Vol. 47: 83–90, 2007 | AQUATIC MICROBIAL ECOLOGY Aquat Microb Ecol | Published April 3

Degradation of gallic acid and hydrolysable polyphenols is constitutively activated in the freshwater plant-associated bacterium *Matsuebacter* sp. FB25

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ABSTRACT: Hydrolysable polyphenols are present in *Myriophyllum spicatum* L. at high concentrations of up to 25% of dry matter and are also excreted. Bacteria associated with the submerged macrophyte *M. spicatum* isolated from the surrounding water column and epiphytic biofilm were tested for their ability to degrade polyphenols. Several bacterial isolates were capable of growing with tannic acid as the sole carbon and energy source, among them *Matsuebacter* sp. FB25, *Agrobacterium vitis* EB26 and *Pseudomonas* sp. FB22. Cell suspensions of *Matsuebacter* sp. precultured on succinate were capable of degrading gallic acid, while those of *A. vitis* were not, indicating the constitutive presence of gallate-degrading enzymes in the former. When cells were precultured on gallic or tannic acid, cell suspensions of both strains exhibited an enhanced degradation rate of gallic acid. *M. spicatum*-derived hydrolysable polyphenols, which are comparable in structure to tannic acid, resulted in the same enhanced degradation rate of gallic acid or tellimagrandin II, the major *M. spicatum* polyphenol, by cell suspensions of *Matsuebacter* sp. FB25. The presence of polyphenol-degrading bacteria in the vicinity of *M. spicatum* explains the observed fast disappearance of tellimagrandin II and other hydrolysable polyphenols after excretion and has implications for allelochemical interference with competitors, herbivores and potential pathogenic microorganisms. The presence of *Matsuebacter* sp. and other polyphenol-degrading strains in such environments suggests a sufficiently strong effect of *M. spicatum* exudates to bring about selection in favour of highly specialised bacteria.

KEY WORDS: Hydrolysable polyphenol · Submerged macrophyte · Allelochemistry · Biofilm · Betaproteobacteria · Burkholderiales · *Myriophyllum spicatum* · Tannin

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INTRODUCTION

The freshwater dicotyledonous plant *Myriophyllum spicatum* L. (Haloragaceae: Rosidae) produces and excretes bioactive hydrolysable polyphenols interfering with competitors and herbivores. On average, 7 to 10% of the plant dry mass (dm) are polyphenols, among them tellimagrandin II (β-1,2,3-tri-*O*-galloyl-4,6-(S)-hexahydroxydiphenoyl-D-glucose). Apical shoots can even comprise more than 20% of polyphenols based on dry matter. Tellimagrandin II and other hydrolysable polyphenols inhibit algae and cyanobacteria by interference with extracellular alkaline phosphatase (Gross et al. 1996) or photosynthesis (Leu et al. 2002). Hydrolysable polyphenols cause a reduced growth of larvae of the aquatic moth *Acentria ephemerella* (Choi et al. 2002), and inhibit the growth of bacteria isolated from the gut of larvae (Walenciak et al. 2002). Exudates of *M. spicatum* also interfere with zooplankton (Linden & Lehtiniemi 2005). Hydrolysable polyphenols are actively excreted by *M. spicatum* (Gross 2003), but the fate of individual

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polyphenols, e.g. tellimagrandin II, is difficult to assess and depends on multiple biotic (microbial degradation) and abiotic (oxidation, photodegradation) factors.

Since the allelochemical activity of *Myriophyllum spicatum* depends on the presence of tellimagrandin II and other hydrolysable polyphenols, a better knowledge of the role of microorganisms in the degradation of these polyphenols is needed. In polyphenols, the ester bonds between the sugar (often glucose) and gallic or ellagic acid are cleaved by tannase (tannin acyl hydrolase, EC 3.1.1.20), an enzyme frequently found in fungi or bacteria (Chowdhury et al. 2004, Li et al. 2006) and even in plants (Niehaus & Gross 1997). The microbial degradation of gallic acid is possible by several distinct mechanisms (Li et al. 2006). Under aerobic conditions, gallic acid can be degraded either (1) by following the β-ketoadipate pathway starting with a NADH-dependent reduction (Armstrong & Patel 1992); or (2) by conversion into pyruvate and oxalacetate feeding into the citrate cycle, initiated by the action of gallate dioxygenase (Kasai et al. 2005, Nogales et al. 2005). The anaerobic degradation starts with gallate decarboxylase to yield pyrogallol, which is further degraded to acetate (Zeida et al. 1998). In aquatic systems, only a few strains (e.g. *Pelobacter acidigallici* from aquatic sediments: Schink et al. 2000) have been investigated for their capacity to degrade phenolic compounds, including gallic acid. Aerobic degradation is most plausible for epiphytic bacteria or bacteria living in the water surrounding *M. spicatum*.

The aims of the present study were (1) to investigate whether bacteria capable of growing with tannic acid or hydrolysable polyphenols can be isolated from the environment of *Myriophyllum spicatum*; (2) to then identify bacterial strains that are capable of growing solely on tannic or gallic acid; and (3) to investigate how these strains degrade tannic acid, gallic acid and tellimagrandin II.

MATERIALS AND METHODS

Origin of bacterial isolates and aquatic plants. Bacterial samples were collected from *Myriophyllum spicatum* stands in mesocosms at the Limnological Institute, University of Konstanz (9.192° N, 47.694° E) during winter 2004/2005. The mesocosms are 2 × 2 × 1 m (depth) concrete basins, filled with a 7 to 10 cm layer of lake sediment, flushed constantly with water from Lake Constance, and planted with *M. spicatum,* which is wintergreen in these basins.

Pelagic bacteria were sampled in the vicinity of plant stands in the mesocosms. Epiphytic bacteria were retrieved from the upper 20 cm of plant shoots (measured from the apex) and placed directly in polypropylene tubes filled with sterile water. Samples were transported in coolers to the laboratory and processed immediately. Pelagic bacteria were used immediately in enrichment cultures. Epiphytic bacteria were detached by ultrasonication at maximum output (Laboson 200 ultrasonic bath, Bender & Hobein) from 3 to 20 *Myriophylllum spicatum* leaves in 2 ml sodium pyrophosphate (0.1 M $Na_4P_2O_7 \times 10\ H_2O$) for 2 × 30 s with a 10 s break.

Isolation and enrichment of polyphenol-degrading bacteria. Water samples (2 ml) or detached epiphytic bacteria (2 ml) were incubated under sterile conditions in 250 ml Erlenmeyer flasks with 100 ml modified Medium B (Hempel 2004: 5 mM NH_4Cl, 0.5 mM $MgSO_4 \times 7\ H_2O$, 14 mM KCl, 7.2 mM NaCl; instead of 10 mM HEPES, 10 mM TRIS were used as buffer, medium adjusted to pH 7). Directly before use, 1 ml of 1000-fold concentrated trace element solution SL10 (Widdel et al. 1983), 0.1 ml of 100 mM Ca_2Cl and 0.3 ml 0.15 mM Na^+/K^+-hydrogenphosphate solution (pH 7) were added. Instead of 0.05% tryptone and 0.0005% yeast extract, we offered 294 µM tannic acid as the sole source of carbon and energy. Cultures were incubated in the dark at 16°C and 200 rpm on an orbital shaker and growth was followed by measuring optical density (OD) at 600 nm. Growing cultures were diluted after 3 to 7 d in a series from 10^{-1} to 10^{-6} and plated on Medium B agar plates solidified with 1.5% agar, containing 0.05% tryptone and 0.0005% yeast extract, but no tannic acid. This change in carbon source was used to allow a faster growth of colonies. The plates were cultivated at 16°C in the dark. After 4 d, colonies were picked and transferred to new plates. This procedure was repeated 3 times to single out colonies.

Each isolate was again tested for growth on tannic acid as sole carbon and energy source. Five microlitres of an actively growing culture of each strain were diluted in 295 µl Medium B with 294 µM tannic acid in 96-well microtitre plates. Growth was recorded in a microplate reader at 595 nm (Genios, Tecan). Only strains exhibiting significant growth (OD_{595} > 0.09) were used further.

Identification of polyphenol-degrading strains. Selected strains were identified by DNA extraction, PCR amplification of the 16S rDNA gene, sequencing and BLAST searches using standard protocols. Growing isolates were harvested by centrifugation at 12 000 rpm (14 800 × *g*) and 4°C for 15 min. The pellets were washed twice with sterile ultrapure water to break the cells by hypoosmotic shock. The final pellet was shock-frozen in liquid nitrogen and ultrasonicated at maximum output for 2 × 30 s with a 10 s break. Cell debris was removed after resuspension of the pellet in 100 µl sterile ultrapure water and centrifugation. The supernatant contained the DNA.

One PCR reaction to amplify the 16S-rDNA gene contained 42.8 µl PCR buffer (*Taq* buffer, Eppendorf), 5 µl dNTPMix (500 mM, Eppendorf), 0.5 µl forward primer at 50 pmol $µl^{-1}$, 27f 5'AGAGTTTGATCCTG GCTCAG-3', 0.5 µl reverse primer at 50 pmol $µl^{-1}$, 1492r 5'-TACGG(CT)TACC TTACGACTT-3', 1 µl of DNA-template, and finally 1 U *Taq* polymerase (0.2 µl). We used a Thermocycler T-Gradient (Biometra) and the following protocol: (1) 3 min at 94°C; (2) 30 s at 55°C; (3) 1 min at 72°C; (4) 30 s at 94°C; (5) 30 s at 55°C; (6) 1 min at 72°C; repeat Steps 4 to 6 33×; (7) 7 min at 72°C. The PCR products were checked by loading 5 µl sample with 1 µl 6× loading buffer on 1% agarose gels in TAE buffer containing 40 mM TRIS, 1 mM EDTA and 20 mM sodium acetate, pH 8 adjusted with glacial acetic acid. Separation was performed at 130 V and 400 mA for 30 min. The gels were stained for 30 to 40 min in 2.5 µM ethidium bromide and DNA bands were identified under UV light with a BioDoc Analyzer (Biometra). Positive samples were purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced by GATC (Konstanz). Sequences were compared with the NCBI database (www.ncbi.com) using the BLAST search tool.

Chemical analyses. HPLC calibration was performed by triplicate injection of concentration series of gallic acid (Sigma G7384) in the range 0 to 1 mM, or 0 to 0.2 mM of purified tellimagrandin II (own source, 98% HPLC-pure). Polyphenols were identified on a Jasco HPLC system using a Kromasil C18 column (250 × 4 mm), Solvent A (1% [v/v] acetic acid) and B (methanol), at a flow rate of 1 ml min^{-1} and photodiode array detection set at the primary wavelengths 254 and 280 nm. Gallic acid was analysed using isocratic conditions (8% Solvent B and 92% Solvent A, 15 min) and tellimagrandin II using a linear gradient of 0 to 15 min 8 to 60% Solvent B, 15 to 17 min 60 to 100% Solvent B, 17 to 20 min 100% Solvent B.

Tannic acid concentrations were determined photometrically by precipitation with bovine serum albumin and staining with $FeCl_3$ (Hagerman & Butler 1978). Besides quantification by HPLC, gallic acid was also quantified with the rhodanine method (Inoue & Hagerman 1988) modified using citrate buffer instead of sulphuric acid (Sharma et al. 2000). A parallel analysis both photometrically and by HPLC confirmed that the 2 methods yield comparable results, with HPLC exhibiting slightly lower values (c[Hplc] = 0.88 c[rhodanin]; r^2 = 0.9995). Glucose was analysed on 500-fold concentrated medium by HPLC following the method in Klebensberger et al. (2006).

In order to test the induction of tannic acid and gallic acid degradation in isolates by *Myriophyllum spicatum* polyphenols, we used crude extracts and a solid-phase extraction (SPE) fraction containing tellimagrandin II (>80%) isolated from apical shoots of *M. spicatum.* Plant material was extracted with aqueous acetone (1:1 [v/v]; 2 × 2 h, 14°C, constant stirring) followed by SPE on C18 cartridges (Phenomenex; 2 g sorbens) using a step-gradient elution with increasing methanol concentrations in water (Leu et al. 2002). The fraction containing tellimagrandin II eluted with 25% [v/v] methanol.

Growth experiments. *Matsuebacter* sp. FB25 was pregrown on Medium B with 294 µM tannic acid. This strain was selected because of its high growth rate on tannic acid in first experiments. Cells were harvested and washed twice in Medium B without carbon source. Three different treatments were used: 3 Erlenmeyer flasks each containing 100 ml of Medium B supplemented with either 294 µM glucose, 294 µM tannic acid or gallic acid were inoculated with cells at an initial $OD_{600\ nm}$ of 0.008. Controls without bacteria were incubated in parallel. Culture conditions were the same as above. Samples were taken at regular intervals for 96 h, and growth of the culture as well as substrate (glucose, gallic acid, tannic acid) concentrations of the medium were analysed.

Induction experiments. Three of the isolated strains were tested for their capability to degrade gallic acid or tannic acid with or without previous exposure to hydrolysable polyphenols. Four different treatments were set up. In each, cells were pregrown in Medium B supplemented with either 10 mM succinate, 10 mM succinate plus 294 µM tannic acid, 294 µM tannic acid or 1 mM gallic acid. An initial $OD_{600\ nm}$ of 0.02 was used to shorten the lag phase. At the end of the exponential growth phase, cells of the different treatments were harvested by centrifugation, and adjusted to an $OD_{600\ nm}$ of ca. 1.0 in 5 ml Medium B containing 1 mM gallic acid in triplicates. Cell suspensions were stirred with 550 rpm at 22°C on a magnetic stirrer. At 0, 30, 60, 120 and 240 min each, 200 µl were taken and trichloroacetic acid was added at a final concentration of 0.1 M to stop enzyme reactions. After centrifugation (13 000 rpm [16 000 × *g*], 4°C, 5 min), 10 µl each were analysed for gallic acid by HPLC.

We then tested whether *Myriophyllum spicatum* polyphenols also induce or accelerate the degradation of gallic acid and tellimagrandin II. *Matsuebacter* sp. FB25 was pregrown in Medium B containing either 75 µM tannic acid (~125 mg l^{-1}) or crude plant extract (containing hydrolysable polyphenols measured as tannic acid equivalents of 125 mg l^{-1}) using the same conditions as given above. Cells harvested at the end of the exponential growth phase were incubated with either 0.2 mM tellimagrandin II or 1 mM gallic acid in triplicates. We also performed controls testing the stability of gallic acid or tellimagrandin II in Medium B under the same environmental conditions (pH, light,

oxygen). The sampling protocols for gallic acid and tellimagrandin II were extended to 5 or 30 h. Degradation rates were calculated based on protein content, derived from the $OD_{660\ nm}$ of cell suspensions used. One litre of a suspension of 1 $OD_{660\ nm}$ was equivalent to 250 mg dry mass, of which we estimated 50% as protein content.

RESULTS

Isolation and enrichment of strains

From the enrichment cultures on tannic acid, 96 isolates were obtained, 32 derived from the mesocosm water surrounding *Myriophyllum spicatum* (designated 'FB' for 'free bacteria' followed by strain number) and 64 from the plant biofilm (designated 'EB' for 'epiphytic bacteria', followed by strain number). Growth curves on 294 µM tannic acid as sole carbon source were obtained with all isolates in microtitre plates. Most isolates reached an $OD_{595\ nm}$ of at least 0.05 after 160 h. We selected the 15 best growing isolates, all exhibiting an $OD_{595\ nm}$ of >0.09, among these were 11 from the biofilm.

All 15 isolates were further characterised by sequencing the first 750 bp of the 16S rDNA. Isolates FB19, FB24 and FB25 showed a 98 to 99% homology with *Matsuebacter* sp. 9 (AB024305) according to a BLAST search. FB22 exhibited 99% similarity to *Pseudomonas* sp. (e.g. AY599719), and all EB isolates (EB3, 4, 22, 23, 25, 26, 28, 33, 40, 53, 54) revealed several *Agrobacterium vitis* strains (e.g. AB247599) as closest relatives, with 96 to 99% similarity. Full sequences (>1500 bp) of the 16S rDNA of Strains FB22, FB25 and EB26 were obtained, confirming the abovementioned identifications with >99% homology. These 3 isolates were used in further experiments. They are all rod- shaped bacteria. The 16S rDNA sequence of *Matsuebacter* sp. FB25 has been submitted to GenBank (EF110621). GenBank taxonomists indicate the change in genus name from '*Matsuebacter*' to '*Mitsuaria*'.

Growth experiment

Matsuebacter sp. FB25 exhibited the best growth on tannic acid and was therefore selected to study the details of tannic acid and gallic acid utilisation. Since tannic acid consists of multiple (usually 8 and up to 11) gallic acid residues esterified to glucose, we grew *Matsuebacter* sp. FB25 on either 294 µM tannic acid or 294 µM glucose for 90 h compared to sterile medium controls. Both controls also showed a slight increase in $OD_{600\ nm}$. In the case of tannic acid this most probably resulted from chemical reactions with Medium B constituents and subsequent precipitation. *Matsuebacter* sp. FB25 exhibited growth on both substrates, but grew faster and to a higher final density on tannic acid (Fig. 1A,B). We had offered an equimolar glucose concentration to test whether cells would use the sugar and not gallic acid after hydrolysis of tannic acid. The higher growth on tannic acid indicates that gallic acid is used as substrate. The uptake of glucose by *Matsuebacter* sp. FB25 could not be followed by HPLC because, even after concentration of the medium, glucose was below detection limit. Tannic acid declined in both control and the *Matsuebacter* sp. FB25 culture (Fig. 1C) to 181 and 105.5 µM after 90 h, respectively. The remaining

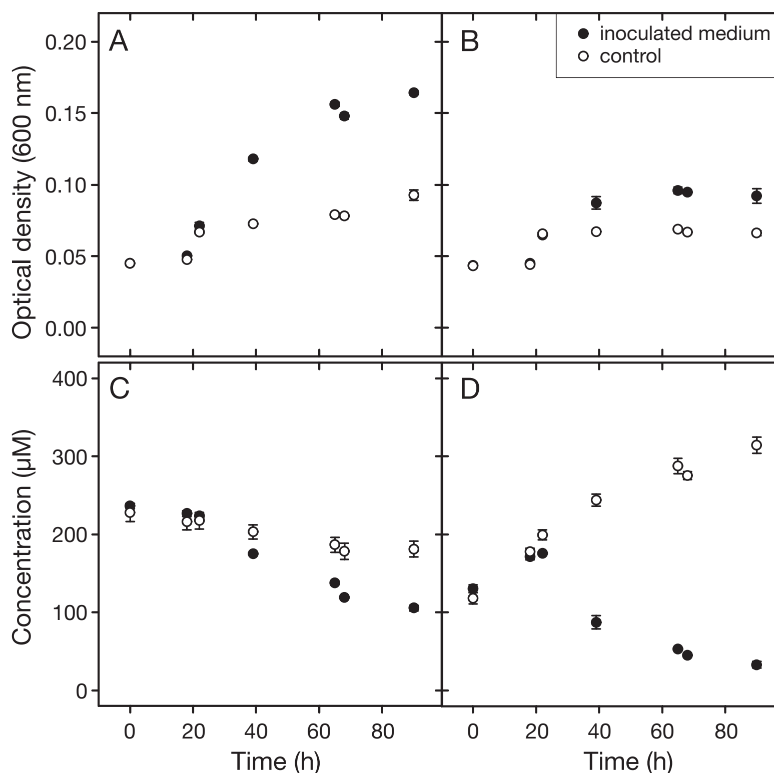

Fig. 1. *Matsuebacter* sp. Growth and substrate utilisation of Strain FB25 derived from water surrounding *Myriophyllum spicatum* in experimental mesocosms. (A) $OD_{600\ nm}$ of culture growing with 294 µM tannic acid; (B) $OD_{600\ nm}$ of culture growing with 294 µM glucose; (C) concentration of tannic acid in the culture with tannic acid as substrate; (D) concentration of gallic acid in the culture with tannic acid as substrate. Data are means ± SE, n = 3 (some error bars are smaller than symbol size)

tannic acid concentrations in the *Matsuebacter* sp. FB25 culture differed significantly from the control after 39 h (2-way ANOVA and post-hoc Bonferroni t-test: $p < 0.04$). Gallic acid in the control medium increased constantly from $t_{0\ h}$ = 118 µM to $t_{90\ h}$ = 314 µM, indicating spontaneous autolysis of tannic acid. In the *Matsuebacter* sp. FB25 culture, gallic acid increased for the first 22 h from 130 to 176 µM and then declined to 33 µM at 90 h (Fig. 1D), indicating an active degradation of this substrate.

Induction of gallic acid degradation with or without precultivation on polyphenols

In a second series of experiments, we initially cultured 3 isolates (*Pseudomonas* sp. FB22, *Matsuebacter* sp. FB25, *Agrobacterium vitis* EB26) on 4 different media with or without polyphenols to investigate whether the degradation of gallic acid is inducible. *Pseudomonas* sp. FB22 did not grow on tannic acid or gallic acid alone, thus the experiment was performed only with *Matsuebacter* sp. FB25 and *Agrobacterium vitis* EB26. Interestingly, *Matsuebacter* sp. FB25 could grow on 1 mM but not on 2.35 mM gallic acid (data not shown).

Cell suspensions of *Matsuebacter* sp. FB25 were able to degrade gallic acid in all 4 treatments (Fig. 2A). The fastest degradation occurred when cells had been precultured on gallic acid (181.9 nmol gallic acid min^{-1} mg^{-1} protein within the first 30 min), followed by those grown on tannic acid (132.3 nmol gallic acid min^{-1} mg^{-1} protein) or succinate plus tannic acid (55.3 nmol gallic acid min^{-1} mg^{-1} protein). Although at a much slower rate, even cells pregrown on succinate were able to degrade gallic acid (4.8 nmol gallic acid min^{-1} mg^{-1} protein).

Cell suspensions with *Agrobacterium vitis* EB26 exhibited a much slower degradation of gallic acid than *Matsuebacter* sp. FB25 in all 4 treatments (Fig. 2B). Cells pregrown on succinate or succinate plus tannic acid exhibited almost no degradation of gallic acid within 4 h, while those induced with either tannic acid or gallic acid slowly degraded gallic acid (10.1 or 15.7 nmol gallic acid min^{-1} mg^{-1} protein within the first 30 min, respectively).

Induction of gallic acid or tellimagrandin II degradation in cells precultured on tannic acid or plant-extracted polyphenols

First, we tested whether polyphenols extracted from *Myriophyllum spicatum* would also induce the degradation of gallic acid in *Matsuebacter* sp. FB25 comparable to that observed with tannic acid. Cell suspensions pretreated with succinate plus either tannic acid or plant extract containing polyphenols at equivalent concentrations rapidly and completely degraded gallic acid within 5 h. Slight, but significant differences in the initial degradation rate were observed (tannic acid or extract: 30.6 or 13.1 nmol gallic acid min^{-1} mg^{-1} protein within the first 60 min, respectively, $p = 0.002$), reaching maximum rates of 87.2 and 41.9 nmol gallic acid min^{-1} mg^{-1}, respectively (Fig. 3A). We then investigated the degradation of tellimagrandin II by either gallic acid or extract induced cell suspensions. Tellimagrandin II was degraded in both treatments at rates of 0.8 to 4.5 nmol tellimagrandin II min^{-1} mg^{-1} protein by induced cells (Fig. 3B). Without bacterial cells, tellimagrandin II declined at a rate of only 0.04 nmol min^{-1}. No difference in the mean degradation rate of either gallic acid or tellimagrandin II between tannic acid or plant extract induced cells was observed (paired t-tests: gallic acid: $p = 0.760$; tellimagrandin II: $p = 0.743$).

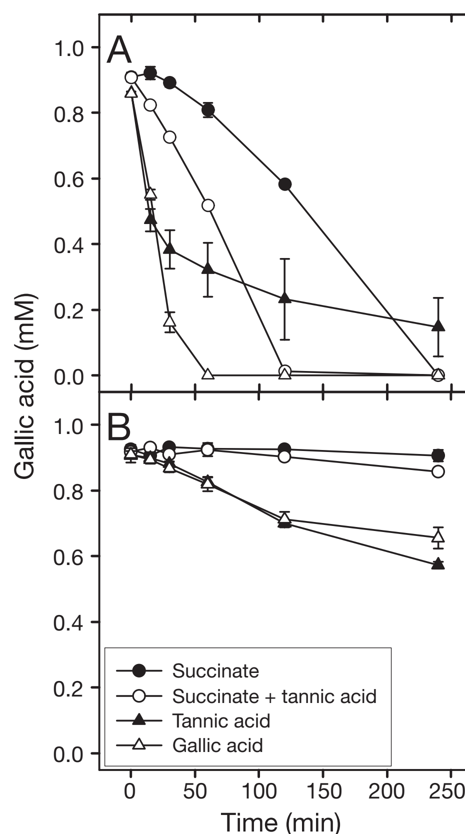

Fig. 2. Induction of gallate degradation in 2 bacterial strains isolated from *Myriophyllum spicatum*. (A) *Matsuebacter* sp. Strain FB25; (B) *Agrobacterium vitis* Strain EB26, derived from the epiphyton on *Myriophyllum spicatum* in experimental mesocosms. Data are means ± SE (n = 3)

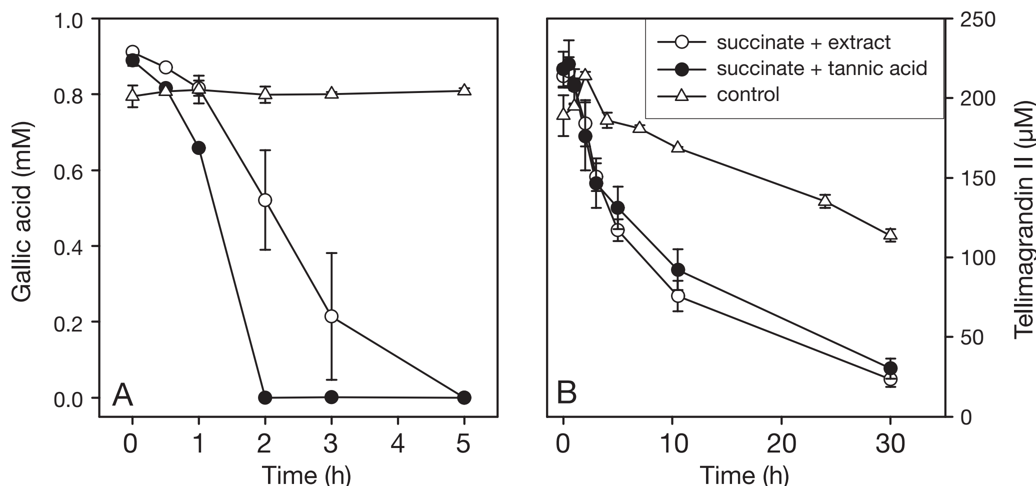

Fig. 3. *Matsuebacter* sp. Strain FB25. Cell suspension experiments. Cell suspensions offered (A) 1 mM gallic acid and (B) 0.2 mM tellimagrandin II. Data are means ± SE, n = 3 (some error bars are smaller than symbol size)

DISCUSSION

Several bacterial strains isolated via enrichment cultures on tannic acid from the environment of the polyphenol-producing submerged macrophyte *Myriophyllum spicatum* were able to degrade tannic acid, gallic acid or plant-derived tellimagrandin II, and could even use these substrates as sole carbon and energy source. To our knowledge, this is the first study investigating the role of bacteria in degrading *Myriophyllum*-derived polyphenols. Previous studies of *Myriophyllum*-associated bacteria focused on potential pathogens useful for the biological control of *M. spicatum* (Chand et al. 1992) or on rhizosphere bacteria involved in nitrogen metabolism (Blotnick et al. 1980). So far, the majority of studies focusing on tannin-degrading bacteria are either from environments exposed to industrial tannery waste (Chowdhury et al. 2004, Franco et al. 2005) or from investigations of intestinal bacteria in tannin-feeding invertebrates and vertebrates (Goel et al. 2005, Smith et al. 2005), while little evidence exists for such specialised bacteria in natural waters, most of them from anaerobic environments (Mahadevan & Muthukumar 1980, Schink et al. 2000). In contrast to other major submerged aquatic angiosperms, *M. spicatum* produces and releases high concentrations of hydrolysable polyphenols (Smolders et al. 2000, Choi et al. 2002, Gross 2003). Indirect evidence already suggested a limited persistence of released polyphenols in water (Gross & Sütfeld 1994, Nakai et al. 1999), and herein we provide evidence for the bacterial degradation of these allelochemicals.

The 3 major strains isolated and identified in our study (*Pseudomonas* sp. FB22, *Agrobacterium vitis* EB26 and *Matsuebacter* sp. FB25) have not directly been associated with tannin degradation so far. Many *Pseudomonas* strains are able to degrade a wide range of aromatic hydrocarbons, yet our isolate *Pseudomonas* sp. FB22 did not grow as pure culture only on tannic or gallic acid, suggesting that this strain used other carbon sources. Eleven isolates derived directly from the biofilm on *Myriophyllum spicatum* were identified as strains of the Gram-negative Alphaproteobacteria *A. vitis.* Usually *A. vitis* strains are found on grapevines, where they may prove serious pathogens. In that environment, they may come in contact with and be able to degrade grapevine polyphenols, comparable to the polyphenol-degrading capacity of the strain *A. vitis* EB26 we isolated from the biofilm on *M. spicatum*.

Matsuebacter sp. FB25 was the most interesting among our tannin-degrading isolates since it was capable of using gallic acid without prior induction. Two of our other isolates (FB19, FB24) were also affiliated to *Matsuebacter*. Other relatives to *Matsuebacter* sp. FB25 are *Herbaspirillum* spp., e.g. *H. chlorophenolicum* CT2 (AY702478), which has been isolated from the rhizosphere of emergent macrophytes grown in a tannery effluent exposed wetland (Franco et al. 2005).

Matsuebacter sp. FB25 seems to be a specialist for hydrolysable polyphenols; this strain grew even faster on tannic acid than on glucose. When grown with tannic acid, a lag time of approx. 40 h was observed, suggesting that either tannase needed for the hydrolytic cleavage had to be induced or cells could only grow when sufficient tannic acid underwent autolysis to yield free gallic acid. A direct comparison of degradation rates of gallic acid between *Matsuebacter* sp. FB25 and *Agrobacterium vitis* EB26 clearly shows the faster adaptation of the former when exposed to gallic or tannic acid. Cell suspensions of *A.*

vitis EB26 precultured on succinate or even succinate plus tannic acid did not exhibit a significant degradation of gallic acid within 4 h, and even when precultured on tannic or gallic acid, only about 30 to 40% of the substrate (gallic acid) was degraded during that time. In contrast, cell suspensions of *Matsuebacter* sp. FB25 degraded gallic acid at higher rates and, in most cases, completely used the offered substrate, although cells incubated earlier with succinate exhibited somewhat slower initial degradation rates. Tellimagrandin II from *Myriophyllum spicatum* was also degraded by *Matsuebacter* sp. FB25, but at a slower rate. It might be that the ellagic acid (a dilactone of gallic acid) derived from the hexahydroxydiphenoic moiety in tellimagrandin II interferes with the degradation of this hydrolysable polyphenol and gallic acid. Ellagic acid may have antimicrobial activity, as shown for extracted phenolic compounds from the floating macrophyte *Nuphar variegatum* (Nishizawa et al. 1990). Ellagic acid and tannic acid had an antagonistic effect on AHL-dependent quorum-sensing systems in *Escherichia coli* MT102 (pSB403) (Huber et al. 2003).

The gallic acid degradation in both *Matsuebacter* sp. FB25 and *Agrobacterium vitis* EB26 occurs under oxic conditions, but we do not know yet which pathway is used. Most probably gallate dioxygenases are involved, comparably to gallate-degrading enzymes found in *Pseudomonas putida* KT2440 or *Sphingomonas paucimobilis* SYK-6 (Kasai et al. 2005, Nogales et al. 2005). Further biochemical studies are needed to clarify the degradation pathway and intermediate metabolites.

At present, we cannot say how abundant tannin-degrading strains are in the biofilm or water surrounding *Myriophyllum spicatum*. It is quite likely that other, yet unidentified and uncultured, strains with the same metabolic capacity to degrade hydrolysable polyphenols are present. In a parallel study of epiphytic bacteria on submerged macrophytes, we isolated a strain closely related to *Pantoea agglomerans* from *M. spicatum*. This strain (formerly named *Enterobacter agglomerans*) is known to possess tannase and gallic acid decarboxylase (Zeida et al. 1998).

The presence of tannin-degrading bacteria as epiphytes of tannin-producing plants might even be beneficial to other, non-adapted bacteria. The inhibition of bacteria by tannins is mainly based on their complexation with essential polymers and minerals (Scalbert 1991, Smith et al. 2005), thus affecting cell-bound or extracellular proteins or the uptake of nutrients. Tannin-tolerant bacteria, in contrast, have evolved different mechanisms to overcome these limitations (Smith et al. 2005), e.g., the modification or degradation of tannins, the dissociation of tannin–substrate complexes, tannin inactivation by high-affinity binders, membrane modifications or repair, and metal ion sequestration. Epiphytic microorganisms are 3-dimensionally enmeshed with hydrated mucopolysaccharide materials excreted by epiphytic bacteria and algae. Such a matrix might bind *Myriophyllum spicatum*-released polyphenols and render them less toxic to epiphytic bacteria (Ervin & Wetzel 2003).

Bacteria often influence allelopathically active compounds. Juglone from walnut trees is only active after hydrolysis by soil bacteria. Several phenolic compounds released by *Polygonella myriophylla,* an allelopathically active shrub of the Florida sand pine scrub community, undergo rapid degradation or conversion in non-sterile soil compared to sterile conditions (Weidenhamer & Romeo 2004). Whether the presence of bacteria diminishes the allelopathic activity is difficult to say, because this depends on the rate of production and release of active compounds and the rate of conversion/degradation. Similarly, the degradation of polyphenolic allelochemicals by associated microorganisms has important implications for the chemical ecology of *Myriophyllum spicatum*. First, exuded polyphenols have only a limited half-life and do not accumulate. This would be expected, since a constant rise in such inhibitory compounds would not allow the plant to regulate the active concentration in its vicinity. However, if exuded polyphenols are degraded too fast, their deleterious effect on competing phytoplankton or epiphytic algae and cyanobacteria should decline. In short-term experiments, tellimagrandin II is detectable in axenic (bacteria-free) cultures (Gross & Sütfeld 1994), and other compounds, possibly oxidation products, increase over time. When non-axenic plants are incubated in water, tellimagrandin II and other hydrolysable polyphenols decline even faster, suggesting microbial break-down. Bacterial degradation together with photochemical conversion probably accounts for the failure to detect tellimagrandin II in exudates of field *M. spicatum* (Glomski et al. 2002). Nevertheless, *M. spicatum* exudates are inhibitory to cyanobacteria and algae, but the exposure to such allelopathically active compounds has to be (semi)continuous (Nakai et al. 1999).

Acknowledgements. We acknowledge the technical assistance of C. Feldbaum and helpful discussions with H. P. Grossart and B. Schink. This project was supported by DFG German Science foundation in project CRC454, A2 to E.M.G. and B9 to B.P., and by the University of Konstanz, AFF to E.M.G. and B.P.

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Editorial responsibility: Ruben Sommaruga, Innsbruck, Austria

Submitted: December 4, 2006; Accepted: January 30, 2007
Proofs received from author(s): March 1, 2007