

Iron effects on colonization behavior, motility, and enzymatic activity of marine bacteria

Kam W. Tang and Hans-Peter Grossart

Abstract: Iron availability in the ocean has been shown to affect the growth and production of phytoplankton and free-living bacteria. A large fraction of marine bacteria are specialized in colonizing and living on particles and aggregates, but the effects of iron limitation on these bacteria are not fully known. We conducted laboratory experiments to study the effects of iron availability on particle colonization behavior, motility, and enzymatic activities of 4 strains of marine bacteria. Iron depletion reduced the bacterial particle colonization rate by 1.7%–43.1%, which could be attributed to reduced swimming speeds in 2 of the 4 strains. Protease activity was not affected by iron availability. However, attached bacteria did show higher protease activities than their free counterparts. Our results suggest that iron limitation in the ocean could in some cases reduce bacteria–particle interactions by reducing bacterial motility and colonization rate.

Key words: iron, bacterial motility, bacterial colonization, enzymatic activity.

Résumé : La disponibilité du fer de l'océan affecte la croissance et la production de phytoplancton et de bactéries libres. Une grande proportion des bactéries marines sont spécialisées dans la colonisation et la croissance sur des particules ou des agrégats, mais les effets de la limitation en fer sur ces bactéries ne sont pas bien connus. Nous avons réalisé des expériences en laboratoire afin d'étudier les effets de la disponibilité en fer sur la colonisation des particules, la motilité et l'activité enzymatique de quatre souches de bactéries marines. La déplétion en fer a réduit le taux de colonisation de particules de 1,7 % à 43,1 %, ce qui pourrait être attribué à une vitesse de nage réduite chez 2 des 4 souches. L'activité protéase n'était pas affectée par la disponibilité du fer. Cependant, les bactéries attachées n'ont pas démontré d'activité protéase plus élevée que leurs contreparties libres. Nos résultats suggèrent que la limitation en fer de l'océan pourrait dans certains cas réduire les interactions bactérie–particules en réduisant la motilité et le taux de colonisation.

Mots-clés : fer, motilité bactérienne, colonisation bactérienne, activité enzymatique.

[Traduit par la Rédaction]

Introduction

There is growing interest and concern over iron fertilization of the ocean as means to enhance carbon sequestration and mitigate greenhouse effects (Chisholm et al. 2001). Iron has been shown to be the limiting factor for phytoplankton growth in many oceanic regions (Martin and Fitzwater 1988; Coale et al. 1996; Hutchins and Bruland 1998; Boyd et al. 2000), although its role in regulating marine bacterial production and activity is less well known. Tortell et al. (1996) estimated that marine bacteria, on a per biomass basis, contain more iron than phytoplankton and are responsible for a large fraction of the biological iron uptake in some areas. A number of studies have shown that in iron-depleted waters bacterial growth rate and growth efficiency appear to be limited by iron after their organic carbon demand has been satisfied (Church et al. 2000; Kirchman et al. 2000, 2003; Cochlan 2001; Arrieta et al. 2004). Similar iron-regulated growth and production is also observed in *Escherichia coli* (Appenzeller et al. 2005) and *Campylobacter*

jejuni (Palyada et al. 2004). A large portion of the marine bacteria are associated with particles and aggregates instead of being free living (Azam 1998), and these attached bacteria play a central role in aggregate formation, degradation, and remineralization (Simon et al. 2002). The microbial dynamics on particles and aggregates necessarily begin with the bacteria encountering and attaching to the surfaces. These initial steps of the colonization process are regulated in part by the particle size, ambient bacterial concentration, bacterial motility, probability of attachment, and growth upon encounter (Kjørboe et al. 2002). In medical studies it has been shown that bacterial biofilm formation can be suppressed under iron-limiting conditions (Palyada et al. 2004; Weinberg 2004). Presently, there is a lack of knowledge of how iron limitation affects interactions between bacteria and particles in the marine environment, and the consequences on organic matter fluxes. We conducted laboratory experiments to investigate how iron affects the colonization behavior, motility, and enzymatic activity of marine bacteria. Our goal was to better under-

Received 14 February 2007. Revision received 8 May 2007. Accepted 10 May 2007. Published on the NRC Research Press Web site at cjm.nrc.ca on 31 August 2007.

K.W. Tang.¹ Virginia Institute of Marine Science, 1208 Greates Road, Gloucester Point, Virginia 23062, USA.

H.-P. Grossart. Leibniz Institute of Freshwater Ecology and Inland Fisheries, Alte Fischerhütte 2, D-16775, Stechlin, Germany.

¹Corresponding author (e-mail: kamtang@vims.edu).

Table 1. Phylogenetic affiliation of bacterial strains used in this study.

Strain	Acc. No.	Most closely related isolate (acc. No.)	Sequence homology
HP4	AY241549	<i>Saccharospirillum impatiens</i> (AJ315983)	98%
		<i>Arhodomonas</i> sp. strain EL-201 (AJ315984)	98%
HP11	AY241557	<i>Flexibacteraceae</i> bacterium JL007 (DQ869296)	98%
HP15b	AY241552	<i>Marinobacter</i> sp. strain WMQ_A (DQ841245)	99%
HP33	AY239010	<i>Rhodobacteraceae</i> bacterium ROS7 (AY841781)	99%

stand how iron limitation in nature may affect the bacterial community and its interactions with particulate organic matter such as particle colonization and degradation.

Materials and methods

Bacteria

Four strains of bacteria originating from marine snow aggregates (HP4, HP11, HP15b, and HP33; Grossart et al. 2004) were used for this study; their phylogenetic affiliation is presented in Table 1. The bacteria were maintained on 50% marine broth agar (Difco MB2216) until use. Before the experiments, an inoculum of each isolate was resuspended in 1.25% marine broth and allowed to regain motility and growth to high density. To induce iron limitation, we used the chelator desferrioxamine B (DFOB; Sigma-Aldrich, St. Louis, Missouri) to remove free dissolved iron (Hutchins et al. 1999; Wells 1999). Actively growing bacteria were transferred to filter-sterilized and aged seawater (1 year old North Sea water) in duplicate sets of test tubes. To one set of test tubes, DFOB was added to a final concentration of 1000 nmol·L⁻¹. Hutchins et al. (1999) calculated that 100 nmol·L⁻¹ DFOB is sufficient to chelate almost all free iron in natural seawater. Because a small amount of residual marine broth nutrients would have been transferred to the test tubes, we used 10-fold higher concentration of DFOB to remove the free iron; this set was labeled as “no-iron” treatment. Preliminary tests showed that the residual iron in the experimental test tubes was no more than 800 nmol·L⁻¹; as such the amount of DFOB added should have been enough to chelate all of the free iron. The other set of test tubes without DFOB addition was labeled as “iron” treatment. After 1 day of inoculation, aliquots were taken for colonization experiments, motility observations, and enzymatic activity measurements as described later in the text.

Colonization experiments

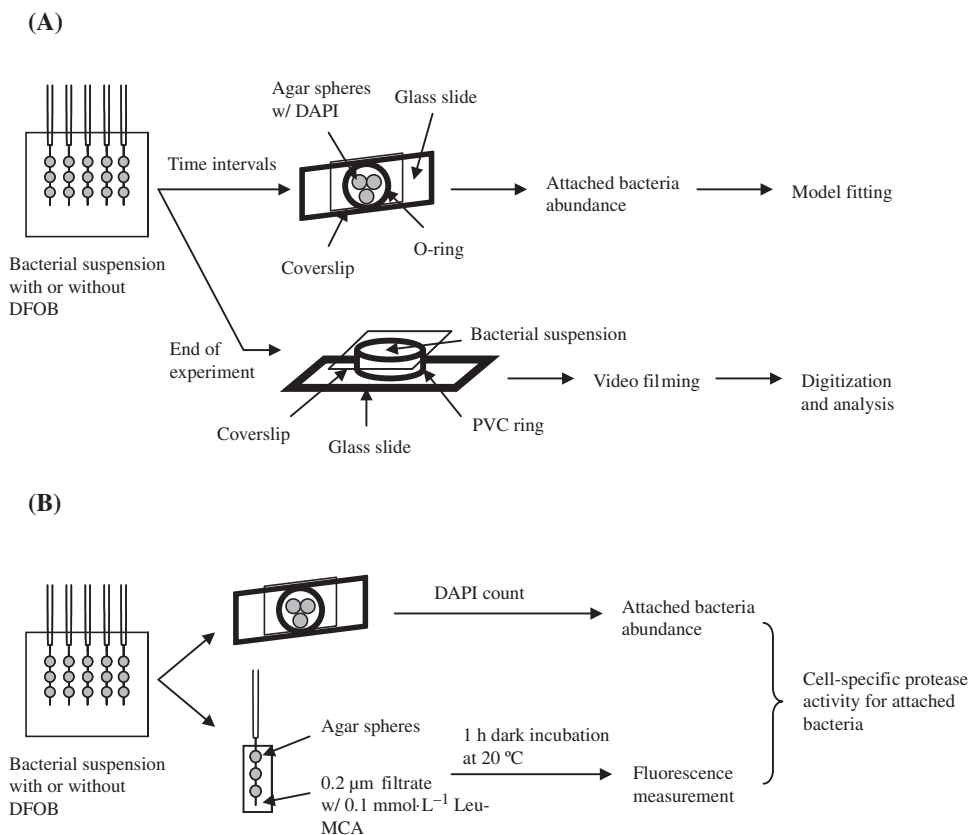
The bacterial colonization rate was measured using 4 mm diameter model particles made of ultra-pure (DNA–RNAase-free) agar (Bioline, Randolph, Massachusetts) as described previously (Kjørboe et al. 2002; Fig. 1). The use of ultra-pure agar minimized the introduction of iron impurities during the experiments. Bacteria from iron and no-iron treatments were, respectively, diluted with sterile seawater and sterile seawater plus 1000 nmol·L⁻¹ DFOB in 500 mL beakers to a concentration of approximately 10⁶ cells·mL⁻¹. Agar spheres were suspended on glass needles and immersed in the beaker for up to 180 min. At certain time intervals triplicate agar spheres were transferred onto a counting chamber made of a rubber O-ring glued to a glass

slide, and a drop of DAPI was added directly to the spheres. A cover slip was put on the spheres to create a flat surface for epifluorescence observation and bacterial counts. This method does not result in any measurable loss of attached bacteria from the sphere’s surface (Kjørboe et al. 2002). Counted bacteria per unit surface area were then scaled up to the entire surface of the sphere to obtain the total number of attached bacteria. Aliquots of ambient water were taken at the beginning and the end of the experiments for quantifying free bacteria by DAPI direct count. Attached bacterial abundance per sphere was normalized to ambient bacterial concentration, and the mathematical model of Kjørboe et al. (2002) was fitted to the observations to calculate bacterial diffusivity owing to random-walk motility (D) and detachment rate of recently attached bacteria (δ). Because these 2 parameters are not independent of each other in the model, we calculated a colonization coefficient R as the numerical value of D/δ such that R characterizes the net accumulation of bacteria on the spheres as a result of encounter and detachment, providing a way to compare the different treatments.

Motility observations

At the end of each colonization experiment, bacteria in ambient water were transferred to an observation chamber made of a polyvinyl chloride ring (height 0.5 cm, diameter 1.6 cm) attached to a microscopic slide (Kjørboe et al. 2002; Fig. 1). The chamber was closed by a cover slip without air bubbles. The motility pattern of each strain was recorded by a digital camera at 100× magnification under dark field. Two-dimensional projections of the swimming tracks were digitized by ImagePro[®] and analyzed for speed (= distance·time⁻¹) and linear velocity (= displacement·time⁻¹). The 3-dimensional swimming speeds were estimated as $\sqrt{(3/2)}$ times the 2-dimensional speeds (Kjørboe et al. 2002). Heating from the microscope created a small background flow, which was corrected for by using the trajectories of abiotic objects (e.g., dust particles). Marine bacteria typically swim in a run-and-tumble mode, which results in an overall non-linear swimming path (Berg 1993). Instead of using the more elaborate procedure of Kjørboe et al. (2002) to estimate the average turn angle of the swimming path, we simply calculated the ratio of speed to velocity, which is sufficient to detect changes in swimming behavior owing to iron depletion. A ratio of 1 would indicate a straight swimming path and a ratio substantially higher than 1 would indicate a more convoluted swimming path. Swimming speeds and speed-to-velocity ratios between iron and no-iron treatments for each strain were tested for significant differences by the Mann–Whitney rank sum test.

Fig. 1. Schematic of experimental designs for colonization and motility measurements (A), and protease activity measurements (B). See text for details.



Protease activity

Bacteria that had been growing under iron or no-iron condition for 1 day were collected for measurement of their protease activity. Bacteria were diluted with sterile seawater with or without 1000 nmol·L⁻¹ DFOB to a concentration of approximately 10⁵ cells·mL⁻¹, and ultra-pure agar spheres were suspended in the bacterial suspensions for 3 h. Afterwards, aliquots from the bacterial suspensions were filtered through 0.2 μm membrane filters and 3 mL of each filtrate was added to a 1 cm² cuvette. To measure attached bacterial protease activity, triplicates of 3 precolonized agar spheres for each strain were removed and suspended on glass needles in the cuvettes with the filtrate (Fig. 1). Aliquots of the bacterial suspensions were added directly to a separate set of square cuvettes (3 mL in triplicate) for measurement of free bacterial protease activity (Hoppe 1983) using the fluorogenic substrate analogue L-leucine – methyl coumarinyl amide (Leu–MCA) at a final concentration of 0.1 mmol·L⁻¹, which was enough to maximize hydrolysis based on saturation kinetics. The cuvettes were incubated at 20 °C in the dark for 1 h. Controls were sterile agar spheres (control for attached bacteria) and aliquots of sterile filtered seawater (control for free bacteria including free extracellular enzymes) incubated in the same manner. At the end of the incubation the glass needles were removed and the fluorescence of the cuvette contents was read using a fluorometer with an excitation of 365 nm and emission of 455 nm (Kontron, Poway, California). A range of analogue concentrations (0–100 μmol·L⁻¹) were measured for their fluorescence to generate a calibration curve for the enzyme assay.

Additional spheres and aliquots of bacterial suspensions were collected for DAPI direct counts of attached bacteria and free bacteria, respectively, as described earlier in the text. To obtain cell-specific activity, the measured protease activity per agar sphere was normalized to the number of attached bacteria per sphere, and protease activity in the suspension was normalized to free bacterial concentration. Cell-specific protease activities between free and attached bacteria were compared using Student's *t* test.

Results

Colonization rate

In all treatments the colonization process followed the pattern described by Kjørboe et al. (2002): bacterial abundance on spheres increased curvilinearly until it approached a plateau (Fig. 2). Among the no-iron treatments, strain HP15b had the highest colonization rate and the corresponding *R* value (0.22), followed by strains HP11 (0.17), HP33 (0.10), and HP4 (0.07) (Table 2). Iron had a slight effect on strain HP11 and the colonization curves were similar between iron and no-iron treatments (Fig. 2). Strains HP4, 15b, and 33 colonized the agar spheres at a considerably higher rate in the iron treatments (Fig. 2) and the calculated *R* was 58%–75% higher relative to the no-iron treatment (Table 2).

Motility pattern

Forty-nine or more swimming tracks were analyzed per treatment except for the strain HP33 no-iron treatment,

Fig. 2. Colonization of agar spheres in still water by 4 strains of marine snow bacteria in iron (●) and no-iron (○) treatments. Bacterial abundances on spheres were normalized to ambient bacterial concentrations. Error bars represent standard deviations of 10 measurements. Lines represent model fits based on Kjørboe et al. (2002).

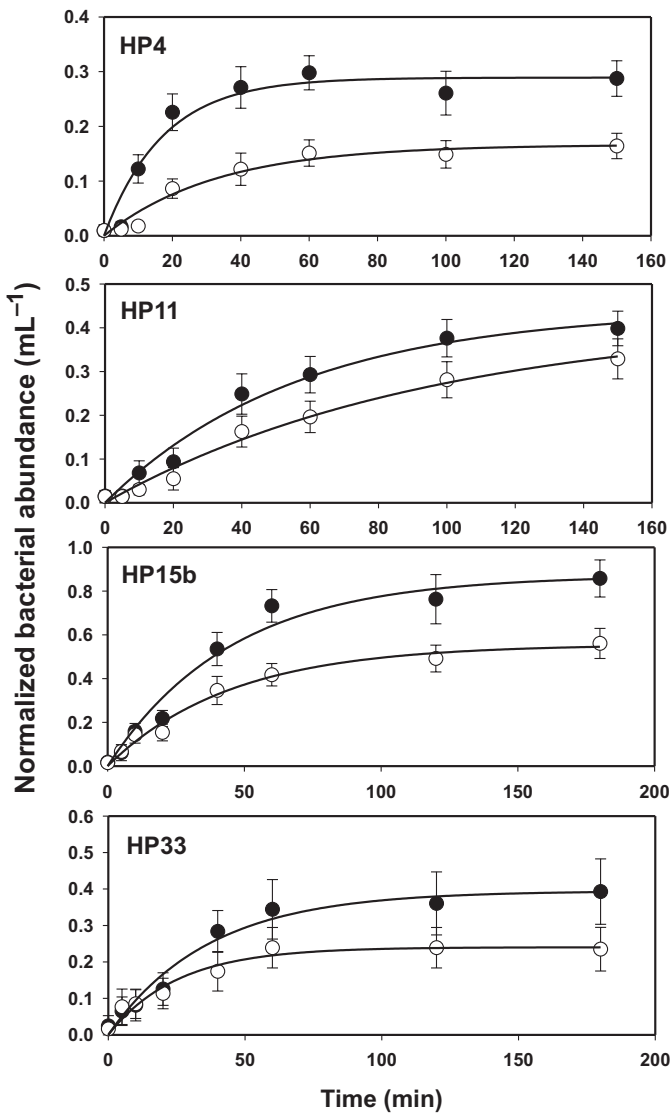
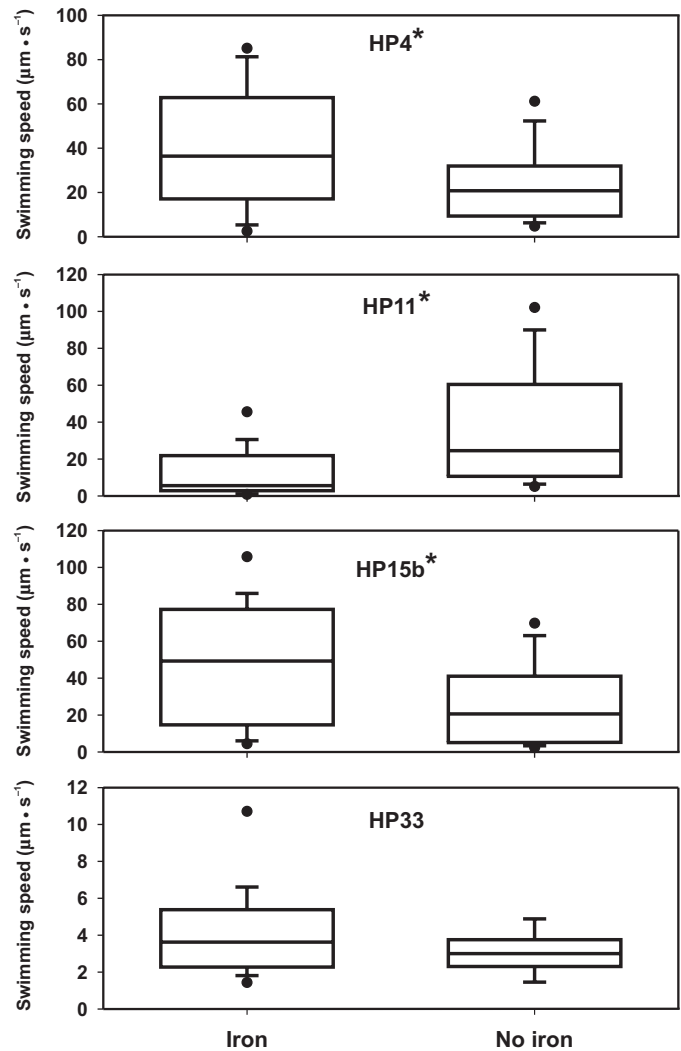


Table 2. Calculated *R* for 4 strains of marine snow bacteria in iron and no-iron treatments.

Strain	Iron	No iron
HP4	0.116	0.066
HP11	0.176	0.173
HP15b	0.348	0.221
HP33	0.158	0.096

where only 16 swimming tracks were successfully retrieved from the video footage (Fig. 3). Among strains HP4, 11, and 15b, swimming speed varied by 1 to 2 orders of magnitude, even within a population: some individuals were nearly stationary but showed slight vibration or rotation, whereas

Fig. 3. Box plots of swimming speeds of 4 strains of marine snow bacteria in iron and no-iron treatments. Each plot shows 5, 10, 25, 50, 75, 90, and 95 percentiles. Asterisks indicate significant differences between treatments (Mann–Whitney test, $P < 0.05$).



some individuals swam at $>100 \mu\text{m}\cdot\text{s}^{-1}$. In comparison, strain HP33 was overall much less motile than the other strains. Most of the bacteria were swimming at close to a straight line as indicated by a speed-to-velocity ratio close to 1. Nevertheless, a small fraction of the populations had highly convoluted swimming paths, resulting in a speed-to-velocity ratio as high as 12.09 (Table 3).

The swimming speeds of strains HP4 (average $40.6 \mu\text{m}\cdot\text{s}^{-1}$) and HP15 (average $49.7 \mu\text{m}\cdot\text{s}^{-1}$) were significantly higher in the iron treatment than in the no-iron treatment (average 23.7 and $26.4 \mu\text{m}\cdot\text{s}^{-1}$, respectively) (Mann–Whitney test, $P < 0.05$). In contrast, strain HP11 had a significantly higher swimming speed in the no-iron treatment (average $37.7 \mu\text{m}\cdot\text{s}^{-1}$) than in the iron treatment (average $13.2 \mu\text{m}\cdot\text{s}^{-1}$). Strain HP33 did not show significant difference in swimming speed between treatments (average 4.0 and $3.1 \mu\text{m}\cdot\text{s}^{-1}$).

Speed-to-velocity ratio was not different between treatments except for strain HP11, where iron treatment had a significantly higher ratio than no-iron treatment (Mann–Whitney test, $P < 0.05$).

Table 3. Speed-to-velocity ratios for 4 strains of marine snow bacteria in iron and no-iron treatments.

	Strain HP4		Strain HP11*		Strain HP15b		Strain HP33	
	Iron	No iron	Iron	No iron	Iron	No iron	Iron	No iron
Mean	1.31	1.64	1.97	1.45	1.59	1.43	1.13	1.23
Maximum	5.38	6.44	12.09	11.35	8.49	4.02	3.18	3.04
Minimum	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Median	1.09	1.06	1.25	1.06	1.07	1.09	1.03	1.06
<i>n</i>	57	50	59	75	49	50	50	16

*Significant difference between treatments (Mann–Whitney test; $P < 0.05$).

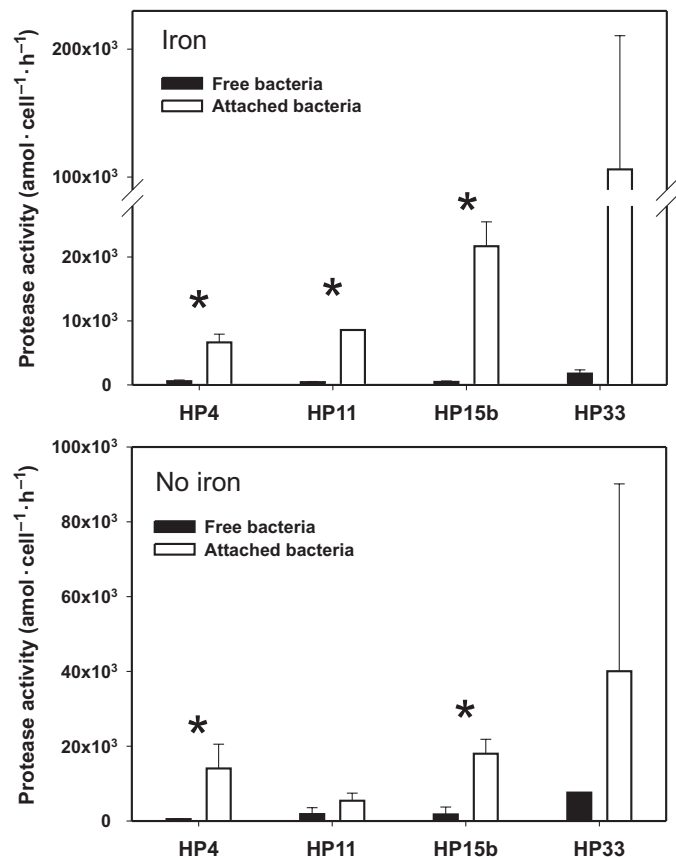
Protease activity

For strains HP4, 11, and HP15b, protease activity of free bacteria ranged from 0.43×10^3 to 1.84×10^3 amol·cell⁻¹·h⁻¹; no significant difference was detected between the iron and no-iron treatments (Student's *t* test; $P > 0.05$). For strain HP33 protease activity of free bacteria in the no-iron treatment (7.59×10^3 amol·cell⁻¹·h⁻¹) was 4.3 times that in the iron treatment ($P < 0.05$). Protease activity of attached bacteria ranged from 5.40×10^3 to 106.04×10^3 amol·cell⁻¹·h⁻¹ among the 4 strains with no significant difference between iron and no-iron treatments. Among the iron treatments, strains HP4, HP11, and HP15b showed significantly higher protease activity when attached than in free suspension ($P < 0.05$); the exception was strain HP33 where no significant difference was detected between attached and free bacteria (Fig. 4, upper panel). Similarly, among the no-iron treatments, the protease activity of attached bacteria was significantly (9–27 times) higher than that of free bacteria for strains HP4 and HP15b ($P < 0.05$) but not for strains HP11 or HP33 (Fig. 4, lower panel).

Discussion

Numerous studies have demonstrated that bacteria are an integral part of marine snow aggregates in the ocean, and their activities on and around the aggregates significantly affect the fate of the aggregates (Simon et al. 2002). Phytoplankton is not the only group of organisms that could be limited by iron in the ocean. It has been shown that low iron availability may directly or indirectly affect growth rate, production rate and growth efficiency of free bacteria (Hutchins et al. 1999; Kirchman et al. 2003). For marine bacteria that specialize in living on particles and aggregates, however, it is unknown whether iron availability may affect their rate of encounter and colonization of particles and aggregates. By removing free iron with the chelator DFOB we show that agar sphere colonization rate of marine snow bacteria are reduced by 1.7%–43.1% (based on calculated *R* values; Table 2). This reduction can be explained partly by a change in the bacterial swimming speeds under iron limitation. For example, the population swimming speeds of strains HP4 and HP15 were significantly reduced in the no-iron treatment, although not all individuals of the populations were equally affected. This reduction in population swimming speeds resulted in a lower diffusivity and subsequently a lower colonization rate. This explanation does not apply to strain HP33 where swimming speed did not differ

Fig. 4. Cell-specific protease activity of 4 strains of marine snow bacteria in iron and no-iron treatments (mean ± standard deviation; $n = 3$). Asterisks indicate significant difference between free and attached bacteria (Student's *t* test, $P < 0.05$).



between treatments; hence, a different effect of iron availability may have occurred. In the case of strain HP11, no-iron treatment actually resulted in a higher population swimming speed and a lower speed-to-velocity ratio; both are expected to result in higher diffusivity and colonization rate. The observed lower colonization rate in the no-iron treatment for strains HP11 and HP33 implies that a lower portion of the populations successfully attaches upon encounter with the agar spheres. A clinical study has shown that iron depletion alters the cell surface property of pathogenic bacteria and lowers their attachment to surfaces (Harjai et al. 1996). It is therefore possible that iron limita-

tion reduces the ability of marine snow bacteria to attach to agar sphere surfaces upon encounter, leading to a lower colonization rate. In addition to iron limitation, other laboratory experiments have shown that organic nutrient limitation would also reduce the motility and colonization rate of marine snow bacteria (K.W. Tang and H.-P. Grossart, unpublished data). As such, low iron availability may limit bacteria–particle interactions by reducing both the organic carbon production by phytoplankton and the bacteria colonization rate.

Elevated enzymatic activity on aggregates is commonly observed in aquatic environments (Karner and Herndl 1992; Middelboe et al. 1995; Smith et al. 1995; also see Martinez et al. 1996; Karrasch et al. 2004), and may contribute to rapid disintegration and solubilization of particulate organic matters (Smith et al. 1992; Grossart and Simon 1998). Interestingly, iron availability did not appear to have an effect on protease activity of attached bacteria in our experiments, suggesting that protease production by the bacteria is not dependent on iron. Nevertheless, in the iron treatment protease activity of attached bacteria was 10–47 times higher than that of free bacteria for strains HP4, 11, and 15b. Because both free and attached bacteria were collected from the same experimental vessels for enzyme measurements, these observations suggest that the bacteria rapidly turn on their enzyme production upon attachment to surfaces. Taylor and Gulnick (1996) showed that addition of inert, organic-free surfaces (glass beads) enhance proteolytic enzyme activities and leucine incorporation by salt marsh bacteria. Our previous study also consistently showed that marine snow bacteria increase protease activity upon attachment to surfaces even in the absence of a protein source (Grossart et al. 2007). Because encountering an aggregate could be a rare event in the open ocean (Kjørboe et al. 2002), the ability to up-regulate enzyme production would allow marine snow bacteria to rapidly exploit aggregate resources upon attachment while conserving energy between successful encounters with aggregates.

Iron limitation may affect not only growth and production by marine bacteria (Hutchins et al. 1999; Kirchman et al. 2003) but also motility and surface colonization behavior of the bacteria (this study). Because marine snow aggregates are often highly enriched with nutrients, possibly including iron, relative to ambient water (Azam and Long 2001), successful colonization of these aggregates may help alleviate iron and other nutrient limitations on the bacteria, and allow them to attain high growth and production rate relative to free bacteria.

Acknowledgements

This study was supported by US NSF OCE-0352125 (K.W.T.) and by the Leibniz Foundation. We thank Uta Mallok and Maureen Lynch for technical assistance. Comments from the editors and anonymous reviewers helped improve the manuscript. Contribution No. 2857 of the Virginia Institute of Marine Science.

References

Appenzeller, B.M.R., Yanez, C., Jorand, F., and Block, J.C. 2005.

- Advantage provided by iron for *Escherichia coli* growth and cultivability in drinking water. *Appl. Environ. Microbiol.* **71**: 5621–5623. doi:10.1128/AEM.71.9.5621-5623.2005. PMID: 16151163.
- Arrieta, J.M., Weinbauer, M.G., Lute, C., and Herndl, G.J. 2004. Response of bacterioplankton to iron fertilization in the Southern Ocean. *Limnol. Oceanogr.* **49**: 799–808.
- Azam, F. 1998. Microbial control of oceanic carbon flux: the plot thickens. *Science (Washington, D.C.)*, **280**: 694–696. doi:10.1126/science.280.5364.694.
- Azam, F., and Long, R.A. 2001. Sea snow microcosms. *Nature (London)*, **414**: 495–498. doi:10.1038/35107174. PMID: 11734832.
- Berg, H.C. 1993. *Random walks in biology*. Princeton University Press, New Jersey, USA.
- Boyd, P.W., Watson, A.J., Law, C.S., Abraham, E.R., Trull, T., Murdoch, R., et al. 2000. A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization. *Nature (London)*, **407**: 695–702. doi:10.1038/35037500. PMID: 11048709.
- Chisholm, S.W., Falkowski, P.G., and Cullen, J.J. 2001. Discrediting ocean fertilization. *Science (Washington, D.C.)*, **294**: 309–310. doi:10.1126/science.1065349. PMID:11598285.
- Church, M.J., Hutchins, D.A., and Ducklow, H.W. 2000. Limitation of bacterial growth by dissolved organic matter and iron in the Southern Ocean. *Appl. Environ. Microbiol.* **66**: 455–466. doi:10.1128/AEM.66.2.455-466.2000. PMID:10653704.
- Coale, K., Johnson, K.S., Fitzwater, S.E., Gordon, R.M., Tanner, S., Chavez, F.P., et al. 1996. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. *Nature (London)*, **383**: 495–501. doi:10.1038/383495a0.
- Cochlan, W.P. 2001. The heterotrophic bacterial response during a mesoscale iron enrichment experiment (IronEx II) in the eastern equatorial Pacific Ocean. *Limnol. Oceanogr.* **46**: 428–435.
- Grossart, H.-P., and Simon, M. 1998. Significance of limnetic organic aggregates (lake snow) for the sinking flux of particulate organic matter in a large lake. *Aquat. Microb. Ecol.* **15**: 115–125.
- Grossart, H.-P., Schlingloff, A., Bernhard, M., Simon, M., and Brinkhoff, T. 2004. Antagonistic activity of bacteria isolated from organic aggregates of the German Wadden Sea. *FEMS Microbiol. Ecol.* **47**: 387–396.
- Grossart, H.-P., Tang, K.W., Kjørboe, T., and Ploug, H. 2007. Comparison of cell-specific activity between free-living and attached bacteria using isolates and natural assemblages. *FEMS Microbiol. Lett.* **266**: 194–200. doi:10.1111/j.1574-6968.2006.00520.x. PMID:17233730.
- Harjai, K., Chhibber, S., and Sharma, S. 1996. Alteration in the adhesion properties of uropathogenic *Escherichia coli* in an iron-limited environment. *J. Adhesion Sci. Tech.* **10**: 391–398.
- Hoppe, H.G. 1983. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. *Mar. Ecol. Prog. Ser.* **11**: 299–308.
- Hutchins, D.A., and Bruland, K.W. 1998. Iron-limited diatom growth and Si:N uptake ratios in a coastal upwelling regime. *Nature (London)*, **393**: 561–564. doi:10.1038/31203.
- Hutchins, D.A., Franck, V.M., Brzezinski, M.A., and Bruland, K.W. 1999. Inducing phytoplankton iron limitation in iron-replete coastal waters with a strong chelating ligand. *Limnol. Oceanogr.* **44**: 1009–1018.
- Karner, M., and Herndl, G. 1992. Extracellular enzymatic activity and secondary production in free-living and marine-snow-associated bacteria. *Mar. Biol.* **113**: 341–347.

- Karrasch, B., Ullrich, S., Mehrens, M., and Zimmermann-Timm, H. 2004. Free and particle-associated extracellular enzyme activity and bacterial production in the lower Elbe estuary, Germany. *Acta Hydrochim. Hydrobiol.* **31**: 297–306.
- Kjørboe, T., Grossart, H.-P., Ploug, H., and Tang, K. 2002. Mechanisms and rates of bacterial colonization of sinking aggregates. *Appl. Environ. Microbiol.* **68**: 3996–4006. doi:10.1128/AEM.68.8.3996-4006.2002. PMID:12147501.
- Kirchman, D.L., Meon, B., Cottrell, M.T., Hutchins, D.A., Weeks, D., and Bruland, K.W. 2000. Carbon versus iron limitation of bacterial growth in the California upwelling regime. *Limnol. Oceanogr.* **45**: 1681–1688.
- Kirchman, L., Hoffman, K.A., Weaver, R., and Hutchins, D.A. 2003. Regulation of growth and energetics of a marine bacterium by nitrogen source and iron availability. *Mar. Ecol. Prog. Ser.* **250**: 291–296.
- Martin, J.H., and Fitzwater, S.E. 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. *Nature (London)*, **331**: 341–343. doi:10.1038/331341a0.
- Martinez, J., Smith, D.C., Steward, G.F., and Azam, F. 1996. Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. *Aquat. Microb. Ecol.* **10**: 223–230.
- Middelboe, M., Sondergaard, M., Letarte, Y., and Borch, N.H. 1995. Attached and free-living bacteria — production and polymer hydrolysis during a diatom bloom. *Microb. Ecol.* **29**: 231–248. doi:10.1007/BF00164887.
- Palyada, K., Threadgill, D., and Stintzi, A. 2004. Iron acquisition and regulation in *Campylobacter jejuni*. *J. Bacteriol.* **186**: 4714–4729. doi:10.1128/JB.186.14.4714-4729.2004. PMID:15231804.
- Simon, M., Grossart, H.-P., Schweitzer, B., and Ploug, H. 2002. Microbial ecology of organic aggregates in aquatic ecosystems. *Aquat. Microb. Ecol.* **28**: 175–211.
- Smith, D.C., Simon, M., Alldredge, A.L., and Azam, F. 1992. Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature (London)*, **359**: 139–142. doi:10.1038/359139a0.
- Smith, D.C., Steward, G.F., Long, R.A., and Azam, F. 1995. Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm. *Deep-Sea Res. Part II*, **42**: 75–97. doi:10.1016/0967-0645(95)00005-B.
- Taylor, G.T., and Gulnick, J.D. 1996. Enhancement of marine bacterial growth by mineral surfaces. *Can. J. Microbiol.* **42**: 911–918.
- Tortell, P.D., Maldonado, M.T., and Price, N.M. 1996. The role of heterotrophic bacteria in iron-limited ocean ecosystems. *Nature (London)*, **383**: 330–332. doi:10.1038/383330a0.
- Weinberg, E.D. 2004. Suppression of bacterial biofilm formation by iron limitation. *Med. Hypotheses*, **63**: 863–865. doi:10.1016/j.mehy.2004.04.010. PMID:15488661.
- Wells, M.L. 1999. Manipulating the availability of Fe in nearshore waters. *Limnol. Oceanogr.* **39**: 1481–1486.