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Differential effect of three polyunsaturated aldehydes on marine bacterial isolates

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Abstract

Bioactive polyunsaturated aldehydes (PUAs) are produced by several marine phytoplankton (mainly diatoms) and have been shown to have a detrimental effect on a wide variety of organisms, including phytoplankton and invertebrates. However, their potential impact on marine bacteria has been largely neglected. We assess here the effect of three PUAs produced by marine diatoms: 2*E*,4*E*-decadienal, 2*E*,4*E*-octadienal and 2*E*,4*E*-heptadienal, on the growth of 33 marine bacterial strains, including 16 strains isolated during a bloom of the PUA-producing diatom *Skeletonema marinoi* in the Northern Adriatic Sea. A concentration-dependent growth reduction was observed for 19 bacterial strains at concentrations ranging from 3 to 145 μmol L^{-1}. Surprisingly, *Eudora adriatica* strain MOLA358 (*Flavobacteriaceae*) and *Alteromonas hispanica* strain MOLA151 (*Alteromonadaceae*) showed growth stimulation upon exposure to PUAs at concentrations between 13 and 18 μmol L^{-1}. The remaining 12 strains were unaffected by even very high PUA concentrations. Strains isolated during the diatom bloom showed remarkable resistance to PUA exposures, with only two out of 16 strains showing growth inhibition at PUA concentrations below 106, 130, and 145 μmol L^{-1} for 2*E*,4*E*-decadienal, 2*E*,4*E*-octadienal and 2*E*,4*E*-heptadienal, respectively. No correlation between taxonomical position and sensitivity to PUA was observed. Considering that many bacteria thrive in close vicinity of diatom cells, it is likely that these compounds may shape the structure of associated bacterial communities by representing a selection force. This is even more relevant during the final stages of blooms, when senescence and nutrient limitation increase the potential production and release of aldehydes.

Keywords: Allelochemicals; Diatoms; Polyunsaturated aldehydes; Toxicity; Growth; Marine bacteria; LOEC

1. Introduction

Diatoms are successful autotrophic microorganisms and major contributors to global carbon fixation (Dugdale and Wilkerson, 1998). Several diatom species produce a wide range of secondary metabolites, including volatile polyunsaturated aldehydes (here abbreviated as PUAs), such as 2*E*,4*E*/*Z*-heptadienal, 2*E*,4*E*/*Z*-octadienal, 2*E*,4*E*/*Z*,7*Z*-octatrienal, 2*E*,4*E*/*Z*-decadienal and 2*E*,4*E*/*Z*,7*Z*-decatrienal (Wichard et al., 2005). The production and release of PUAs result from the lipoxygenase-mediated degradation of free polyunsaturated fatty acids released by phospho- and galactolipids hydrolysis (Pohnert, 2002; d'Ippolito et al., 2004). This enzymatic cascade is activated immediately after cell integrity is broken by lysis or mastication by grazers (Pohnert, 2000). PUA production in diatoms varies quantitatively and qualitatively according to the species (Wichard et al., 2005) and to the physiological state of the cell (Ribalet et al., 2007a). These compounds, including 2*E*,4*E*-decadienal, which has been widely used as a model aldehyde, have been shown to have a detrimental effect on cell proliferation of human cancer cell lines, egg hatching success and cell division of marine invertebrate embryos (Ianora et al., 2006), seed germination of plants (Eom et al., 2006), and growth of fungi (Adolph et al., 2004) and phytoplankton (Casotti et al., 2005; Ribalet et al., 2007b). Many studies have reported an inhibitory or stimulatory effect of diatom extracts on pathogenic bacteria, suggesting the presence of active molecules responsible for these effects (e.g. Bell et al., 1974; Naviner et al., 1999). More recently, it has been shown that 2*E*,4*E*-decadienal reduces the growth of pathogenic and non-marine bacteria (Bisignano et al., 2001; Adolph et al., 2004). No marine bacteria have been tested so far, despite their importance in marine ecosystems

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and their presence in close vicinity to diatoms and phytoplankton at sea. Although bacteria and phytoplankton dynamics are closely linked in coastal marine environments, relatively little is known about how these communities interact with each other at the species composition level. Phytoplanktonic cells may represent selective forces that gradually drive community succession towards a phylogenetic composition that differs from that of the surrounding water. Marine diatom species are known to harbour distinct bacterial communities (Grossart et al., 2005) but the selection mechanisms are mostly unknown.

The aim of this study is to examine the effect of three diatom-derived PUAs on the growth of 33 marine bacteria belonging to different taxonomical groups, including 16 strains isolated in the Northern Adriatic Sea during a late-winter bloom of the PUA-producing marine diatom *Skeletonema marinoi*.

2. Materials and methods

2.1. Cultures and experimental design

Thirty-three marine bacterial strains from the culture collection MOLA (Microbial Observatory Laboratoire Arago, http://microbial.obs-banyuls.fr/) (Table 1) were grown in marine broth medium (Marine Broth 2216, Difco Laboratories, Detroit, USA) at room temperature in the dark. Exponentially growing cultures (1.5 mL) in duplicate were exposed to the following final PUA concentrations: 13, 26, 53, 106 μmol L^{-1} of 2*E*,4*E*-decadienal; 16, 32, 65, 130 μmol L^{-1} of 2*E*,4*E*-octadienal; and 18, 36, 73, 145 μmol L^{-1} of 2*E*,4*E*-heptadienal. Initial experiments showed that lower PUA concentrations had no effect on the growth of the bacteria tested, except for *Enterococcus faecium* MOLA195.

Controls were represented by cultures inoculated with methanol only. To assess the specificity of the PUAs, ethanal (which bears the aldehydic group) and decanoic acid (which has a carbon chain as long as the 2*E*,4*E*-decadienal) were tested on each culture at the same highest concentrations used for the three PUAs (150 μmol L^{-1}). These compounds had no effect on any strain tested (data not shown).

Growth was monitored by measuring light absorbance at 450 nm using a spectrophotometer connected to a multiplate reader (Victor3 multilabel counter, PerkinElmer, Waltham, USA). Initial cell concentrations were measured by flow cytometry (FACScalibur, Becton Dickinson Biosciences, Franklin Lakes, USA) on fixed (2% formaldehyde) and stained cells (SYBR Green I, 0.025% final concentration in DMSO, Molecular Probes, Leiden, The Netherlands). Yellow-green fluorescent microspheres (0.95 μm Polysciences fluorospheres, Warrington, USA) were used as internal standards.

In order to test the hypothesis that PUAs could be used as an alternative source of carbon and energy, a culture of *Alteromonas hispanica* MOLA151 (which showed stimulation of growth upon inoculation of PUAs, see Section 3) was grown in minimal medium (Widdel et al., 1983), alone or in medium with 2*E*,4*E*-decadienal (final concentration 106 μmol L^{-1}), 2*E*,4*E*-octadienal (final concentration 130 μmol L^{-1}) or 2*E*,4*E*-heptadienal (final concentration 145 μmol L^{-1}). As a further control, a culture of the same strain was grown in minimal medium containing, in addition, 1% mannitol, 0.1% pyruvate and 0.1% proline as carbon substrates.

2.2. Preparation of chemicals

2*E*,4*E*-Heptadienal, 2*E*,4*E*-octadienal, 2*E*,4*E*-decadienal, ethanal and decanoic acid were obtained commercially from Sigma–Aldrich Inc. (Milano, Italy). Working solutions were prepared by diluting the stock in absolute methanol (Sigma–Aldrich Inc., Milano, Italy) at room temperature. The effective aldehyde concentration of the working solution was assessed spectrophotometrically by measuring absorption at 274 nm using a specific absorption coefficient (per mole of compound) of 31000 (Pippen and Nonaka, 1958). Toxicity of the methanol solvent was tested for all bacterial strains and resulted to start above 7 μL of pure methanol per mL of culture (data not shown) and therefore the amount of aldehyde solution in each test was kept always below this threshold.

2.3. Statistical analyses

Normal distribution and equal variance of the data were tested and a Dunnett's Multiple Comparison post-test (Prism 4, GraphPad Software, San Diego, USA) was used to assess the PUA concentrations inducing a significant effect when compared to the controls. The lowest observed effect concentration (LOEC) was estimated as the lowest PUA concentration inducing an effect different than the control with 95% significance. We have not used the minimal inhibitory concentration (MIC), in order to account for the non-inhibitory (positive) effect observed on some strains.

3. Results

A group of 12 strains showed no difference compared to the control when inoculated with PUA concentrations up to 106, 130, and 145 μmol L^{-1} of 2*E*,4*E*-decadienal, 2*E*,4*E*-octadienal and 2*E*,4*E*-heptadienal, respectively. These included 3 out of 6 γ-Proteobacteria, 5 out of 15 α-Proteobacteria and 4 out of 8 Bacteroidetes (Table 1). 2*E*,4*E*-Heptadienal had no effect on Bacteroidetes except for *Muricauda aquimarina* MOLA110 (Table 1), while *Microscilla pacifica* MOLA120 and *Pibocella ponti* MOLA312 showed growth inhibition only at the highest concentrations of 2*E*,4*E*-decadienal and 2*E*,4*E*-octadienal. The response of the Bacteroidetes *Roseivirga echinicomitans* MOLA389 and the γ-Proteobacteria *Alteromonas marina* MOLA3 to PUAs exposure is given as an example (Fig. 1). Most of the strains isolated during a bloom of the PUA-producing diatom *Skeletonema marinoi* (see Table 1) showed no effect upon PUA inoculation, and only *Pseudomonas oryzihabitans* MOLA331 and *Paracoccus alcaliphilus* MOLA325 were inhibited at PUA concentrations below 106, 130, and 145 μmol L^{-1} for 2*E*,4*E*-decadienal, 2*E*,4*E*-octadienal and 2*E*,4*E*-heptadienal, respectively.

A concentration-dependent growth reduction was observed for 16 strains exposed to 2*E*,4*E*-decadienal, 17 strains exposed to