 coral commensals utilized *Acropora palmata* mucus *in vitro.* The white pox pathogen *Serratia marcescens* PDL100 rapidly downregulates some of its enzymatic activities depending on the availability of simple sugars, and the catabolite repression was relieved within the 2 to 18 h incubation. Feedback inhibition of enzymatic activities occurred during extended incubation (18 h) in the presence of simple sugars. In the commensal bacteria, catabolite repression occurred during the extended incubation (2 to 18 h); neither glucose nor *N*-acetyl-glucosamine had a catabolite repressive effect in the coral commensal *Photobacterium mandapamensis*. These differences in the preferred substrates may explain how *S. marcescens* and other opportunistic pathogens become established within the coral surface mucus layer.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *Serratia marcescens* PDL100 is an isolate associated with white pox of *Acropora palmata* (Patterson et al. 2002). *Photobacterium mandapamensis* 33C12 and *Halomonas meridiana* 33E7 were isolated from mucus of *A. palmata* based on their ability to grow on a medium supplemented with coral mucus (Ritchie 2006). *S. marcescens* ATCC43422 (isolated from human throat) was purchased from the American Type Culture Collection (ATCC, Manassas, Virginia, USA).

Serratia isolates were grown in Luria-Bertani (LB) broth (Fisher Scientific). Bacteria isolated from *Acropora palmata* mucus were routinely grown in glycerol artificial seawater (GASW) broth (356 mM NaCl, 40 mM $MgSO_4$, 20 mM $MgCl_2 \cdot 6H_2O$, 8 mM KCl, 60 µM K_2HPO_4, 33 µM Tris, 7 µM $FeSO_4$, with 0.05% peptone, 0.2% yeast extract and 2.0% glycerol; pH 7.0) or on GASW medium solidified with 1.5% agar (Fisher Scientific) (Ritchie 2006). Unless otherwise stated in text, seawater used in the experiments was collected at Crescent Beach, Florida, USA (29° 45′ 56″ N, 81° 15′ 11′ W) and sterilized through a 0.22 µm filter. As needed for experiments, seawater was buffered with 10 mM HEPES to pH 7.

Coral mucus was collected from 2 asymptomatic *Acropora palmata* colonies at Looe Key Reef, Florida (24° 33′ 75″ N, 81° 24′ 05″ W) in August and September 2006 with a needleless syringe as in Ritchie (2006). Mucus was stored as aliquots at −80°C. As needed, aliquots were thawed and exposed to UV irradiation in plastic tubes (254 nm) for 20 min. Preparations were then filtered through a glass fiber GFC filter, followed by filter-sterilization through 0.45 and 0.22 µm filters. Mucus was then separated into low and high molecular weight fractions with VisaSpin-15 spin dialysis assemblies (VivaScience), following manufacturer's instructions. The low molecular weight fraction was not used in the studies presented here. The high molecular weight fraction was brought up to volume in artificial seawater (Instant Ocean).

Artificial mucus was made by supplementing artificial seawater with 169.2 g l^{-1} L-arabinose, 64.8 g l^{-1} D-mannose, 104.4 g l^{-1} *N*-acetyl-glucosamine, 3.6 g l^{-1} corn starch, 7.2 g l^{-1} D-galactose (all sugars from Acrōs Organics) and 300 g l^{-1} casamino acids (Sigma Aldrich). This mixture crudely approximates the composition of *Acropora* mucus reported previously (Meikle et al. 1988).

Enzymatic assays. Two overnight cultures of each isolate were grown in LB or in GASW broths to an optical density at 600 nm (OD_{600}) of 2.0. Cells were pelleted, washed in filter-sterilized seawater and resuspended in the same volume of HEPES-buffered seawater. Bacteria were starved in buffered seawater at 30°C while shaking for 3 d. Following starvation, 1 ml of the cell suspension in buffered seawater was added to 2 ml of high molecular weight coral mucus preparation with 0.1% (w/v) of one the following sugars: D-glucose, *N*-acetyl-D-glucosamine, D-mannose, L-arabinose or D-galactose (Acrōs Organics). A negative control (coral mucus and buffered seawater without supplementation with sugars) was carried out in parallel. Enzymatic assays were conducted using chromogenic *p*-nitro-phenyl substrates (Sigma Aldrich) after 2 or 18 h of incubation in mucus (at 30°C) following published protocols (Miller 1972). All enzymatic reactions were conducted for exactly 24 h; cellular debris and unused enzymatic substrate were then pelleted at 16 000 × *g*. The supernatants were then transferred to a clear polystyrene 96-well plate and absorbance at 405 nm (A_{405}) was measured on a Victor-3 plate reader (Perkin Elmer). Buffered seawater and coral mucus were included in each plate as blanks. Enzymatic activities in the coral mucus treatment were measured and subtracted from all other treatments and the activity was then calculated using modified Miller Units (A_{405}/A_{590}).

Biofilm assays. For the biofilm assays, bacterial cultures were grown overnight to an approximate OD_{600} of 2.0. Biofilms were set up either in 3-N-morpholino-2-hydroxpropane sulfonic acid (MOPS) buffered colony-forming antigen (CFA) medium or in seawater on polystyrene surfaces coated with crude coral mucus. Buffered CFA medium contained 10 g l^{-1} casamino acids, 1.5 g l^{-1} yeast extract, 50 mg l^{-1} $MgSO_4$ and 5 mg l^{-1} $MnCl_2$, buffered with 0.1 M MOPS (pH 7). To test biofilm formation in CFA, overnight bacterial cultures were washed and then resuspended in 0.1 M MOPS-buffered CFA, and 100 µl of the cell suspension were added to the wells of a 96-well polystyrene

microtiter plate (Fisher Scientific). After 48 h of incubation, biofilms were stained with 25 µl 1% crystal violet and biofilm formation was quantified as before (Jackson et al. 2002, Teplitski et al. 2006a).

To test the role of individual mucus monomers in attachment to coral mucus-coated surfaces, 50 µl of the mucus preparation (buffered with 10 mM HEPES to pH 7) was incubated in each well of a polystyrene microtiter plate for 1 h, after which 40 µl of the liquid were aspirated and discarded, and the residue was dried at 30°C overnight in a microbiological hood, as in Bavington et al. (2004). The inoculum was prepared by growing bacterial cultures overnight in LB or in GASW broth at 30°C, then washing them twice in filter-sterilized buffered seawater. One hundred microliters of the suspension were added to the wells coated with mucus. As indicated, assays were supplemented with 0.1 or 1% (w/v) of individual sugars that occur in coral mucus (D-mannose, L-arabinose, D-galactose, *N*-acetyl-D-glucosamine). As a control, biofilm formation in wells coated with artificial mucus was measured in parallel. The effects of simple sugars on biofilm formation were analyzed through 1-way ANOVA and Tukey's Honestly Significant Diffference (HSD) for post hoc comparison of means. All statistical tests were performed using SAS (SAS Institute).

RESULTS AND DISCUSSION

Enzymatic degradation of the high molecular weight fraction of coral mucus

As shown in Table 1, α-D-glucopyranosidase, *N*-acetyl-α-D-galactosaminidase, β-D-galactopyranosidase and α-L-arabinopyranosidase were strongly and constitutively active in *Serratia marcescens* PDL100. The corresponding substrates are present within the mucus polymer of acroporid corals (Meikle et al. 1987). Incubation with the high molecular weight fraction of coral mucus induced only D-mannopyranosidase activity in this organism as compared to starved cells without mucus. During early growth on mucus, mannosidases were not induced in the coral commensals or the human mucosal pathogen *S. marcescens* ATCC43422, and the constitutive levels of mannosidase activities were also low in these bacteria (Table 1). Because mannose residues connect oligosaccharide decorations to the polypeptide backbone of the mucus glycoprotein (Meikle et al. 1987), these results may suggest that to utilize coral mucus, the white pox pathogen cleaves off the oligosaccharide side

Table 1. Enzymatic activities during growth on the high molecular weight fraction of mucus from *Acropora palmata*. Enzymatic activities that increased (*italics*) or decreased (**bold**) at least 2-fold after incubation (2 or 18 h) with the high molecular weight fraction of mucus from *A. palmata* are indicated

Enzyme	Starved	2 h	18 h
***Serratia marcescens* PDL100**			
N-acetyl-β-D-galactosaminidase	256.21 ± 18.46	171.64 ± 24.00	130.35 ± 5.22
α-D-galactopyranosidase	6.91 ± 0.58	13.14 ± 1.11	**1.98 ± 0.64**
β-D-galactopyranosidase	13.60 ± 1.07	16.91 ± 1.97	9.11 ± 0.14
α-D-glucopyranosidase	91.70 ± 12.91	60.26 ± 6.04	161.77 ± 1.99
β-D-glucopyranosidase	17.03 ± 1.58	16.90 ± 0.44	**4.45 ± 0.80**
α-L-arabinopyranosidase	13.02 ± 1.43	17.86 ± 0.31	11.83 ± 0.08
β-L-arabinopyranosidase	4.61 ± 0.33	6.44 ± 0.96	**1.86 ± 0.30**
α-L-fucopyranosidase	7.70 ± 0.43	10.14 ± 1.18	**0.62 ± 0.21**
β-D-fucopyranosidase	10.81 ± 1.11	17.24 ± 2.65	**1.32 ± 0.33**
α-D-mannopyranosidase	3.06 ± 0.70	*6.83 ± 1.79*	1.72 ± 0.39
β-D-mannopyranosidase	9.22 ± 1.71	*33.22 ± 5.46*	**2.89 ± 0.85**
***Serratia marcescens* 43422**			
N-acetyl-β-D-galactosaminidase	63.99 ± 4.50	*146.86 ± 5.76*	*160.83 ± 6.53*
α-D-galactopyranosidase	0.65 ± 0.03	0.22 ± 0.63	0.31 ± 0.53
β-D-galactopyranosidase	4.90 ± 0.20	*9.80 ± 0.23*	*10.31 ± 0.57*
α-D-glucopyranosidase	40.03 ± 1.92	*152.33 ± 6.25*	*135.59 ± 2.50*
β-D-glucopyranosidase	0.36 ± 0.06	*3.68 ± 0.96*	*3.49 ± 1.00*
α-L-arabinopyranosidase	2.48 ± 0.15	*11.17 ± 0.02*	*10.18 ± 0.31*
β-L-arabinopyranosidase	1.16 ± 0.05	**0.29 ± 0.26**	1.30 ± 0.22
α-L-fucopyranosidase	0.34 ± 0.03	0.39 ± 0.27	0.50 ± 0.23
β-D-fucopyranosidase	0.82 ± 0.05	0.55 ± 0.39	0.85 ± 0.34
α-D-mannopyranosidase	0.91 ± 0.04	**0.27 ± 0.34**	0.97 ± 0.31
β-D-mannopyranosidase	0.88 ± 0.03	1.75 ± 0.82	2.12 ± 0.77
***Photobacterium mandapamensis* 33C12**			
N-acetyl-β-D-galactosaminidase	8.53 ± 0.24	8.25 ± 0.68	*49.08 ± 1.25*
α-D-galactopyranosidase	0.85 ± 0.03	0.65 ± 0.52	*5.07 ± 0.42*
β-D-galactopyranosidase	0.74 ± 0.02	1.03 ± 0.08	*2.44 ± 0.01*
α-D-glucopyranosidase	1.29 ± 0.06	0.84 ± 0.34	*3.88 ± 0.22*
β-D-glucopyranosidase	0.94 ± 0.07	*4.19 ± 0.94*	*3.69 ± 0.60*
α-L-arabinopyranosidase	0.69 ± 0.04	1.19 ± 0.34	*2.68 ± 0.28*
β-L-arabinopyranosidase	0.99 ± 0.09	1.54 ± 0.27	*3.39 ± 0.21*
α-L-fucopyranosidase	0.71 ± 0.01	0.56 ± 0.25	*2.23 ± 0.26*
β-D-fucopyranosidase	0.74 ± 0.03	*1.49 ± 0.39*	*3.02 ± 0.58*
α-D-mannopyranosidase	0.85 ± 0.25	1.27 ± 0.39	*3.40 ± 0.29*
β-D-mannopyranosidase	0.99 ± 0.04	2.42 ± 0.80	2.52 ± 0.82
***Halomonas meridiana* 33E7**			
N-acetyl-β-D-galactosaminidase	2.33 ± 0.24	4.52 ± 0.54	*6.16 ± 0.30*
α-D-galactopyranosidase	0.88 ± 0.00	*2.13 ± 0.34*	*2.71 ± 0.62*
β-D-galactopyranosidase	0.77 ± 0.01	**0.11 ± 0.07**	0.93 ± 1.04
α-D-glucopyranosidase	313.81 ± 1.67	257.22 ± 13.03	220.89 ± 4.21
β-D-glucopyranosidase	1.53 ± 0.00	2.05 ± 0.73	1.28 ± 0.44
α-L-arabinopyranosidase	1.19 ± 0.03	**0.39 ± 0.31**	**0.47 ± 0.07**
β-L-arabinopyranosidase	1.33 ± 0.00	0.87 ± 0.27	1.00 ± 1.14
α-L-fucopyranosidase	0.91 ± 0.00	0.67 ± 0.33	*2.98 ± 0.88*
β-D-fucopyranosidase	0.90 ± 0.04	0.73 ± 0.06	1.18 ± 0.29
α-D-mannopyranosidase	0.87 ± 0.00	1.06 ± 0.26	0.65 ± 0.29
β-D-mannopyranosidase	1.14 ± 0.02	1.99 ± 0.81	1.07 ± 0.41

chains. The cleavage of the oligosaccharide side chains may facilitate enzymatic access to the polypeptide backbone. Oligosaccharides resulting from this cleavage are likely taken up by the cells and/or degraded by constitutively active glycosidases.

In coral commensal bacteria, incubation with the high molecular weight fraction of the *Acropora palmata* mucus induced several new activities (Table 1). The 2 h incubation with the high molecular weight fraction of mucus resulted in only modest (2- to 3-fold) upregulation of 2 enzymatic activities in *Photobacterium mandapamensis* 33C12. Ten new activities were induced in this organism during the extended incubation on mucus. Only 4 new activities were upregulated in *Halomonas meridiana* within the same time frame (Table 1). The α-D-glucopyranosidase enzyme was constitutively and strongly active in *H. meridiana* (Table 1). This enzyme is induced by the incubation on mucus in *P. mandapamensis* and *S. marcescens* ATCC43422, suggesting that α-D-glucopyranosidase may be involved in the degradation of coral mucus.

The differences in the pattern of enzymatic activities induced during growth of commensals and pathogens on the high molecular weight fraction of coral mucus suggest that they rely on different catabolic activities to establish within their preferred ecological niche. The enzymatic hydrolysis of the preferred bonds would release simple sugars, and this in turn may have a regulatory effect on the enzymatic activities involved in mucus degradation. The regulatory effects of various carbon sources on gene expression in bacteria through catabolite repression are well documented (Deutscher 2008, Gorke & Stulke 2008). Therefore, we tested a potential regulatory role of simple sugars in the degradation of the mucus polymer.

Catabolite control of coral mucus degradation by simple sugars

Commensal and pathogenic bacteria utilize components of host mucus using different strategies, and these differences may help them occupy different ecological niches. For example, commensal *Escherichia coli* and enterohaemorrhagic *E. coli* O157:H7 are capable of utilizing essentially the same carbon sources (Chang et al. 2004, Fabich et al. 2008). However, temporal regulation of enzymatic activities and timing of carbon source utilization during growth on the components of mouse intestinal mucus were different in these 2 conspecifics. Mutational inactivation of the corresponding pathways affected fitness of the bacteria during colonization of the mouse gut. The same mutations in the commensal and enterohaemorrhagic *E. coli* had different effects on fitness of the 2 strains (Fabich et al. 2008). These results suggested that the pattern and temporal regulation of host mucus utilization are central to the ability of commensals and pathogens to establish within the available ecological niches of the host mucus layer.

To begin learning how various components of coral mucus are utilized by commensals and a model pathogen, the present study tested the effect of simple sugars present in coral mucus on the enzymatic activities induced in the white pox pathogen *Serratia marcescens* PDL100, a human pathogen *S. marcescens* ATCC43422 and 2 coral commensals (*Photobacterium mandapamensis* 33C12 and *Halomonas meridiana* 33E7). Glucose, *N*-acetyl-glucosamine, mannose, arabinose and galactose (at 0.1% w/v) were selected for 3 reasons: (1) the corresponding residues are known to occur in the mucus glycoprotein of acroporid corals (Meikle et al. 1987); (2) arabinose, galactose and *N*-acetyl-glucosamine are terminal residues in the oligosaccharide decorations of the mucus glycoprotein, while the mannose residue invariably connects the oligosaccharide side chain to the polypeptide backbone (Meikle et al. 1987); and (3) glucose is a known catabolite repressor of gene regulation in enterobacteria and photobacteria (Nealson et al. 1972, Jackson et al. 2002, Teplitski et al. 2006b, Deutscher 2008, Gorke & Stulke 2008).

As shown in Table 2, the addition of simple sugars to mucus-grown cultures of *Serratia marcescens* PDL100 had a strong effect during the first 2 h, and the catabolite repression appears to be relieved — at least partially — after 18 h of incubation with the simple sugars (Table 3). The 2 h incubation on the mucus polymer in the presence of glucose strongly repressed activities of 8 (out of 11 tested) enzymatic activities by 2- to 144-fold in *S. marcescens* PDL100. Activities of fucopyranosidase and mannopyranosidase were repressed the strongest. The addition of *N*-acetyl-glucosamine or mannose had similarly strong repressive effects on enzymatic activities in *S. marcescens* PDL100. Treatment with arabinose or galactose had a lesser effect on the enzymatic activities in *S. marcescens* PDL100 (Table 2) and the treatment with glucose, arabinose or galactose strongly upregulated α-D-glucopyranosidase during the first 2 h. These results suggest that arabinose and galactose are not the preferred carbon sources. *N*-acetyl-galactosaminidase and α-D-glucopyranosidase were not subject to catabolite repression (Table 2). These results suggest a mechanism by which *S. marcescens* PDL100 may outcompete the native microbiota: during the starvation period it strongly upregulates several enzymatic activities and — when the substrates are present — these enzymes hydrolyze the corresponding substrates. The availability of preferred carbon sources downregulates other enzymatic

Table 2. Enzymatic activities detected during the 2 h incubation on the high molecular weight fraction of mucus (M) from *Acropora palmata* in the presence of simple sugars. Simple sugars added: galactose (Gal), glucose (Glu), arabinose (Ara), mannose (Man) and *N*-acetyl-glucosamine (NGlu). Enzymatic activities that increased (*italics*) or decreased (**bold**) at least 2-fold after incubation are indicated

Enzyme	M	M + Gal	M + Glu	M + Ara	M + Man	M + NGlu
***Serratia marcescens* PDL100**						
N-acetyl-β-D-galactosaminidase	171.64 ± 24.00	176.45 ± 5.36	158.72 ± 5.22	177.44 ± 0.29	97.45 ± 3.76	118.42 ± 5.44
α-D-galactopyranosidase	13.14 ± 1.11	**1.44 ± 0.62**	**0.74 ± 0.64**	**0.90 ± 0.64**	**0.19 ± 0.61**	**0.85 ± 0.41**
β-D-galactopyranosidase	16.91 ± 1.97	20.96 ± 0.46	**8.29 ± 0.04**	24.96 ± 0.30	**2.39 ± 0.12**	**3.27 ± 0.33**
α-D-glucopyranosidase	60.26 ± 6.04	*163.10 ± 5.02*	*244.83 ± 2.01*	*166.55 ± 2.75*	36.69 ± 0.12	40.52 ± 15.81
β-D-glucopyranosidase	16.90 ± 0.44	*4.07 ± 0.95*	**3.33 ± 1.01**	**4.05 ± 1.02**	**2.91 ± 0.89**	**8.03 ± 0.20**
α-L-arabinopyranosidase	17.86 ± 0.31	17.36 ± 0.11	11.46 ± 0.20	18.85 ± 0.18	**0.90 ± 0.29**	**1.73 ± 0.11**
β-L-arabinopyranosidase	6.44 ± 0.96	**1.58 ± 0.28**	**1.22 ± 0.29**	**1.63 ± 0.32**	**0.33 ± 0.24**	**0.08 ± 0.13**
α-L-fucopyranosidase	10.14 ± 1.18	**0.76 ± 0.27**	**0.07 ± 0.29**	**0.81 ± 0.29**	**2.88 ± 0.19**	**4.32 ± 0.29**
β-D-fucopyranosidase	17.24 ± 2.65	**0.90 ± 0.36**	**0.78 ± 0.40**	**0.92 ± 0.41**	**0.54 ± 0.38**	**0.07 ± 0.24**
α-D-mannopyranosidase	6.83 ± 1.79	**1.49 ± 0.38**	**1.01 ± 0.38**	**1.55 ± 0.41**	**0.97 ± 0.40**	**0.41 ± 0.15**
β-D-mannopyranosidase	33.22 ± 5.46	**2.64 ± 0.83**	**2.03 ± 0.82**	**2.70 ± 0.87**	**2.04 ± 0.84**	**1.57 ± 0.70**
***Serratia marcescens* 43422**						
N-acetyl-β-D-galactosaminidase	146.86 ± 5.76	165.29 ± 1.23	106.16 ± 1.78	168.07 ± 0.20	191.86 ± 6.18	110.79 ± 4.38
α-D-galactopyranosidase	0.22 ± 0.63	*1.58 ± 0.62*	0.31 ± 0.59	0.88 ± 0.61	0.72 ± 0.61	0.05 ± 0.12
β-D-galactopyranosidase	9.80 ± 0.23	9.54 ± 0.23	7.19 ± 0.20	8.16 ± 0.01	**4.15 ± 0.05**	7.14 ± 0.15
α-D-glucopyranosidase	152.33 ± 6.25	82.21 ± 4.36	114.81 ± 6.45	82.93 ± 0.97	91.39 ± 2.91	130.16 ± 2.61
β-D-glucopyranosidase	3.68 ± 0.96	4.39 ± 1.00	3.93 ± 0.95	4.37 ± 1.01	4.21 ± 1.02	4.03 ± 0.95
α-L-arabinopyranosidase	11.17 ± 0.02	8.63 ± 0.17	7.58 ± 0.07	7.32 ± 0.80	**3.76 ± 0.22**	7.47 ± 0.05
β-L-arabinopyranosidase	0.29 ± 0.26	*1.50 ± 0.32*	0.50 ± 0.25	*1.42 ± 0.31*	*1.34 ± 0.28*	0.48 ± 0.03
α-L-fucopyranosidase	0.39 ± 0.27	0.80 ± 0.29	0.39 ± 0.27	0.75 ± 0.28	0.71 ± 0.28	0.64 ± 0.26
β-D-fucopyranosidase	0.55 ± 0.39	1.16 ± 0.32	0.77 ± 0.41	0.14 ± 0.63	*1.26 ± 0.42*	0.99 ± 0.37
α-D-mannopyranosidase	0.27 ± 0.34	*1.24 ± 0.40*	0.45 ± 0.40	*1.16 ± 0.37*	*1.05 ± 0.34*	0.65 ± 0.28
β-D-mannopyranosidase	1.75 ± 0.82	2.56 ± 0.85	1.88 ± 0.85	2.45 ± 0.84	2.38 ± 0.84	1.53 ± 0.45
***Photobacterium mandapamensis* 33C12**						
N-acetyl-β-D-galactosaminidase	8.25 ± 0.68	*51.75 ± 0.70*	12.36 ± 0.37	*50.94 ± 0.44*	*51.33 ± 0.38*	11.00 ± 0.57
α-D-galactopyranosidase	0.65 ± 0.52	0.06 ± 0.62	0.37 ± 0.53	*2.36 ± 0.29*	*2.23 ± 0.36*	0.66 ± 0.59
β-D-galactopyranosidase	1.03 ± 0.08	0.99 ± 0.13	0.58 ± 0.01	0.67 ± 0.29	0.93 ± 0.10	1.09 ± 0.11
α-D-glucopyranosidase	0.84 ± 0.34	0.64 ± 0.38	0.64 ± 0.29	0.52 ± 0.35	**0.11 ± 0.11**	0.90 ± 0.40
β-D-glucopyranosidase	4.19 ± 0.94	2.47 ± 0.06	4.04 ± 0.97	3.45 ± 1.00	3.41 ± 0.99	4.23 ± 0.99
α-L-arabinopyranosidase	1.19 ± 0.34	0.96 ± 0.33	1.03 ± 0.36	0.93 ± 0.37	1.01 ± 0.34	1.24 ± 0.37
β-L-arabinopyranosidase	1.54 ± 0.27	1.55 ± 0.32	1.39 ± 0.27	1.43 ± 0.29	1.46 ± 0.31	1.62 ± 0.31
α-L-fucopyranosidase	0.56 ± 0.25	0.26 ± 0.18	0.42 ± 0.27	0.29 ± 0.28	0.23 ± 0.21	0.67 ± 0.28
β-D-fucopyranosidase	1.49 ± 0.39	1.47 ± 0.44	0.62 ± 1.29	1.31 ± 0.41	1.33 ± 0.39	1.57 ± 0.42
α-D-mannopyranosidase	1.27 ± 0.34	1.38 ± 0.84	1.09 ± 0.36	1.24 ± 0.38	1.34 ± 0.39	1.35 ± 0.40
β-D-mannopyranosidase	2.42 ± 0.80	2.21 ± 0.84	2.21 ± 0.82	1.96 ± 0.78	2.18 ± 0.83	2.46 ± 0.85
***Halomonas meridiana* 33E7**						
N-acetyl-β-D-galactosaminidase	4.52 ± 0.54	1.20 ± 2.43	2.53 ± 0.08	3.38 ± 1.02	2.66 ± 1.20	1.73 ± 1.20
α-D-galactopyranosidase	2.13 ± 0.34	**0.51 ± 0.20**	1.14 ± 0.33	0.71 ± 0.49	0.46 ± 0.60	2.21 ± 0.25
β-D-galactopyranosidase	0.11 ± 0.07	*0.60 ± 0.34*	*0.87 ± 0.07*	*1.26 ± 0.07*	*1.05 ± 0.07*	1.88 ± 2.16
α-D-glucopyranosidase	257.22 ± 13.03	164.27 ± 10.66	177.93 ± 21.04	148.72 ± 12.67	161.84 ± 8.56	213.47 ± 3.68
β-D-glucopyranosidase	2.05 ± 0.73	2.67 ± 0.63	3.18 ± 0.81	3.45 ± 0.76	2.45 ± 0.87	1.66 ± 0.18
α-L-arabinopyranosidase	0.39 ± 0.31	0.25 ± 0.03	0.63 ± 0.29	1.02 ± 0.29	0.04 ± 0.34	0.70 ± 0.34
β-L-arabinopyranosidase	0.87 ± 0.27	1.15 ± 0.17	1.15 ± 0.21	1.53 ± 0.22	1.37 ± 0.29	1.40 ± 0.30
α-L-fucopyranosidase	0.67 ± 0.33	0.31 ± 0.14	**0.02 ± 0.05**	0.45 ± 0.15	0.46 ± 0.26	0.39 ± 0.24
β-D-fucopyranosidase	0.73 ± 0.06	1.41 ± 0.29	1.48 ± 0.24	1.64 ± 0.34	1.43 ± 0.30	1.39 ± 0.27
α-D-mannopyranosidase	1.06 ± 0.26	1.40 ± 0.34	0.96 ± 0.26	1.61 ± 0.34	1.31 ± 0.29	1.38 ± 0.37
β-D-mannopyranosidase	1.99 ± 0.81	2.14 ± 0.71	2.12 ± 0.80	2.58 ± 0.76	1.95 ± 0.45	2.37 ± 0.84

activities. This hypothesis is yet to be tested *in situ* using defined mutants.

The 2 coral commensals tested in the present study appear to rely on mucus degradation strategies that are distinct from the white pox pathogen, although this limited survey of coral commensals does not suggest a unifying model by which commensals as a group utilize coral mucus. Based on the results presented in Table 2, it appears that the 2 h incubation of the 2 commensals on mucus with simple sugars did not have a strong catabolite repression effect on the enzymatic activities. Treatment of *Photobacterium mandapamensis* with mannose, arabinose or galactose stimulated *N*-acetyl galactosaminidase, and after 18 h of incubation

Table 3. Enzymatic activities during the 18 h incubation on the high molecular weight fraction of mucus (M) from *Acropora palmata* in the presence of simple sugars. Simple sugars added: galactose (Gal), glucose (Glu), arabinose (Ara), mannose (Man) and *N*-acetyl-glucosamine (NGlu). Enzymatic activities that increased (*italics*) or decreased (**bold**) at least 2-fold after incubation are indicated

Enzyme	M	M + Gal	M + Glu	M + Ara	M + Man	M + NGlu
***Serratia marcescens* PDL100**						
N-acetyl-β-D-galactosaminidase	130.35 ± 3.87	108.6 ± 4.72	**38.16 ± 5.64**	192.98 ± 5.95	111.31 ± 0.73	82.43 ± 0.12
α-D-galactopyranosidase	1.98 ± 0.64	1.79 ± 0.63	1.31 ± 0.63	0.94 ± 0.65	1.31 ± 0.63	1.69 ± 0.64
β-D-galactopyranosidase	9.11 ± 0.14	*45.61 ± 0.01*	**2.11 ± 0.03**	14.42 ± 0.15	5.85 ± 0.07	**2.75 ± 0.08**
α-D-glucopyranosidase	161.77 ± 1.99	**13.84 ± 0.44**	**76.16 ± 3.77**	**15.60 ± 1.48**	**9.61 ± 0.10**	**7.28 ± 0.18**
β-D-glucopyranosidase	4.45 ± 0.80	4.79 ± 0.97	2.69 ± 0.94	4.24 ± 0.98	4.63 ± 0.99	4.93 ± 1.01
α-L-arabinopyranosidase	11.83 ± 0.08	*40.88 ± 0.01*	**2.40 ± 0.28**	15.62 ± 0.07	6.07 ± 0.31	**3.05 ± 0.28**
β-L-arabinopyranosidase	1.86 ± 0.30	1.27 ± 0.26	1.18 ± 0.28	0.92 ± 0.30	1.44 ± 0.29	1.69 ± 0.29
α-L-fucopyranosidase	0.62 ± 0.21	1.02 ± 0.29	0.62 ± 0.08	0.82 ± 0.29	0.89 ± 0.25	1.02 ± 0.30
β-D-fucopyranosidase	1.32 ± 0.33	**0.57 ± 0.41**	1.25 ± 0.33	0.94 ± 0.40	1.40 ± 0.37	1.77 ± 0.42
α-D-mannopyranosidase	1.72 ± 0.39	1.59 ± 0.40	1.62 ± 0.38	1.21 ± 0.40	1.43 ± 0.37	1.70 ± 0.40
β-D-mannopyranosidase	2.89 ± 0.85	2.92 ± 0.85	2.81 ± 0.86	2.48 ± 0.86	2.80 ± 0.85	2.99 ± 0.83
***Serratia marcescens* 43422**						
N-acetyl-β-D-galactosaminidase	160.83 ± 6.53	181.11 ± 10.59	88.83 ± 3.88	174.67 ± 6.27	147.03 ± 5.11	**68.07 ± 0.08**
α-D-galactopyranosidase	0.31 ± 0.53	1.48 ± 0.58	0.65 ± 0.54	0.82 ± 0.57	1.15 ± 0.62	1.46 ± 0.55
β-D-galactopyranosidase	10.31 ± 0.57	10.62 ± 0.87	**0.67 ± 0.03**	8.54 ± 0.32	**2.76 ± 0.02**	**3.76 ± 0.20**
α-D-glucopyranosidase	135.59 ± 2.50	90.47 ± 9.16	**0.33 ± 0.27**	86.16 ± 3.69	70.33 ± 2.45	**16.62 ± 1.79**
β-D-glucopyranosidase	3.49 ± 1.00	4.25 ± 0.92	4.02 ± 0.84	4.31 ± 0.96	4.59 ± 1.02	4.69 ± 0.85
α-L-arabinopyranosidase	10.18 ± 0.31	9.62 ± 0.41	**0.43 ± 0.30**	7.59 ± 0.76	**2.45 ± 0.24**	**3.78 ± 0.02**
β-L-arabinopyranosidase	1.30 ± 0.22	1.39 ± 0.26	1.57 ± 0.28	1.37 ± 0.27	1.65 ± 0.29	2.22 ± 0.31
α-L-fucopyranosidase	0.50 ± 0.23	0.74 ± 0.25	0.63 ± 0.26	0.72 ± 0.25	0.89 ± 0.28	1.17 ± 0.29
β-D-fucopyranosidase	0.85 ± 0.34	1.02 ± 0.24	1.43 ± 0.40	0.01 ± 0.76	1.55 ± 0.42	1.90 ± 0.42
α-D-mannopyranosidase	0.97 ± 0.31	1.12 ± 0.33	1.12 ± 0.36	1.11 ± 0.33	1.36 ± 0.36	1.96 ± 0.41
β-D-mannopyranosidase	2.12 ± 0.77	2.45 ± 0.79	2.36 ± 0.80	2.39 ± 0.79	2.70 ± 0.85	3.27 ± 0.86
***Photobacterium mandapamensis* 33C12**						
N-acetyl-β-D-galactosaminidase	49.08 ± 1.25	66.60 ± 3.21	54.16 ± 4.07	53.67 ± 6.77	59.01 ± 2.44	68.98 ± 1.59
α-D-galactopyranosidase	5.07 ± 0.42	**2.37 ± 0.10**	8.07 ± 1.07	3.92 ± 0.68	**2.07 ± 0.24**	*18.58 ± 0.94*
β-D-galactopyranosidase	2.44 ± 0.01	1.17 ± 1.53	2.74 ± 0.24	0.36 ± 0.00	**0.74 ± 0.06**	2.57 ± 0.18
α-D-glucopyranosidase	3.88 ± 0.22	**0.38 ± 0.01**	5.16 ± 0.41	**0.67 ± 0.39**	**0.04 ± 0.25**	3.82 ± 0.10
β-D-glucopyranosidase	3.69 ± 0.60	**1.28 ± 0.09**	2.99 ± 0.17	2.95 ± 0.60	3.03 ± 0.84	3.42 ± 0.42
α-L-arabinopyranosidase	2.68 ± 0.28	**0.59 ± 0.27**	*8.37 ± 2.26*	**0.77 ± 0.26**	**0.71 ± 0.23**	3.45 ± 0.83
β-L-arabinopyranosidase	3.39 ± 0.21	0.98 ± 0.22	2.43 ± 0.26	**0.50 ± 1.26**	**1.23 ± 0.17**	2.29 ± 0.03
α-L-fucopyranosidase	2.23 ± 0.26	1.49 ± 1.01	2.41 ± 0.05	**0.30 ± 0.16**	**0.02 ± 0.09**	4.28 ± 0.48
β-D-fucopyranosidase	3.02 ± 0.58	**0.87 ± 1.06**	2.09 ± 0.06	**0.94 ± 0.10**	**0.41 ± 0.17**	5.44 ± 2.00
α-D-mannopyranosidase	3.40 ± 0.29	**0.62 ± 0.29**	2.75 ± 0.28	4.82 ± 3.31	**1.18 ± 0.32**	2.14 ± 0.12
β-D-mannopyranosidase	2.52 ± 0.82	1.52 ± 0.60	1.86 ± 0.62	1.33 ± 0.26	1.27 ± 0.34	3.63 ± 0.68
***Halomonas meridiana* 33E7**						
N-acetyl-β-D-galactosaminidase	6.16 ± 0.30	**2.57 ± 0.79**	**1.05 ± 0.96**	3.10 ± 1.00	3.65 ± 0.97	3.40 ± 1.18
α-D-galactopyranosidase	2.71 ± 0.62	0.56 ± 0.28	**0.27 ± 0.60**	1.72 ± 0.41	1.00 ± 0.58	1.18 ± 0.28
β-D-galactopyranosidase	0.93 ± 1.04	1.01 ± 1.00	0.70 ± 0.06	**0.06 ± 0.63**	1.28 ± 0.10	0.58 ± 0.02
α-D-glucopyranosidase	220.89 ± 4.21	**93.78 ± 15.78**	**100.47 ± 21.58**	124.70 ± 4.31	110.93 ± 12.40	188.85 ± 30.67
β-D-glucopyranosidase	1.18 ± 0.44	2.15 ± 0.00	*3.09 ± 0.84*	*3.78 ± 0.99*	*3.76 ± 0.98*	2.58 ± 0.92
α-L-arabinopyranosidase	0.47 ± 0.07	0.23 ± 0.05	**0.05 ± 0.03**	0.88 ± 0.27	0.29 ± 0.81	0.25 ± 0.32
β-L-arabinopyranosidase	1.00 ± 1.14	1.24 ± 0.17	2.52 ± 0.187	1.59 ± 0.20	1.58 ± 0.12	0.96 ± 0.09
α-L-fucopyranosidase	2.98 ± 0.88	**0.05 ± 0.26**	**0.28 ± 0.12**	**0.42 ± 0.08**	**0.78 ± 0.27**	**0.53 ± 0.07**
β-D-fucopyranosidase	1.18 ± 0.29	1.11 ± 0.06	1.61 ± 0.37	1.62 ± 0.36	1.85 ± 0.43	1.39 ± 0.41
α-D-mannopyranosidase	0.65 ± 0.29	1.25 ± 0.27	1.27 ± 0.27	*1.65 ± 0.36*	*1.62 ± 0.26*	0.66 ± 0.01
β-D-mannopyranosidase	1.07 ± 0.41	2.48 ± 0.70	1.66 ± 0.35	2.74 ± 0.84	2.73 ± 0.84	1.66 ± 0.67

the induction was relieved (Table 3). Neither glucose nor *N*-acetyl-glucosamine had a strong catabolite repression effect on *P. mandapamensis* (Tables 2 & 3). This suggests that neither glucose nor *N*-acetyl-glucosamine is the preferred carbon source for this bacterium. Both galactosidase and *N*-acetyl-galactosidase appear to be co-upregulated within the first 2 h (Table 2), suggesting that the corresponding substrates maybe preferentially co-utilized by the bacterium. Within the 18 h incubation, the catabolite repression effects of galactose, arabinose and mannose on the enzymatic activities in this bacterium were the strongest; galactosidases, arabinosidases and α-D-mannopyranosidase appear to be controlled by the

feedback inhibition in the presence of sugars that could result from the activity of these enzymes (Table 3). Besides *N*-acetyl-glucosamine, mannose, arabinose and galactose are the 3 most abundant sugars in the mucus polymer of acroporid corals (Meikle et al. 1987). Arabinose and galactose occur as terminal residues, while mannose links the oligosaccharide side chain to the polypeptide backbone (Meikle et al. 1987). These sugars are most likely released during the enzymatic hydrolysis of the polymer through the actions of endo- and exo-glycanases *in situ*. The strong, but delayed, repressive effect of these sugars may, in part, explain why growth of *P. mandapamensis* 33C12 on coral mucus plateaus early despite the fact that it initially grows fast on coral mucus (Krediet et al. 2009). The abundance of simple sugars resulting from hydrolysis of the mucus polymer may signal to this commensal that its habitat (coral mucus surface layer) is degraded, and the bacterium switches to a lower metabolic rate. Most pathogens, on the other hand, in response to nutrient limitation, upregulate virulence-related genes and thus may switch from saprophytic growth to virulence (Teplitski et al. 2006b, Gorke & Stulke 2008).

In another coral commensal, *Halomonas meridiana* 33E7, galactose and glucose had some catabolite repression effect after 2 h of incubation on mucus, although the number of repressed activities and the magnitude of repression were less than in *Serratia marcescens* PDL100 (Table 2). With the exception of *N*-acetyl-glucosamine, the addition of all monosaccharides promoted β-galactosidase activity (Table 2) after 2 h. These results suggest that galactose and glucose may be preferred carbon sources based on their strong catabolite repression.

The 4 tested bacteria had a different set of preferred carbon sources when grown on the mucus polymers; they also differed in the strength of their enzymatic activities. These differences may help explain how coral mucus-associated microbial communities are structured *in situ*, and how pathogens may be able to establish with native coral microbiota. Further mutational analyses are needed to establish the role for these activities *in situ*.

Attachment to coral mucus

Adhesion of a coral pathogen *Vibrio shiloi* to mucus of its coral host was shown to be one of the steps involved in pathogenesis (Banin et al. 2001, Rosenberg & Falkovitz 2004). In the reef environment, coral mucus is colonized by microbes and microscopic eukaryotes (Vacelet & Thomassin 1991). These observations suggest that binding to mucus and establishment within coral mucus are important to the outcome of coral–bacteria interactions. Because bacteria utilize mucus as a growth substrate, we tested bacterial biofilm formation on mucus in the presence of monosaccharides that would result from the enzymatic hydrolysis of mucus polymers.

Biofilm assays were set up in polystyrene microtiter plates coated with either crude coral mucus or with artificial mucus (a solution containing carbon and nitrogen sources to approximate the composition and viscosity of coral mucus). The addition of sugars at 0.1% had no effect on biofilm formation (data not shown). As shown in Fig. 1A, *Serratia marcescens* PDL100 formed biofilms on artificial mucus and on crude *Acropora palmata* mucus, regardless of the addition of simple sugars to the sample. Biofilm formation by a human pathogen *S. marcescens* 43422 was stronger on artificial mucus than on any combination of coral mucus (Fig. 1B). In control experiments, crude mucus of *A. palmata* also modestly inhibited biofilm formation in CFA medium (data not shown), suggesting that an unknown component of coral mucus inhibits biofilm formation in some bacteria. This is reminiscent of a study in which mucus of some echinoderms contained substances capable of inhibiting attachment by bacteria (Bavington et al. 2004).

In *Photobacterium mandapamensis* there was no difference in biofilm formation on mucus-coated surfaces (vs. surfaces coated with artificial mucus). As shown in Fig. 1D, biofilm formation on mucus-coated surfaces and those formed on mucus-coated surfaces in the presence of simple sugars was similar (although statistically significant, 2-fold differences were observed between biofilms formed on mucus-coated surfaces with the addition of 1% arabinose or galactose, compared to the biofilms formed in the presence of glucose, *N*-acetyl glucosamine or mannose). Biofilm formation by *Halomonas meridiana* 33E7 on surfaces coated with mucus was somewhat lower compared to the biofilm formation on surfaces coated with the artificial mucus (Fig. 1C). With the exception of glucose, none of the simple sugars had a statistically significant effect on biofilm formation. Interestingly, glucopyranosidase (an enzyme that makes glucose available to the cell) is the strongest constitutively active enzyme in *H. meridiana* 33E7. It is, therefore, possible that the availability of the preferred carbon source (glucose for *H. meridiana*, galactose or arabinose for *Photobacterium mandapamensis*, based on the strongest catabolite repression activity by these sugars) indicates a preferred ecological niche for this organism and stimulates settlement and biofilm formation. An alternative explanation for the observed effect could be based on the reports that bacteria bind to specific carbohydrate receptors present in coral mucus (Banin et

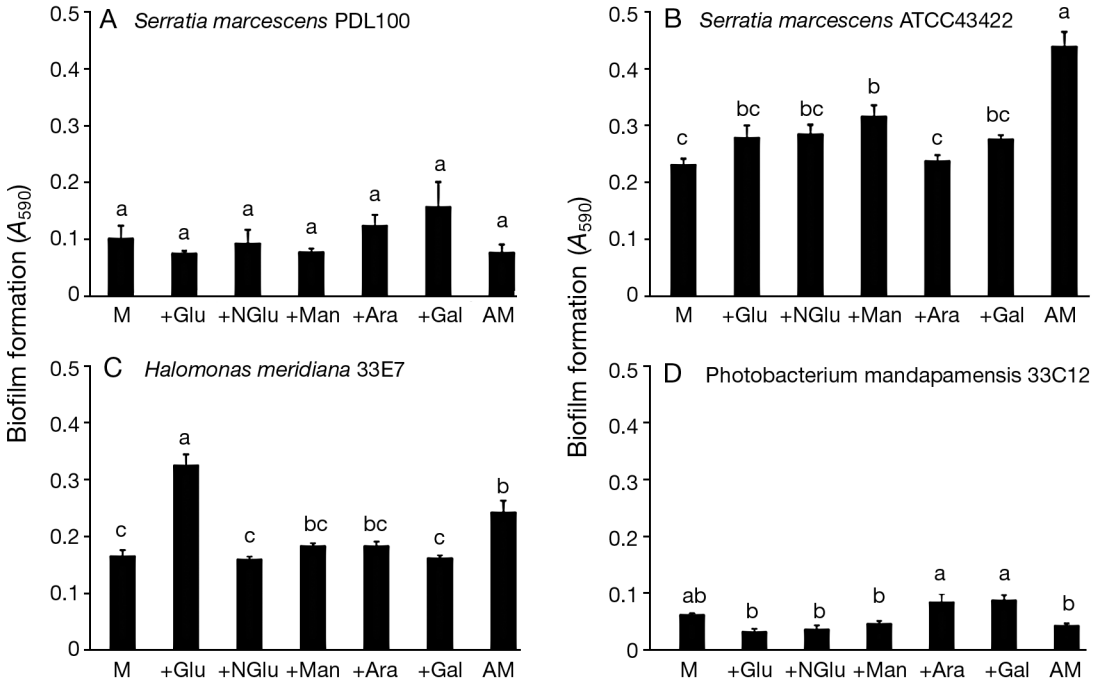

Fig. 1. Biofilm formation on surfaces coated with mucus of *Acropora palmata* in the presence of simple sugars. (A) White pox pathogen *Serratia marcescens* PDL100, (B) human pathogen *S. marcescens* ATCC43422, coral commensals (C) *Halomonas meridiana* 33E7 and (D) *Photobacterium mandapamensis* 33E12. A_{590}: absorbance at 590 nm. Man: D-mannose; Ara: L-arabinose; Gal: D-galactose; NGlu: *N*-acetyl-D-glucosamine; Glu: glucose. Error bars represent SE; data points that are statistically different are indicated by different lowercase letters

al. 2001) and the addition of simple sugars may either facilitate or inhibit these interactions.

CONCLUSIONS

It is becoming increasingly clear that nutrients and signals found in the coral surface mucopolysaccharide layer dictate, at least in part, the structure and the composition of the associated microbiota (Ritchie 2006, Sharon & Rosenberg 2008, Teplitski & Ritchie 2009). The present study suggests that to colonize mucus of its coral host, *Acropora palmata*, a model pathogen *Serratia marcescens* and 2 representative coral commensals relied on different strategies. Upon exposure to coral mucus, mannopyranosidases were induced in *S. marcescens*, these enzymes hydrolyze oligosaccharide side chains from the polypeptide backbone of the mucus glycoprotein. At least 5 glycosidases were strongly expressed in the starved cultures of this bacterium, and these enzymes may help degrade mucus oligosaccharides that are released by mannosidases. Most of the glycosidases in *S. marcescens* were subject to catabolite repression early, and this suggests that the ability to efficiently downregulate enzymatic activities during colonization of coral mucus may help this pathogen establish within the coral mucus layer. Catabolite repression was relieved within 18 h of incubation on mucus, suggesting that as nutrients become less available, this pathogen becomes more aggressive. This would be consistent with the observations in other pathogens that upregulate their virulence genes when preferred carbon sources become limiting (Deutscher 2008, Gorke & Stulke 2008, Teplitski et al. 2006b).

A comparison of enzymatic activities induced in the commensals during growth on the mucus polymer suggested that they relied on different strategies to colonize the coral surface mucopolysaccharide layer. In *Photobacterium mandapamensis* at least 10 new glycosidases were induced at various stages of *in vitro* growth on the mucus polymers. The availability of galactose, mannose or arabinose had a strong repressive affect on enzymatic activities after 18 h of growth on mucus. In another commensal bacterium, *Halomonas meridiana*, α-D-glucopyranosidase was strongly and constitutively produced and 4 new glycosidases were induced during growth on the high molecular weight fraction of coral mucus. Interestingly, in both commensals, catabolite repression effects were strongest after 18 h of incubation, while in the white pox pathogen, the catabolite repression effects were largely relieved within the same time frame. It may be possible (while not yet experimentally tested) that this catabolite repression by sugars may arrest overgrowth

of commensals, which could be detrimental to the coral host. Elucidation of the differences in the strategies that coral commensals and opportunistic pathogens will help define the mechanisms of coral disease and may lead to research on defining potential approaches for managing or treating coral diseases.

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