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Nodularia spumigena and Its Attribute to Bloom Formation in the Baltic Sea

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N. spumigena is a dominant cyanobacterial species found in the Baltic Sea. It forms extensive bloom in late summer in the areas of the Baltic Sea with high phosphorus concentrations and moderate salinity. Both environmental and manmade factors are involved in bloom formation. This review also elucidates the physiological and molecular aspects of nitrogen fixation, heterocyst formation and nodularin production in *N. spumigena*.

Key words: *Nitrogen fixation, heterocyst, summer bloom, nodularin.*

1. Introduction

Nodularia spumigena is a filamentous and heterocystous cyanobacterium commonly found in brackish water systems, capable of forming cyanobacterial toxic blooms in the Baltic Sea. *N. spumigena* belongs to oxygenic photoautotrophic prokaryotes having thick-walled akinetes. The toxic summer blooms are mainly dominated by filamentous, heterocystous cyanobacteria, *N. spumigena, Aphanizomenon* sp. and *Anabaena* sp. The cyanobacterial summer blooms in the Baltic Sea contribute significantly to the nutrient input, aggravating the effects of eutrophication, and blooms dominated by *N. spumigena* are particularly problematic due to their toxicity.

Harmful algal blooms form an increasing problem in many aquatic environments, both freshwater and marine. They develop in response to increasing eutrophication of aquatic systems but also because of shifts in the equilibrium of ecosystems (Kononen, 2001). Blooms are defined as mass occurrences of microalgae. They are usually undesirable and are often a nuisance both to the environment and to man. While several bloom forming algae are toxic, non-toxic algal blooms may also exert a negative influence on the environment. Decaying algal blooms may deplete the water of O_2, initiating the death of fish and other animals and causing bad smells and low quality water. In addition, in marine environments, O_2 depletion will lead to the formation of extremely toxic sulfide (Welsh et al., 2001). In addition, beaches become filthy because of the deposition of surface accumulations of algal blooms, decreasing the recreational and economic value of these waters and their shorelines. The formation of massive water blooms has a further detrimental impact on the biodiversity of the environment where they occur, since blooms are composed of a single, or no more than a few, species (Lucas et al., 2003). However, despite these problems, it should not be forgotten that microalgae form a normal component of aquatic ecosystems and, as primary producers; they form the basis of the food web of these ecosystems (Welsh et al., 2001). In order to understand the development of cyanobacterial blooms it is essential to know their physiological and molecular features and genetic diversity across the Baltic Sea along with the factors that determine this diversity. We also necessitate precise knowledge of its physiological and molecular responses behind bloom formation.

2. Factors affecting bloom formation

The Baltic Sea is the largest brackish water ecosystem in the world. Reasons of the Baltic Sea eutrophication and its consequences have been extensively studied and are now well documented (Lucas et al., 2003; Savchuk, 2005). It is known that increased eutrophication and cyanobacterial blooms are due to discharges of human sewage, industrial wastes and riverine input (Bonsdorff et al., 2002). Eutrophication arises when excessive amounts of nutrients, mainly nitrogen (N) and phosphorus (P), build up in aquatic ecosystems and cause accelerated growth of algae and plants, often resulting in undesirable effects. It is now well documented that diazotrophic cyanobacterial blooms develop only in areas where the N:P ratio is well below the Redfield ratio of 16 (Lucas et al., 2003). Not only the ratio of N:P but also the adequate concentration is an important factor for bloom formation. Local variation of phosphate concentration is believed to cause patchy development of cyanobacterial blooms (Lucas et al. 2003). Phosphorus is required for the synthesis of many cellular components such as ATP and nucleic acids and also for regulation of protein activities. In addition, N_2-fixing cyanobacteria have an elevated requirement for iron. Iron works as a component of nitrogenase itself and of ferredoxin, which acts as an electron donor to nitrogenase. The supply of iron may vary, due to local inputs or hydrodynamic conditions, giving rise to patchy development of blooms. Furthermore, it has been reported that high sulphate concentrations rather than high salinity are factors that limit the formation of diazotrophic blooms (Lucas et al., 2003). Although water temperature has often been mentioned as an important factor for the development of diazotrophic cyanobacterial blooms, its effect is indirect by establishing conditions that provide sufficient light for productive growth. Even in summer, the daily irradiance may be insufficient to allow net photosynthesis of the cyanobacterial community (Stal and Walsby, 2000).

3. Nitrogen fixation

Nitrogen fixation is a physiological process in cyanobacteria by which nitrogen (N_2) in the atmosphere is converted into ammonia (NH_3). Cyanobacteria are able to utilize a variety of inorganic and organic sources of combined nitrogen. Nitrogenase is the enzyme responsible for nitrogen fixation, and nifH, the gene encoding the dinitrogenase reductase subunit of nitrogenase, is often used as a marker for nitrogen-fixation activity (El-Shehawy et al., 2003; Short & Zehr, 2007). Owing to the high-energy requirements of N_2-fixation, fixation in heterocystous cyanobacteria is often coupled with photosynthesis and, therefore, with light (Gallon et al., 2002). In contrast, the oxygen sensitivity of the nitrogenase will drive an uncoupling with photosynthetic activity. Factors controlling N_2 fixation can be grouped into physical, biological and biogeochemical factors. Light is a very important controlling factor, because it provides energy for primary production as well as N_2 fixation. It is estimated that nitrogen-fixing cyanobacteria are responsible for approximately 50 % of the annual nitrogen input in the Baltic Sea (Stal et al., 2003).

Response of *N. spumigena* to nitrogen and ammonium supplementation, with a focus on N_2-fixation behaviour, has been analysed (Vintila and El-Shehawy, 2007). Vintila and El-Shehawy, (2007) reported that expression of nifH (encoding the dinitrogenase reductase component of the nitrogenase enzyme) was suppressed and the levels of NifH protein decreased dramatically in response to treatment with ammonium. It is generally known that ammonium and nitrate repress N_2 fixation, with the latter being less effective and strain-dependent (Guerrero & Lara, 1987). It has been reported that Cyanobacteria like *Nodularia* can utilize different sources of nitrogen, such as ammonia, nitrate and nitrogen gas. The preferred source of nitrogen is ammonium ion because it is readily incorporated into the carbon skeleton through the glutamine synthetase-glutamate synthase (GS-GOGAT) cycle (Flores & Herrero, 2005).

4. Heterocyst differentiation

The heterocyst is a terminally differentiated cell that protects the nitrogenase complex under oxic conditions by thick cell envelope and increased rate of respiration, providing a microaerobic environment (Haselkorn, 2007). *N. spumigena* fixes atmospheric nitrogen (N_2) in heterocysts. The formation and maintenance of a pattern of heterocysts along the filaments of heterocystous cyanobacteria are complex processes, not yet fully understood, which involve many genes in a developmental programme (Wolk, 2000). The heterocyst possesses an active photosystem I and, through anoxygenic photosynthesis, can provide the energy for nitrogenase. Filaments of *N. spumigena* contain on average 5% heterocysts; A. flos-aquae only1% (Lucas et al., 2003). The heterocysts have a frequency of approx. 5-10 %, depending on the species, along the filaments. They provide the vegetative cells with fixed-nitrogen and receive carbohydrates, possibly through narrow cellular junctions (microplasmodesmata) located at the polar regions of the cells.

Reduction of atmospheric nitrogen is performed by the nitrogenase complex yielding ammonium as an end product. The reductants used by nitrogenase are generated from metabolism of a sugar, provided by vegetative cells, via the oxidative pentose pathway in heterocysts yielding 2-OG, which is transported to vegetative cells. ATP is produced by cyclic phosphorylation, using photosystem I, and the respiratory electron transport chain. The ammonium produced by nitrogenase is incorporated into glutamine by glutamine synthethase (GS) and is

rapidly transported to the vegetative cells where it is used together with 2-OG to yield two glutamate (Figure 1). The reaction is catalysed by glutamate synthase (GOGAT). These sequential reactions are commonly known as the GS-GOGAT pathway (Flores & Herrero, 2005; Thiel, 2004). The signalling pathway of this response is not fully understood, but the cells seem to sense the carbon/nitrogen balance and the redox status of the cells. Nevertheless, there have been reports of heterocyst differentiation during nitrogen supplementation (Thiel, 2004).

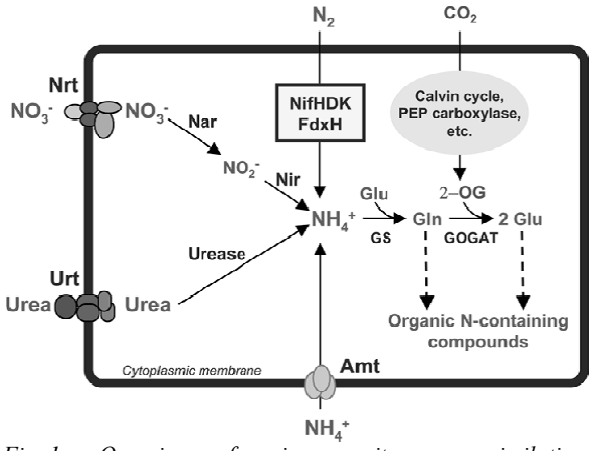

Fig. 1. Overview of primary nitrogen assimilation pathways in cyanobacteria (Adapted from Flores & Herrero, 2005)

One of the key genes involved in heterocyst differentiation is ntcA, which encodes the transcription factor NtcA, belonging to the cyclic AMP receptor family (CRP-family) of bacterial regulators (Vega-Palas et al., 1992) and its activity is required for the development and function of mature heterocysts. NTcA indirectly activates the expression of hetR, which encodes a serine-type protease with DNA-binding activity and is expressed early during heterocyst differentiation (Ehira & Ohmori, 2006). HetR is another crucial gene which is involved with the differentiation process. The hetR is positively autoregulated and has a positive effect on ntcA transcription (Herrero et al., 2001).

5. Toxin production

N. spumigena produces the hepatotoxin nodularin, a cyclic pentapeptide. Seven naturally-occurring isoforms of nodularin have been reported to date. The toxin has been reported to have detrimental effects on numerous organisms within the ecosystem, including invertebrates and fish, but may have no effect on other organisms (Karjalainen et al., 2007). The consumption of water containing toxic *N. spumigena* blooms has led to the death of domestic and native animals by massive liver haemorrhage. Nodularin may also act as a carcinogen via the initiation and promotion of liver cell division (Ohta et al., 1994). Nodularin production by *N. spumigena* is influenced by irradiance, temperature, salinity, concentration of inorganic phosphorous and growth phase (Ohta et al., 1994). Though the environmental factors are important for the toxicity of any given *N. spumigena* population, the main concern for water managers will be the potentially very rapid accumulations or dispersions of cyanobacteria that may change cell densities of *Nodulari*a by several orders of magnitude within a short time.

The nodularin biosynthesis gene cluster, nda-cluster, has been sequenced and characterised in *N. spumigena* NSOR10 (Moffitt & Neilan, 2004). The 48 kb region of the genome consists of nine ORFs (ndaA to ndaI) transcribed from a bidirectional regulatory promoter region and includes NRPS modules, PKS modules and tailoring enzymes (Figure 2). The proposed pathway for nodularin biosynthesis is similar to that for microcystin. More recently, elucidation of the nda cluster has provided an opportunity to monitor transcriptional regulation of the biosynthetic pathway (Jonasson et al., 2008).

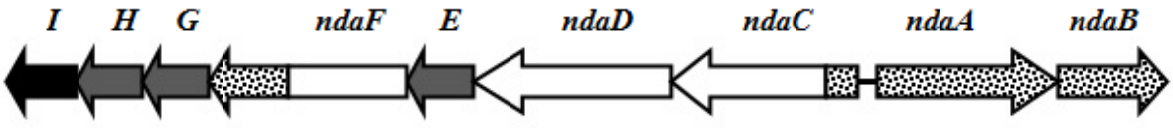

Fig. 2. Structure of hepatotoxin gene clusters in N. spumigena strain NSOR10, showing genes encoding ABC-transporters (black), tailoring enzymes (grey), non-ribosomal peptide synthetases (dotted), polyketide synthases (white)

The effects of ammonia, nitrogen and phosphate starvation were analysed on the expression of the nda genes. Jonasson et al. (2008) reported that the *nda* cluster appears to be constitutive, phosphate starvation resulted in an approximately two-fold increase in expression, while ammonia supplementation decreased the expression two-fold. Despite the changes in the expression, intracellular and extracellular nodularin concentration remained stable (Jonasson et al., 2008).

These results are in harmony with Repka et al. (2001), who demonstrated that in chemostat cultures of Nodularia strain GR8b, the phosphate concentration did not have a statistically significant effect on the nodularin production rate. Analysis of the transcriptional pattern of these genes is critical in elucidating the factors regulating toxin biosynthesis, the upstream signaling pathways that target toxin synthesis, and the molecular mechanism(s) by which toxin production is controlled.

6. Conclusions

Summer bloom is a problem in the entire Baltic Sea area, but the effects and consequences vary among sub-basins. Positive measurements and potential recovery require action both on basinwide and regional scales. Given the strong links between eutrophication abatement and protection of marine biodiversity, the improving eutrophication status will also result in significant improvements in habitat quality and conservation status in many parts of the Baltic Sea.

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