Strains of the toxic and bloom-forming *Nodularia spumigena* (cyanobacteria) can degrade methylphosphonate and release methane

Jonna E. Teikari 1 · David P. Fewer 1 · Rashmi Shrestha 1 · Shengwei Hou 2 · Niina Leikoski 1 · Minna Mäkelä 3 · Asko Simojoki 3 · Wolfgang R. Hess 2 · Kaarina Sivonen 1

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Abstract

*Nodularia spumigena* is a nitrogen-fixing cyanobacterium that forms toxic blooms in the Baltic Sea each summer and the availability of phosphorous is an important factor limiting the formation of these blooms. Bioinformatic analysis identified a phosphonate degrading (*phn*) gene cluster in the genome of *N. spumigena* suggesting that this bacterium may use phosphonates as a phosphorus source. Our results show that strains of *N. spumigena* could grow in medium containing methylphosphonic acid (MPn) as the sole source of phosphorous and released methane when growing in medium containing MPn. We analyzed the total transcriptomes of *N. spumigena* UHCC 0039 grown using MPn and compared them with cultures growing in P$_i$-replete medium. The *phnJ*, phosphonate lyase gene, was upregulated when MPn was the sole source of phosphorus, suggesting that the expression of this gene could be used to indicate the presence of bioavailable phosphonates. Otherwise, growth on MPn resulted in only a minor reconstruction of the transcriptome and enabled good growth. However, *N. spumigena* strains were not able to utilize any of the anthropogenic phosphonates tested. The phosphonate utilizing pathway may offer *N. spumigena* a competitive advantage in the P$_i$-limited cyanobacterial blooms of the Baltic Sea.

Introduction

Phosphorus is an essential macronutrient for life, being a key component in organic biomolecules, such as DNA, proteins, and phospholipids. The most preferable form of phosphorus for the uptake by cyanobacteria is orthophosphate ions H$_2$PO$_4$$^-$$^-$, HPO$_4$$^{2-}$, and PO$_4$$^{3-}$ (P$_i$), which occur at an oxidation state of +5 in nature and these orthophosphates dominate the pool of dissolved inorganic phosphorus (DIP) [1]. Dissolved organic phosphorus (DOP) comprises another pool of phosphorus in the water ecosystems and includes two important bond classes, ester (C-O-P) and carbon-phosphorus (C-P) bonds. Phosphoesters are degraded by alkaline phosphatase and measurement of alkaline phosphatase activity has been used generally as an indicator for P$_i$ deficiency [2, 3] (Van Wambeke et al. 2002). Organic phosphonates, derivatives of phosphorus acid where the phosphorus is at the oxidation state of +3, are poorly studied even though they have proposed to constitute up to 25 % of the total DOP pool in the oceans [4–6]. Many of the phosphonates in the DOP pool are natural metabolites but some have an anthropogenic origin [7–9]. Phosphonates are recalcitrant to degradation, due to the presence of the C-P bond, and are generally thought to particulate and sediment [1].

P$_i$ is usually found at very low concentrations in environment and therefore lack of P$_i$ is the main growth-limiting factor for nitrogen-fixing and phototrophic cyanobacteria during blooms in aquatic ecosystems [10–12]. Bacteria have
evolved specific strategies to enhance phosphorus availability under P-limiting conditions [13, 14]. The high-affinity phosphate transport system, encoded in the pstABCS operon, is the most studied system for P uptake. The pstABCS operon belongs to the pho regulon, which is activated by autophosphorylation when the Pi concentration is low [15–17]. The PstABC complex thus ensures rapid and effective scavenging of Pi in phosphorus-limiting conditions.

Many heterotrophic bacteria possess a phosphonate degrading (phn) gene cluster required for transport and assimilation of phosphonates that enables them to cope better with DIP-limited conditions by allowing them to use phosphonates as a source of phosphorus [18, 19]. The phn gene cluster is also part of the pho regulon and it consists of a phosphonate transporter complex (phnC-E) and the multi-subunit C-P lyase complex (phnG-P), which cleaves the C-P bond in phosphonates [20–22]. The PhnN, PhnO, and PhnP proteins are not required for C-P bond cleavage but they most probably have a role as accessory proteins or regulators [23]. The PhnF protein acted as a repressor of phnP gene cluster in the genome of N. spumigena CCY 9414 suggested that this strain might be able to degrade and use phosphonates as an alternative source of phosphorus [35]. However, the presence of a phn gene cluster in the genome of N. spumigena CCY 9414 suggested that this strain might be able to degrade and use phosphonates as an alternative source of phosphorus [36].

### Materials and methods

**Screening for phnJ genes**

The BlastP algorithm was used to identify PhnJ phosphonate lyase proteins from cyanobacterial genomes using the PhnJ sequences from Escherichia coli (E. coli) K12 and N.
**spumigena** UHCC 0039 as queries. Genomes encoding the PhnJ protein were downloaded from the NCBI genome database (Table S1). The gene order of the *phn* gene cluster was determined using the Artemis genome browser [39]. A total of 16 strains of the genus *Nodularia* were selected for the screening for the occurrence and distribution of phosphonate lyase (*phnJ*) and transporter (*phnD*) genes (Table S2). *phnJ, phnD, and pstS* primers were designed based on the known *N. spumigena* UHCC 0039 (NCBI accession number, PRJNA352241), CCY 9414 sequences (NCBI accession number, PRJNA13447), and CENA 596 (NCBI accession number, PRJNA315832) (Table 1). *N. spumigena*-specific primers were also used to amplify *phnJ* and *phnD* genes from environmental DNA samples collected after cyanobacterial blooms in August 2016 (Table S3). Genomic DNA was isolated from the cyanobacterial cultures listed in Table S2 using the E.Z.N.A® Plant DNA kit (Omega Biotek) and from environmental samples using AllPrep DNA/RNA mini kit (Qiagen). The PCR reaction mix (20 µL) consisted of 100 ng template genomic DNA, 750 nM of both primers (Oligomer, Thermo Scientific), 2 × PCR reaction mix (200 µL) consisted of 100 ng template genomic DNA, 750 nM of both primers (Oligomer, Table 1), 2 µL of 10× reaction buffer (Thermo Scientific), 200 µM dNTP (Thermo Scientific) and 0.4 U Dynazyme II (Thermo Scientific) in a total volume of 20 µL. Purified water was used as a template in negative controls. The PCR cycling parameters were as follows: 94 °C for 3 min, 30 × (94 °C for 30 s, 60.5 °C for 30 s, 72 °C for 180 s) and 72 °C for 10 min.

**Cyanobacterial strains and cultivation**

*N. spumigena* strains UHCC 0039 (formerly named *N. spumigena* AV1) and UHCC 0060 (formerly named *N. spumigena* HEM) were isolated from the Gulf of Finland [32] and purified into axenic cultures (Table S1). The strains were maintained in continuous batch culture with Z8XS medium containing 17.1 mg L⁻¹ of inorganic phosphate *P*₁, without nitrogen and under continuous illumination of 3.2–3.7 µmol photons m⁻² s⁻¹ [40]. Cultures were starved in phosphorus-free medium (Z8XS-P) for 7 days to deplete the intracellular phosphorus store. Cells, in three biological replicates, were harvested after starvation and transferred to fresh Z8XS-P medium containing either methylphosphonic acid (MPn) (Sigma-Aldrich), ethylphosphonic acid (EPn) (Sigma-Aldrich), 2-aminoethylphosphonic acid (2Apn) (Sigma-Aldrich), etidronic acid monohydrate (Sigma-Aldrich), N-(phosphonomethyl) glycine (Sigma-Aldrich), nitritoltri(methylphosphonic acid) (Sigma-Aldrich) or 2-phosphonobutane-1,2,4-tricarboxylic acid (abcr GmbH) as a source of phosphorus. The phosphorus concentration in each phosphonate medium was adjusted to be equal to the amount of phosphorus in the original Z8XS medium. Z8XS medium containing *P*₁ was used as a positive control to gain proper cellular proliferation and Z8XS-P medium lacking *P*₁ (-*P*₁) was used as a negative control. Cultures were grown under continuous illumination of 3.2–3.7 µmol photons m⁻² s⁻¹ for 12 days. In all, 54 mL of the cultures were moved to 60 mL of fresh medium after 12 days of incubation and further cultured for 41/38 days. All glassware was acid washed using 0.1 M HCl.

**Determination of chlorophyll a concentration, alkaline phosphatase activity, and methane liberation**

Chlorophyll *a* concentration and alkaline phosphatase activity were measured at 4-day intervals during the experiment. In all, 1 mL of culture was filtered through 21 mm glass microfiber filters GF/C (pore size 1.2 µm) (GE HealthCare) and stored in −80 °C for chlorophyll *a* measurements. Chlorophyll *a* was extracted from the filters using 1 mL of 90% acetone for 24 h at −20 °C and chlorophyll *a* concentrations were determined by measuring absorbance at 664, 647, and 630 nm. The chlorophyll *a* concentration was calculated using the Jeffrey and Humphrey [41] equation. Alkaline phosphatase activity was determined fluorometrically using 4-umbelliferyl phosphate as a substrate (Sigma-Aldrich) and 4-methylbelliferone as a standard (Sigma-Aldrich) [42]. Enzyme activity was inhibited by heating the culture to 100 °C for 2 min and this was used as a zero sample in order to eliminate background levels caused by cyanobacterial photopigments. The methane emission rate was determined by transferring 2 mL of *N. spumigena* UHCC 0039 culture to 12 mL Exetainer® vials with Double Wadded Exetainer® Cap (Labco) in 47 replicates, which were incubated for 0–32 h. In total, 2 mL samples of *N. spumigena* UHCC 0039 and UHCC 0060 cultures were also transferred to 12 mL Exetainer® vials with Double Wadded Exetainer® Cap and incubated for 24 h using the original cultivation conditions to measure the methane emission in different stages of the experiment. Methane release to gaseous environment was analyzed by the headspace technique as follows. Gas samples of 8 mL (at atmospheric pressure) were taken from the headspace with a polypropylene syringe attached to a hypodermic needle and injected into helium-flushed and pre-evacuated 3 mL vials. Gas concentrations (cm³ m⁻³) were analyzed using an Agilent GC 7890 custom gas chromatograph equipped with thermal conductivity, flame ionization, and electron capture detectors [43].

**Reverse transcriptase-quantitative PCR (RT-qPCR) and RNA-sequencing**

Samples for transcriptomic studies were collected at days 0, 12, and 24 from the cultures containing no phosphorus, *P*₁,
MPn, and 2APn. RNA samples were fixed with a solution, containing 10 % ethanol and 5 % of phenol and filtered through a 0.22 µm pore diameter polycarbonate filters (GE Water and Process Technologies). RNA was isolated from the filters using RNeasy mini kit (Qiagen) and genomic DNA was degraded using the TURBO DNA-free™ kit (Life Technologies). Ribosomal RNA was removed using MICROBExpress™ Bacterial mRNA enrichment kit (Life Technologies) and complementary DNA (cDNA) libraries were prepared using Bacterial ScriptSeq Complete Kit (Illumina). Total transcriptomes of N. spumigena UHCC 0039 in control (Pi) and MPn treatment on day 12 were sequenced in three replicates of control and in two replicates of treatment, at the Institute for Molecular Medicine Finland (FIMM). Paired-end Illumina HiSeq 2500 RNA-sequencing data were deposited under the accession number of PRJNA388731 and downstream data analysis was performed as described in supplementary information. The nodularia sp. cyanobacteria isolated from the Baltic Sea e and environmental samples from the Baltic Sea d. Detailed description of Nodularia sp. strains and environmental sampling points can be found from Tables S2 and S3.

**Results**

Distribution of *phn* gene cluster

Bioinformatics analysis demonstrates that the phosphonate gene cluster is widely distributed in the cyanobacterial phylum (Fig. 1). A total of 27 out of 500 (5.4%) sequenced cyanobacterial genomes in the NCBI database were found to encode complete or nearly complete sets of genes for phosphonate transport (*phnC-E*) and the C-P lyase complex (*phnG-M*) (Fig. 1a). The *phn* gene clusters lacked clear synteny and the size of the *phn* gene clusters ranged from 8 to 21 kb with the size variation due to gene deletions,
duplications, and accessory/hypothetical proteins located between core genes (Table S2). All three strains of *N. spumigena*, for which genome sequences are available, encoded the *phn* gene cluster (Fig. 1a). The *phnJ* and *phnD* genes were also common in *N. spumigena* strains but were absent from the other species of the *Nodularia* genus tested and from *Aphanizomenon flos-aquae* (Fig. 1b and Table S2). We amplified *phnD* and *phnJ* genes from the environmental samples collected from the Baltic Sea using the *N. spumigena*-specific *phnD* and *phnJ* primers demonstrating that these genes are abundant in the Gulf of Finland and the Baltic Proper (Fig. 1c).

Phosphonates as a sole source of phosphorus

*N. spumigena* UHCC 0039 and UHCC 0060 were both able to grow in medium containing MPn (Figs. 2a, b). Minor growth was observed when *N. spumigena* UHCC 0039 grew in the presence of EPn and *N. spumigena* UHCC 0060 in the medium containing 2APn. None of the tested strains were able to utilize the anthropogenic phosphonates etidronic acid monohydrate, *N*-phosphonomethyl) glycine, nitrilotri(methylphosphonic acid), or 2-phosphonobutane-1,2,4-tricarboxylic acid tested here. Maximal growth rates in studied conditions were obtained in the cultures growing in Pi medium. Moreover, minor growth in -Pi medium was measured indicating that they had not exhausted internal polyphosphate stores (Figs. 2a, b).

Alkaline phosphatase activity was followed during the cultivation experiment. Alkaline phosphatase activity peaked in both strains in the negative control, when phosphorus was omitted from the medium (Figs. 2c, d). Remarkably elevated alkaline phosphatase activity was additionally observed in 2APn cultures but enzyme activity was reduced compared with -Pi medium (Figs. 2c, d). Elevated alkaline phosphatase activity was not observed in MPn supplemented medium despite the absence of Pi from the growth medium (Figs. 2c, d).

Degradation of phosphonates by the C-P lyase complex releases not only phosphate for the use of the cells but also the organic byproduct, e.g. methane. The rate of the methane flux from *N. spumigena* UHCC 0039 culture to the gaseous environment was determined to be 1.84 nmol h\(^{-1}\) per mg of chlorophyll *a*, while prevailing chlorophyll *a* concentration in the culture was 2.4 mg L\(^{-1}\) (Fig. 3a). Release of methane was observed in cultures of both strains (Fig. 3b) but emission rate differed remarkably in different growth phases. Overall, *N. spumigena* UHCC 0060 seemed to release more methane compared *N. spumigena* UHCC 0039.

**Transcriptional remodeling**

MPn and 2APn conditions were selected to analyze the expression levels of phosphorus utilizing genes, compared against the phosphorus-replete condition (Pi), with
phosphorus-deplete condition (-P) as the negative control. Specifically, the gene expression levels of phnD, phnJ, and pstS were investigated in N. spumigena UHCC 0039 and UHCC 0060 using RT-qPCR. Although the C-P lyase complex is thought to belong to the pho regulon, the expression of phnJ was upregulated only in the presence of MPn (Figs. 4). The phnJ gene was upregulated on day 12, in both MPn and 2APn conditions, whereas expression remained upregulated only in MPn treatment for 24 days. This provided further evidence that MPn was a suitable phosphorus source for the studied Baltic Sea N. spumigena cyanobacteria. The phnD gene was upregulated in -P and 2APn conditions (Figs. 4). Upregulation of the phnD gene was minor in MPn treatment compared with Pι treatments. Gene expression of pstS gene was additionally studied due to its suggested suitability as a marker for Pι scarcity but in RT-qPCR analysis differences in pstS gene expression were not found (Figs. 4).

MPn was the only phosphonate tested that enabled good growth and proper cellular functioning of studied N. spumigena strains. We compared the transcriptomes of N. spumigena UHCC 0039 grown in MPn and normal Pι conditions using RNA-Seq to unravel the transcriptomic responses of phosphonate treatment. In all, 84 upregulated and 8 downregulated genes were determined, by applying the false discovery rate cut-off of <0.01, accounting for a relatively small fraction of all genes (1.8 %). This provided further evidence that MPn permitted normal cellular functioning of N. spumigena UHCC 0039 (Fig. 5, Table 2, Table S4). The phn gene cluster in N. spumigena UHCC 0039 contains 14 genes, of which four (phnC-E12) are responsible for phosphonate transport, phnF acts as a regulator and phnG-M compose a complex cleaving the C-P bond (Fig. 1a). The two additional genes within the phn gene cluster of N. spumigena UHCC 0039 may be related to accessory or regulatory proteins. The C-P lyase complex (phnG-M) was heavily upregulated when phosphonate was present and was the strongest differentially expressed (DE) genomic region (Figs. 5a, b). Significant upregulation was also found for phnC-E12 and the two putative regulatory genes, but expression was more inconspicuous. The upstream region of the phnF gene was additionally highly expressed in MPn treatment, whereas expression of the coding region of phnF was weaker. By contrast, increased expression was found for the antisense strand of phnF in the Pi control, suggesting an inhibitory role in gene regulation. The genome of N. spumigena UHCC 0039 contains also a secondary operon for phosphonate transport (phnC-E), which might be involved in the uptake of another forms of phosphorus, for example, organophosphates or phosphites. Transcription and upregulation of phnC and phnD in this secondary operon indicated that they could be functional (Table 2). Among the upregulated DE genes participating in phosphorus metabolism, high-affinity phosphate transport system pstABC (Log2FC 0.99–2.1) and one atypical alkaline phosphatase phoA (BMF81_00431) together with hypothetical protein BMF81_00430, were upregulated (Table 2). Increased expression of pstABC was unexpected, because upregulation of pstS was not found in the RT-qPCR study. This is most probably due to the different cDNA library protocols because initial transcriptome was the same and efficiency of the primers was high (Figure S1).

The MPn treatment seemed to co-induce an iron starvation response and upregulated genes related to light-harvesting complex of photosynthesis (Fig. 5, Table 2).
The iron stress-induced protein (IsiA) is heavily upregulated under iron-limiting conditions in many cyanobacteria [44, 45] and it is usually used as a marker gene for iron scarcity. The isiA/psbC/pcb genes are clustered together in N. spumigena UHCC 0039 and also contain a flavodoxin associated hypothetical protein and a hypothetical protein containing a/b hydrolase domain. Genes belonging to iron starvation and acquisition systems, containing upregulated genes from the isiA/isiB/psb family (BMF81_02614; BMF81_02616; BMF81_02618-20, and BMF81_02622), TonB-dependent receptors (BMF81_01155 and 01958), and Fe³⁺-siderophore transport system permease components (BMF81_01159-61), were induced in MPn treatments (Table 2). In addition, increased expression was found of a genomic region encoding 34 genes (BMF81_00493-00526) (Fig. 5c). The first part of this region includes a non-ribosomal peptide synthetase/polyketide synthase (NRPS/PKS) gene cluster and may encode siderophore. An AraC-type transcriptional regulator (BMF81_03100), which has been suggested to regulate operons involved in iron uptake, was also upregulated.

Discussion

The availability of Pi in the Baltic Sea is scarce during the late summer [3, 34, 46]. Despite limited Pi availability, N. spumigena forms massive toxic blooms in the Baltic Sea every summer [32, 47]. Alkaline phosphatase is a well-known enzyme that degrades organic phosphoesters [2, 3] (Van Wambeke et al. 2002) and this enzyme enhance the fitness of N. spumigena during Pi limitation [35]. However, Nodularia spumigena CCY 9414, isolated from the Baltic Sea, encodes a phn gene cluster indicating it could degrade phosphonates and liberate Pi to meet cellular demand [36]. In this study we found phnD and phnJ, key genes of phn gene cluster, from all 12 investigated N. spumigena strains, as well as from two other previously sequenced N. spumigena CCY 9414 [36] and N. spumigena CENA 596 [48] strains showing that this gene cluster is common among N. spumigena (Fig. 1), but not in other Nodularia spp., Aphanizomenon flos-aquae or Dolichospermum spp. strains isolated from the Baltic Sea (Fig. 1b, unpublished data). The phn gene cluster was additionally found to be ubiquitous in the Baltic Sea using N. spumigena-specific primers probing environmental samples (Fig. 1c). Our bioinformatics analysis further showed that only 5.4% of the sequenced cyanobacteria deposited in GenBank carry this particular genetic region and the lack of clear synteny indicates that cyanobacteria have acquired the phn gene cluster by horizontal gene transfer. This has also been demonstrated by phylogenetic analyses of phnJ gene [26, 49]. The sporadic distribution of the phn gene cluster within the cyanobacterial phylum has also been described earlier [50]. This study confirms the hypothesis that N. spumigena could degrade phosphonates to cope with the lack of available Pi in the Baltic Sea during summer [36] (Nausch, 1998) and may provide N. spumigena an important competitive advantage through utilization of phosphonates as an alternative source of phosphorus.

Previous studies have shown that cyanobacteria harboring phosphonate transport and C-P lyase units of the phn gene cluster can exploit a broad range of phosphonates such as...
methyl- and ethylphosphonates, 2-aminoethylphosphonate and glyphosate [27, 28]. The majority of these strains comprise also the regulatory gene phnF despite phnF is missing from *Trichodesmium erythraeum* IMS101 and *Synechococcus* sp. JA-2-3BA(2–13). However, both strains are nevertheless capable of degrading phosphonates [25, 26]. The small pore size of the PhnC-E channel and intracellular location of C-P lyase complex may be the reasons for limited utilization of phosphonate substrates. Furthermore, variations in amino-acid composition of PhnD proteins affecting the dissociation constants to different phosphonates [51] can also limit the usage of phosphonates. The phn gene cluster is thus not sufficient alone to confer the capacity to degrade phosphonates and physiological studies are needed to complement the genomics data to answer the question, which phosphonates are suitable for cyanobacteria.

Here we examined the growth of *N. spumigena* strains UHCC 0039 and UHCC 0060 carrying the phn gene cluster in seven different phosphate supplemented media. MPn fulfilled the phosphorus demand in both strains and 2-aminoethylphosphonate acted as phosphorus source for *N. spumigena* UHCC 0039 and ethylphosphonic acid to *N. spumigena* UHCC 0060 but two latter substrates enabled only minor growth. Our results suggest that the Baltic Sea *N. spumigena* cyanobacteria can exploit naturally produced phosphonates among which MPn was the preferred form.

MPn is suggested to be produced in oceans by heterotrophic microbes expressing a MpnS-dependent biosynthetic pathway [8] and liberation of MPn to the surrounding environment was estimated to be significant due to the short life cycle of these microbes [8]. MPn may thus serve as a phosphorous reservoir for cyanobacteria capable to C-P bond cleavage and this character may explain more intensive occurrence of *N. spumigena* compared with other diazotrophic cyanobacteria when inorganic phosphorus is absent from the upper water layers.

MPn is additionally a precursor for aerobic methane release [29] and circulation of MPn in the oceans is an important contributor in the methane flux to the atmosphere [4]. Based on our results, blooms of *N. spumigena* may also have an important role in aerobic methane release and explain temporal variation of the methane concentration in the Baltic Sea [37, 38]. In addition, phosphonates may increase N2-fixation in P-limit environment because N2 fixation positively correlates with phosphorus availability [34, 46, 52, 53]. Flux of organic nitrogen to water body [11] further enhance eutrophication in water ecosystems [54]. Finally, nutrient-replete conditions also increase toxin production in *N. spumigena* (Lehtimäki et al. 1997). The anthropogenic phosphonates used in this study were not suitable phosphorus sources for studied *N. spumigena* strains and the riverine load of anthropogenic phosphonate
compounds most probably does not enhance *N. spumigena* blooms in the Baltic Sea ([55], this study). However, light intensity used was low compared with conditions *N. spumigena* encounter in the environment [56] although it is likely that *N. spumigena* experience such low light conditions also in nature. Low light conditions resulted in relatively long doubling times of the cultures. In spite of this, studied *N. spumigena* strains were able to grow in MPn medium and release methane.

Genes assisting cyanobacteria to cope with phosphorus-limited conditions belong to the *pho* regulon, which are activated by \( P_i \) scarcity. One widely used marker for detecting the activation of *pho* regulon and further \( P_i \) scarcity is alkaline phosphatase activity. In our study, alkaline phosphatase activity decreased in both MPn and 2APn conditions compared with -Pi condition. MPn did not induce alkaline phosphatase activity despite the lack of \( P_i \), indicating an alternative phosphorus uptake pathway might be dominating the process. The *phn* gene cluster belongs to the *pho* regulon [16, 26] and thus should be induced in the lack of \( P_i \). The C-P lyase part of the *phn* gene cluster was heavily upregulated in the presence of MPn, whereas upregulation of phosphonate transport system was slighter. RT-qPCR studies further validated that *phnD* gene was upregulated in 2APn and -Pi control but constant upregulation of *phnJ* gene required suitable phosphonate substrate. Due to the bipartite structure of the *phn* gene cluster, *phnD*-E is most probably regulated independently and the role of *phnF* for the regulation of *phnC*-E may be crucial [24]. In addition, *phnJ* gene was found to be expressed only in the presence of suitable substrate (MPn). Chemical detection of phosphonates in saline water ecosystems is challenging and requires specific analytic tools (nuclear magnetic resonance spectroscopy, NMR) [4]. Thus, transcripts of *phnJ* gene may

| Gene name | Function | locus_tag | Log2FC* | p-Value |
|-----------|----------|-----------|---------|---------|
| Phosphorus metabolism | | | | |
| *pstA* | High-affinity phosphate transporter | BMF81_03296 | 1.387 | 5.208e-06 |
| *pstB* | High-affinity phosphate transporter | BMF81_03295 | 0.831 | 4.261e-05 |
| *pstC* | High-affinity phosphate transporter | BMF81_03297 | 1.546 | 2.626e-06 |
| *pstS* | High-affinity phosphate transporter | BMF81_03298 | 2.165 | 6.868e-08 |
| *phnC2* | Phosphonate ABC-transporter | BMF81_03014 | 0.907 | 1.108e-04 |
| *phnD2* | Phosphonate ABC-transporter | BMF81_03015 | 0.769 | 3.924e-05 |
| Hypothetical protein | BMF81_00430 | 2.575 | 1.334e-06 |
| **phoA** | Alkaline phosphatase | BMF81_00431 | 3.607 | 3.972e-07 |
| Photosynthesis | | | | |
| *pbsC1* | Chlorophyll a/b binding light-harvesting protein | BMF81_02614 | 1.542 | 3.072e-05 |
| *pbsC2* | Chlorophyll a/b binding light-harvesting protein | BMF81_02616 | 1.570 | 1.752e-05 |
| Flavodoxin, chain A | BMF81_02618 | 1.642 | 5.378e-06 |
| Hypothetical protein | BMF81_02619 | 1.486 | 1.715e-05 |
| *pbsC3* | Chlorophyll a/b binding light-harvesting protein | BMF81_02620 | 1.768 | 8.771e-06 |
| *pbsC4* | Chlorophyll a/b light-harvesting protein | BMF81_02622 | 1.967 | 1.106e-06 |
| Plastocyanin | BMF81_00249 | 0.996 | 1.400e-04 |
| Iron uptake | | | | |
| Regulatory protein | BMF81_03100 | 1.222 | 2.128e-06 |
| TonB-dependent receptor | BMF81_01155 | 1.435 | 1.970e-05 |
| TonB-dependent receptor | BMF81_01958 | 0.881 | 3.238e-05 |
| ABC-type Fe\(^{3+}\)-siderophore transport system permease component | BMF81_01159 | 1.140 | 1.782e-05 |
| ABC-type Fe\(^{3+}\)-siderophore transport system permease component | BMF81_01160 | 1.020 | 4.858e-05 |
| ABC-type Fe\(^{3+}\)-siderophore transport system permease component | BMF81_01161 | 1.215 | 3.983e-06 |
| Hypothetical protein | BMF81_01162 | 1.053 | 2.535e-04 |

* Log2 fold change
be suitable indicator for phosphonate bioavailability. Other genes of the pho regulon, such as the PhnABCS system, were additionally upregulated in MPn condition showing that even though a suitable phosphorus source was available, the pho regulon remained activated.

Growth in MPn had little effect on the transcriptome of *N. spumigena* UHCC 0039. However, the treatment resulted in the co-induction of genes related to the light-harvesting complex, as well as iron starvation and acquisition. Expression of the genes encoding photopigments are sensitive to environmental changes and fine tuning the pool of photopigments is an important way for cyanobacteria in adapting to new environment [57]. The iron scarcity marker gene *isiA* becomes usually highly expressed under iron starvation [44]. The *isiA* gene in *N. spumigena* is located together with genes encoding CP43/Pcb family proteins [36] and in our study the whole locus was heavily upregulated in the presence of MPn. Siderophore-mediated iron uptake in cyanobacteria is one mechanism used to overcome iron limitation [58]. However, siderophores are not sufficient for efficient iron scavenging, because these molecules need to be transported to the extracellular space and then the iron-siderophore complex back to the cell [59]. TonB-dependent carriers together with ABC-type transporters are crucial in iron transport. In our study, MPn treatment induced expression of TonB-dependent receptors and Fe-siderophore transporters, as well as an AraC-type regulator, which has a role in the regulation of gene expression. No known siderophores were identified from the genome of *N. spumigena* UHCC 0039. However, the strain carries a genetic region similar to *Nostoc* sp. PCC 7120, which has an important role in iron metabolism [60]. This particular gene cluster has also a great similarity to a siderophore-coding cluster in *Agrobacterium tumefaciens* [61]. The gene cluster of this putative siderophore in *N. spumigena* UHCC 0039 was heavily upregulated in the MPn condition, but the product remained elusive in this study. Iron constitute cluster with sulfur in the active site of PhnJ [62] and enhanced production of PhnJ may thus cause demand of iron in the cells. In addition, phosphorus and iron are the major nutrients limiting the growth of diazotrophic cyanobacterial blooms during the summer in the Baltic Sea and co-regulation of the genes suggests a hardwired strategy to deal with nutrient limitation in *N. spumigena*.

**Conclusion**

Here we demonstrate that strains of *N. spumigena* can grow using methylphosphonate as a sole source of phosphorous with only minor remodeling of the transcriptome. Our results show that phn gene clusters are wide-spread in the genomes of the toxic bloom-forming *N. spumigena*. This particular genetic element may enable *N. spumigena* to cope with P_i-limited conditions by degrading phosphonates and liberating phosphate for the cellular usage. Alkaline phosphatase activity, a marker used to indicate P_i starvation, was not detected from the culture growing in the medium supplemented by MPn but a transcriptional response of *pstS*, the other molecular marker used for P_i scarcity, was found. *N. spumigena* may contribute to methane supersaturation in the water column by degrading MPn to release phosphate and methane.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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