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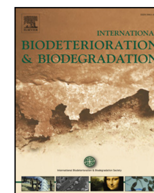

Biodegradation of nodularin and other nonribosomal peptides by the Baltic bacteria

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ABSTRACT

Microcystins (MCs) and nodularin (NOD), the hepatotoxic nonribosomal peptides (NRPs) produced by cyanobacteria, are considered as natural pollutants of many aquatic ecosystems. Removal of the toxins proceeds mainly through biodegradation and/or photolysis. In this study, microbial degradation of NOD and other NRPs produced by *Nodularia spumigena* was investigated. Both, natural bacterial consortia and individual strains from the Southern Baltic Sea were used. Bacterial samples were also screened for the presence of *mlr*A-D genes, previously proved to be involved in the enzymatic breakdown of hepatotoxic cyanopeptides. The Baltic sediments, with natural bacterial consortia, showed NOD-degrading activity throughout the whole year. These samples were also active against other NRPs: anabaenopeptins and spumigins. The time required for NOD biodegradation ranged from 3 to 41 days and depended on the NOD content in the collected sediments. NOD-degrading activity was also revealed in the presence of ten bacterial strains isolated from sediments and three strains isolated from filaments of *N. spumigena* CCNP1401. The *mlr*A-D genes were not found in sediments and only individual genes from the entire *mlr* cluster were detected in five isolated bacteria. The results suggest that in the case of Baltic bacteria other genes are involved in the biosynthesis of NOD-degrading enzymes.

1. Introduction

Eutrophication and massive harmful phytoplankton development have been identified as one of the most serious threats to the Baltic Sea ecosystem. Recently, it has been postulated, that global warming can lead to longer lasting and more toxic cyanobacterial blooms (Huisman et al., 2018). Due to regular annual occurrence, high biomass and toxicity, the blooms formed by filamentous cyanobacterium *Nodularia spumigena* are of special concern. Adverse effects of *N. spumigena* on other bacteria, algae, higher plants, zooplankton, ichthyofauna and terrestrial animals were reported (Koski et al., 1999; Vasconcelos et al., 2001; Algermissen et al., 2011; Lehtimäki et al., 2011; Mazur-Marzec et al., 2015). The cyanobacterium produces a variety of biologically active nonribosomal peptides (NRPs), including hepatotoxic nodularins (NODs), anabaenopeptins (ANPs), aeruginosins (ARGs), pseudoaeruginosins (NSs) and spumigins (SPUs) (Mazur-Marzec et al., 2016). During bloom senescence and decay, NRPs are released from the cells and removed from water through various processes including photochemical degradation, adsorption on particles in suspension or onto sediments, bioaccumulation in the organisms and biodegradation. Kankanpää et al. (2009) calculated that majority of the dissolved NOD released from the cells is lost within 0–4 m layer of surface waters. The intracellular pool of the peptide or compounds adsorbed on the particles suspended in water column, may reach deeper parts. In the surface waters NOD concentrations vary from traces in the open sea to over 40 mg mL^{-1} in scums formed in coastal areas (Mazur-Marzec et al., 2013). In sediments, NOD amounts ranged from 0.4 ng g^{-1} to 75 ng g^{-1} dw (Mazur-Marzec et al., 2007; Kankanpää et al., 2009).

It was reported that degradation of cyanotoxins by associated bacteria leads to their complete removal (Hyenstrand et al., 2003). For the first time, products of MC-LR biodegradation were identified by Bourne et al. (1996). Then, corresponding intermediates were detected during bacterial degradation of different MCs variants and NOD. In case of NOD, the process started with hydrolysis of the Arg-Adda bond and led to formation of linearized peptide (Kato et al., 2007; Mazur-Marzec et al., 2009; Feng et al., 2016). Further hydrolysis gave different tetrapeptides e.g. Adda-Glu-Mdhb-MeAsp, Glu-Mdhb-MeAsp-Arg (Mazur-Marzec et al., 2009). Edwards et al. (2008) suggested that bacteria can also metabolize NOD by demethylation and/or decarboxylation of amino acid components. In most of the studies Adda residue was detected as the final biodegradation product (Imanishi et al., 2005; Kato et al., 2007; Mazur-Marzec et al., 2009; Feng et al., 2016).

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Table 1
NOD degradation by bacterial strains (CCNP0037-0051) isolated from *N. spumigena* CCNP1401 filaments, cultured in different media; identification of *mlr*A-D genes in CCNP0037-0051 bacterial strains, toxic cyanobacterial strains and environmental samples. (* Strains cultured from glycerol stocks, ● Positive result).

Species/genus	Strain	GenBank Accession number	NOD biodegradation in different culture media								Presence of *mlr*A-D genes			
			ZoBell	ZoBell 0.5	ZoBell 0.25	Sterile seawater	M9	M9 without glucose	Z8S	Z8S spend medium	*mlr*A	*mlr*B	*mlr*C	*mlr*D
Pseudomonas putida	CCNP0037	KM219965	–	●	–	–	–	–	–		–	–	–	–
Pseudomonas sp.	CCNP0039	KM219967	–	–	–	–	–	–	–		–	–	–	–
Pseudomonas sp.	CCNP0040	KM219968	–	–	–	–	–	–	–		–	–	–	–
Pseudomonas sp.	CCNP0041	KM219969	–	–	–	–	–	–	–		–	–	–	–
Pseudomonas sp.	CCNP0050B	KM219979	–	–	–	–	–	–	–		–	–	–	–
Pseudomonas putida	CCNP0051	KM219980	–	–	–	–	–	–	–		–	–	–	–
Stenotrophomonas sp.	CCNP0042	KM219970	–	–	–	–	–	–	–		–	–	–	–
Stenotrophomonas sp.	CCNP0047	KM219975	–	–	–	–	–	–	–		–	●	●	●
Stenotrophomonas sp.	CCNP0049	KM219977	–	–	–	–	–	–	–		–	●	●	●
Stenotrophomonas sp.	CCNP0050A	KM219978	–	●	–	●	–	–	●		–	●	●	●
Rheinheimera aquamaris	CCNP0045	KM219973	–	–	–	–	–	–	–		●	–	–	–
Rheinheimera aquamaris	CCNP0048	KM219976	–	–	–	–	–	–	–		●	–	–	–
Ochrobactrum sp.	CCNP0043	KM219971	–	–	–	–	–	–	–		–	–	–	–
Ochrobactrum sp.	CCNP0038	KM219966	–	–	–	–	–	–	–		–	–	–	–
Ochrobactrum sp.	CCNP0046	KM219974	–	–	–	–	–	–	–		–	–	–	–
Chryseobacterium aquaticum	CCNP0044	KM219972	–	●	–	–	–	–	–		–	–	–	–
	CCNP0037-0051		–			●			●	–				
	CCNP0037-0051*		–			●			●	●				
Sphingosinicella microcystinivorans											●	●	●	●
Paucibacter toxinivorans											–	–	–	–
Genomic DNA	**Toxins identified by HPLC**													
N. spumigena bloom (03.07.2008)	NODs										–	–	–	–
N. spumigena bloom (29.06.2009)	NODs										–	–	–	–
N. spumigena CCNP1401	NODs										–	–	–	–
M. aeruginosa CCNP1101	MCs										●	●	●	●
P. aghardii CCNP1325	MCs										–	●	●	●
Sediment (05.05.2009)	NODs										–	–	–	–

The *mlr*A-D gene cluster that encodes hydrolytic enzymes involved in MCs biodegradation was characterized by Bourne et al. (2001). In further studies, the functions of enzymes encoded by *mlr* genes were validated by heterologous expression in *Escherichia coli* (Dziga et al., 2016; Zhu et al., 2016; Wang et al., 2017). The *mlr*-dependent pathway is the only one described in MCs degrading bacteria, but probably it is not the most common in aquatic ecosystems (Mou et al., 2013; Dziga et al., 2017). In NOD-degrading strains the *mlr* genes were detected in few cases (e.g. Edwards et al., 2008; Feng et al., 2016).

Since 1994, more than 40 microcystin-degrading bacterial strains, mostly belonging to Proteobacteria phylum, have been isolated (reviewed by Li et al., 2017). Only 8 of them were active also toward nodularin (Harada et al., 2004; Rapala et al., 2005; Manage et al., 2009; Feng et al., 2016). In fact, data on the presence of NOD degrading bacteria in different environments are scarce. Heresztyn and Nicholson (1997) reported decomposition of the toxin by the natural microbial community from Lake Alexandrina (Australia). Edwards et al. (2008) found that NOD can also be degraded in freshwaters without previous records of *N. spumigena* blooms. Our previous experiments revealed significant role of natural microbial community from the Baltic sediments in NOD removal (Toruńska et al., 2008; Mazur-Marzec et al., 2009). Anaerobic NOD biodegradation by rumen microbial flora from Swedish cows was reported by Manubolu et al. (2014).

In the current work, we aimed to extend the existing knowledge on the process of NOD removal by the Baltic bacteria. For this purpose, surface water samples, sediments and isolated, bacterial strains were used. Within this study we examined, if previous exposure to NOD have any effect on bacterial activity. In addition, the involvement of *mlr* gene cluster in NOD biodegradation was explored.

2. Materials and methods

2.1. Study area and sampling

Surface sediment (top 1 cm layer) and surface seawater samples (0–0.5 m layer) were collected aseptically from the station located in the Gulf of Gdańsk (54°34′ N; 18°47′ E) during cruises of the hydrographic vessel R/V Oceanograf 2. Sampling was performed on the following dates: 3 July and 23 November 2007; 20 August 2008; 18 February, 21 April, 05 May, 30 June, 10 December 2009; 21 February 2013. Van Veen grab was used to collect sediment samples characterized as sandy-silty-clay (particle size < 0.062 mm) (Dadlez et al., 1995). Seawater was collected with bathometer. Sediment samples for NOD analysis were freeze-dried. The remaining material was stored in darkness at 4 °C until the experiments, not longer than for 24 h.

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