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NOTE

Sulphate reduction associated with roots and rhizomes of the marine macrophyte *Zostera marina*

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ABSTRACT: The activity, the location and the oxygen sensitivity of sulphate reducers associated with sediment-free roots and rhizomes of the seagrass *Zostera marina* were investigated. Sulphate reduction rates were determined after treatment of roots and rhizomes with hypochlorite or exposure to air. Untreated roots and rhizomes served as controls. In general, root- as well as rhizome-associated sulphate reduction rates were high (20 to 50 µmol SO_4^{2-} gdw^{-1} d^{-1}). After surface sterilisation, the sulphate reduction rates decreased by about 90%, indicating that most of the sulphate reducers were associated with the surface of the roots and rhizomes. Pre-incubation of roots for 8 h in the presence of 0 to 20% oxygen (in steps of 2%) did not affect the sulphate reduction rates determined posterior under anoxic conditions. This demonstrates a high tolerance of root surface-associated sulphate reducers towards oxygen.

KEY WORDS: Sulphate reducing bacteria · Roots · Rhizomes · Seagrass beds · Oxygen tolerance

The rhizosphere of marine macrophytes has been intensively investigated in recent years. The studies include rate measurements of e.g. bacterial productivity, nitrogen fixation, and sulphate reduction, as well as the identification of microorganisms by application of molecular tools (e.g. Smith & Hayasaka 1982, Moriarty et al. 1985, Glazebrook et al. 1996, Isaksen & Finster 1996, Rooney-Varga et al. 1997, Welsh et al. 1997). In general, the authors focus on interactions between the macrophyte and the associated microorganisms. Recently, Blaabjerg et al. (1998) demonstrated that sulphate reduction rates in a *Zostera marina* bed varied throughout a diel cycle, with significantly higher rates in the light than in the dark. The authors also showed that sulphate reduction responded rapidly when shifting from light to dark and vice versa. This indicates a close physical coupling between the roots/rhizomes and the sulphate reducers.

These observations stimulated the studies on which we report here. We were interested in: (1) determining if sulphate reducers were present on roots and rhizomes and quantifying sulphate reducing activity, (2) roughly localising the sulphate reducers on the roots and rhizomes and (3) determining the tolerance of sulphate reducers towards oxygen. The results given below represent, to our knowledge, the first study of sulphate reduction on isolated subterranean plant compartments.

Material and methods. ***Study site and sampling procedure:*** The sampling site was located in Løgstør Bredning, Limfjorden, Denmark (56° 58' N, 9° 15' E). Sediment with living plants was sampled in plexiglas tubes (10 cm i.d.) and transported to the laboratory in seawater-filled coolers. In the laboratory, the cores were stored in a seawater-filled aquarium at 12°C under shifting light/dark conditions (12 h:12 h). The storage time did not exceed 3 d.

Sample preparation and experimental design: After leaf removal, the upper 6 cm of a sediment core was extruded from the plexiglas tube, cut off with a spatula and transferred to N_2-flushed plastic bags. Roots and rhizomes were sorted out and transferred to a N_2-flushed glass container. Roots were separated from the rhizomes with a forceps and stored in a separate container under nitrogen. All measurements of sulphate reduction rates were carried out in 10 ml screw-cap sealed glass tubes. They were filled with 8 ml of filtered, autoclaved, dithionite-reduced seawater leaving a headspace of 2 ml. Resazurin was added as a redox indicator. The screw caps of the glass tubes contained rubber septa which allowed the addition of ^{35}S-SO_4^{2-} with a syringe and a needle. Approximately, 0.2 to 0.6 g roots and 0.7 to 1.2 g rhizomes (wet weight) were used in each incubation. The experiments were initi-

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ated by the injection of 15 µl 35 to 75 MBq ml^{-1} ^{35}S-SO_4^{2-}. The suspensions were vigorously mixed with a whirlymixer, incubated for 3 to 20 h at 22°C and stopped by injection of 2 ml of a 2% Zn-acetate solution.

Sulphate reduction rates were determined as described by Isaksen & Finster (1996). The dry weight of the incubated roots and rhizomes was determined after incubation at 105°C for 24 h.

The distribution of sulphate reducers between the root/rhizome surface and the cortex was studied by sterilising the surface with hypochlorite. The procedure described by Smith & Hayasaka (1986) was followed. Roots and rhizomes were incubated for 0.5 and 3.5 min in an oxygen-free 1.05% hypochlorite solution and washed afterwards 3 times in N_2-flushed autoclaved seawater prior to incubation. Untreated roots and rhizomes served as controls.

The oxygen sensitivity of root-associated sulphate reducers was investigated by pre-incubating *Zostera marina* roots in screw-cap sealed glass bottles in the presence of 0 to 20% oxygen (in 2% intervals), respectively, for 8 h. The oxygen concentration was measured throughout the incubation by gas chromatography and kept at a constant level.

The sulphate reduction rates were determined for each treatment.

Results and discussion. Our experiments clearly demonstrated that both roots and rhizomes of *Zostera marina* were colonised by sulphate reducers (Fig. 1a, b). Extrapolating from the sulphate reduction rates, the colonisation must have been substantial. Pooling all our experiments, root- and rhizome-associated sulphate reduction rates varied between 20 and 50 µmol gdw^{-1} d^{-1}. These rates were up to 20 times higher than rates determined with an equivalent amount of sediment (Blaabjerg et al. 1998). The rates were well correlated with the quantity of roots/rhizomes incubated (Fig. 1a, b). Assuming that the specific sulphate reduction rate of 10^{-14} to 10^{-15} mol SO_4^{2-} $cell^{-1}$ d^{-1} given in the literature (Ingvorsen & Jørgensen 1984, Jørgensen & Bak 1991) can be extrapolated to the root/rhizome-associated sulphate reducers, the estimated number of cells from the above given rates was 10^7 to 10^9 cells gdw^{-1} or 10^6 to 10^8 cells gww^{-1} (wet weight). The number is 1 to 2 orders of magnitude higher than that which has been reported from unvegetated marine surface sediments (Jørgensen & Bak 1991) but agreed very well with cell numbers found in surface sediment of a *Zostera noltii*-overgrown marine mudflat (Schaub & van Gemerden 1996).

To determine the distribution of sulphate reducers between the surface and the cortex of the roots and rhizomes, a surface-sterilisation experiment was conducted. After surface sterilisation in a 1.05% hypochlorite solution, the root-associated sulphate reduction rates decreased from about 40 µmol gdw^{-1} d^{-1} to about 2 µmol gdw^{-1} d^{-1}, a reduction of 95%. A prolonged sterilisation period of 3.5 min reduced root-associated sulphate reduction rates by 98% (Fig. 2). Similarly, rhizome-associated sulphate reduction rates were reduced by 80% from about 21 µmol gdw^{-1} d^{-1} to 4.5 µmol gdw^{-1} d^{-1} after a sterilisation period of 0.5 min. After 3.5 min, rhizome-associated sulphate reduction rates decreased by 93% compared to a control (Fig. 2). Our results indicate that sulphate reducers were primarily associated with the surfaces of the roots and rhizomes and to a minor extent with the cortex. Similar observations were described by Smith & Hayasaka (1982) when studying nitrogenase activity associated with *Zostera marina* roots. Surface sterilisation reduced C_2H_2 reduction by more than 99% indicating that by far the majority of the nitrogen fixers was associated with the root surface. We confirmed the presence of sulphate reducers in the cortex of roots and rhizomes by enriching sulphate reducers when surface-sterilised roots and rhizomes served as inocula and acetate, propionate, lactate, malate or ethanol served as energy/carbon sources. However, compared to enrichments with untreated root or rhizome inocula,

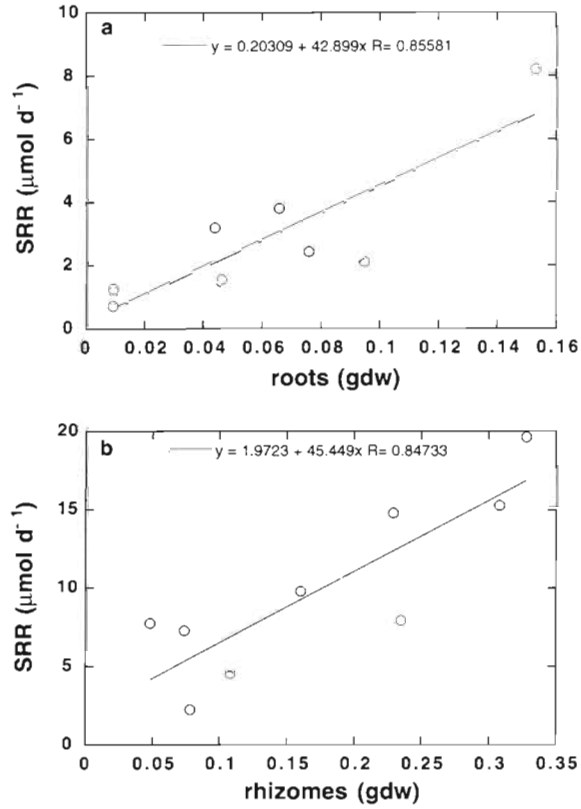

Fig. 1. Sulphate reduction rates (µmol d^{-1}) as a function of (a) roots and (b) rhizome dry weight (g dw)

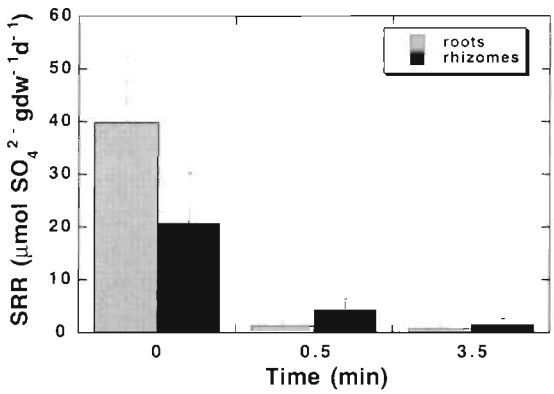

Fig. 2. Sulphate reduction rates (SRR) determined with surface-sterilised roots (grey) and rhizomes (black). Bars give standard errors of mean, n = 3. Roots and rhizomes were surface-sterilised in a 1.05% hypochlorite solution for 0.5 and 3.5 min, respectively. Untreated roots served as a control

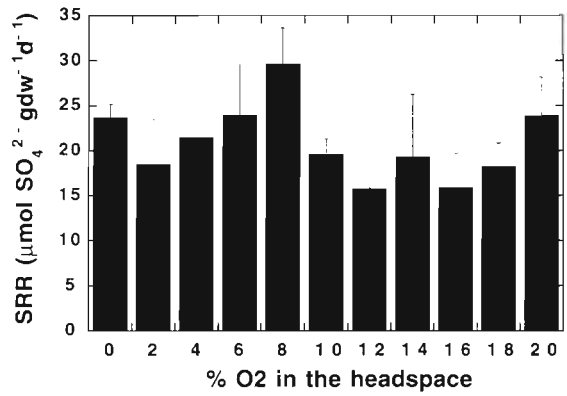

Fig. 3. Sulphate reduction rates measured with roots after an 8 h pre-incubation at constant oxygen concentrations ranging from 0 to 20% in the headspace. The oxygen concentrations were increased at 2% intervals. The bottles were incubated on a bottle roller to ensure homogenous distribution of oxygen. Bars give standard errors of mean, n = 3

bacterial growth and sulphide production were generally delayed when hypochlorite-treated roots or rhizomes were inoculated. With lactate, malate or ethanol as energy and carbon sources the delay was 1 wk. Propionate and acetate enrichments were delayed by 2 and 3 wk, respectively. Lactate, malate or ethanol enriched for *Desulfovibrio*-like sulphate reducers. From the lactate enrichment, a sulphate reducer related to the oxygen-tolerant species *Desulfovibrio salexigens* was isolated (Cypionka et al. 1985). The propionate and acetate enrichment cultures are not identified yet. A detailed characterisation of the cultures is under way.

Recent studies of e.g Sand-Jensen et al. (1982) showed that roots of *Zostera marina* leak oxygen into the environment. Accordingly, an experiment was initiated to examine the oxygen tolerance of sulphate reducers which thrive under these periodically oxic conditions. Interestingly, the root-associated sulphate reduction rates were not affected by 8 h pre-incubations in the presence of molecular oxygen in the range of 0 to 20 % (Fig. 3). From the results obtained in our surface sterilisation experiment we concluded that most of the sulphate reducers were associated with the root surface. Consequently, they were not physically protected by the root tissue but directly exposed to oxygen. The oxygen tolerance of root-associated sulphate reducers is in good agreement with results obtained with pure cultures of sulphate reducers as well as with environmental samples (Hardy & Hamilton 1981, Cypionka et al. 1985, Abdollahi & Wimpenny 1990, Dannenberg et al. 1992, Johnson et al. 1997). The resistance to high oxygen concentrations may, however, not exclusively be a feature of the sulphate reducers per se, but a consequence of the close proximity to an oxygen-consuming matrix, the root tissue or to other oxygen-consuming microorganisms which were also attached to the root surface.

Conclusion. In eelgrass-bearing marine sediments, sulphate reducers colonise both roots and rhizomes as demonstrated by high root-/rhizome-associated sulphate reduction rates. The large standard errors of the mean express the heterogeneity of the root/rhizome material and may be a consequence of different degrees of colonisation and/or variable food quality. The high oxygen tolerance of the root-associated sulphate reducers may either be an inherent capacity of the microorganisms or may result from interactions between aerobic eukaryotic and prokaryotic cells and the sulphate reducers.

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