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ScienceDirect
Harmful Algae 7 (2008) 114–125
HARMFUL ALGAE
www.elsevier.com/locate/hal

The exposure of green turtles (*Chelonia mydas*) to tumour promoting compounds produced by the cyanobacterium *Lyngbya majuscula* and their potential role in the aetiology of fibropapillomatosis

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Received 12 March 2007; received in revised form 23 May 2007; accepted 1 June 2007

Abstract

Lyngbya majuscula, a benthic filamentous cyanobacterium found throughout tropical and subtropical oceans, has been shown to contain the tumour promoting compounds lyngbyatoxin A (LA) and debromoaplysiatoxin (DAT). It grows epiphytically on seagrass and macroalgae, which also form the basis of the diet of the herbivorous green turtle (*Chelonia mydas*). This toxic cyanobacterium has been observed growing in regions where turtles suffer from fibropapillomatosis (FP), a potentially fatal neoplastic disease. The purpose of this study was to determine whether green turtles consume *L. majuscula* in Queensland, Australia and the Hawaiian Islands, USA, resulting in potential exposure to tumour promoting compounds produced by this cyanobacterium. *L. majuscula* was present, though not in bloom, at nine sites examined and LA and DAT were detected in variable concentrations both within and between sites. Although common in green turtle diets, *L. majuscula* was found to contribute less than 2% of total dietary intake, indicating that turtles may be exposed to low concentrations of tumour promoting compounds during non-bloom conditions. Tissue collected from dead green turtles in Moreton Bay tested positive for LA. An estimated dose, based on dietary intake and average toxin concentration at each site, showed a positive correlation for LA with the proportion of the population observed with external FP lesions. No such relationship was observed for DAT. This does not necessarily demonstrate a cause and effect relationship, but does suggest that naturally produced compounds should be considered in the aetiology of marine turtle FP.

Keywords: Green turtle; *Lyngbya majuscula*; Fibropapillomatosis; Lyngbyatoxin A; Debromoaplysiatoxin

1. Introduction

Lyngbya majuscula (Gomont) is a filamentous cyanobacterium found in marine and estuarine environments. It has a pan-tropical distribution (Banner,

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doi:10.1016/j.hal.2007.06.001

1959; Milligan et al., 2000), is commonly found in inshore benthic habitats at depths up to 30 m depth (Banner, 1959) and often forms cosmopolitan assemblages with other filamentous organisms (van den Hoek et al., 1995). This cyanobacterium forms dense blooms, often lasting up to 3–4 months (Dennison et al., 1999) that can impact immotile benthic organisms, such as seagrass and corals, by shading leading to declines in seagrass abundance (Stielow and Ballantine, 2003).

L. majuscula is a prolific producer of secondary metabolites, with over 100 biologically active compounds currently isolated from the organism (Nagle and Paul, 1999; Burja et al., 2001). These compounds may be by-products of physiological processes and many act as deterrents to generalist herbivores (Nagle and Paul, 1999). Lyngbyatoxin A (LA) and debromoaplysiatoxin (DAT) are two compounds that have been isolated from *L. majuscula* as the causative agents in human contact dermatitis, and nausea and vomiting resulting from incidental ingestion (for review, see Mynderse et al., 1977; Cardellina et al., 1979; Moore, 1981; Osborne et al., 2001). Both compounds act as protein kinase C (PKC) activators and are potent tumour promoters in mice (Fujiki et al., 1981, 1983, 1984, 1990).

It has been hypothesised that naturally occurring tumour promoting compounds play a role in the aetiology of fibropapillomatosis (FP), a neoplastic disease observed in marine turtles (Herbst, 1994; Herbst and Klein, 1995; Landsberg et al., 1999; Arthur et al., 2006b). Fibropapillomatosis is characterised by benign tumours found both as external cutaneous lesions and internal visceral masses. While lesions are benign and do not directly lead to death, they may obstruct movement, vision and ability to feed, and can impede organ function. Depending on the size and/or location of the lesion, afflicted turtles may die of starvation or organ failure (Jacobson et al., 1989; Herbst, 1994). However, this disease is not necessarily fatal with spontaneous regression of tumours observed in Hawaii, Florida and Australia (Ehrhart, 1991; Bennett et al., 1999; Limpus et al., 2005).

Fibropapillomatosis has been recorded in green turtle populations throughout the world, however, FP prevalence differs geographically and amongst age classes. Geographic variation is probably a consequence of the high site fidelity demonstrated by green turtles to, and within, their foraging grounds (Taquet et al., 2006), which may lead to chronic exposure to tumour promoting agents and pollutants. A high prevalence of the disease is often associated with increased anthropogenic activity in the surrounding environment (Herbst, 1994). The distribution of turtles suffering from FP also varies between age classes with juveniles (10–30 kg) most effected (Herbst, 1994). Conversely, newly recruited individuals from the pelagic life phase have never been observed with tumours (Ehrhart, 1991; Limpus and Miller, 1994) and adults at nesting grounds have low levels of FP prevalence (George, 1997). The sporadic occurrence of the disease amongst turtles of the same genetic pool over short geographic ranges eliminates a genetic predisposition while the global prevalence of the disease suggests a multifactorial aetiology, the nature of which may vary between sites (Balazs, 1991; Aguirre and Lutz, 2004).

Microscopic evidence of virus-like particles in tumour cells and experimental transmission studies using cell-free homogenate indicate that the infectious agent of fibropapillomatosis is a virus (Herbst, 1994; Herbst et al., 1995). Similarly, direct experimental inoculations have consistently resulted in horizontal transmission of the disease between turtles with tumours developing within a year at the site of inoculation (Herbst et al., 1995, 1996). Although retroviruses have been found in association with FP (Casey et al., 1997; Rufina et al., 1997), recent genetic studies also support the involvement of a novel herpesvirus, fibropapilloma-associated turtle herpesvirus (FPTHV) that has been detected in all tumour tissue samples tested to date using PCR analysis (Van Devanter et al., 1996; Quackenbush et al., 1998, 2001; Lackovich et al., 1999; Greenblatt et al., 2005). However, genetic material from FPTHV has also been found in turtles not displaying external lesions, suggesting that the progression to lesions is multifactorial and potentially involves a tumour promotion phase of development (Quackenbush et al., 2001).

Naturally produced compounds from marine organisms have been vastly overlooked in terms of their potential impact on wildlife (Landsberg et al., 1999). There are many examples of multifactorial carcinomas where oncogenic viruses require an environmental cofactor to express carcinogenicity (Pamukcu et al., 1967; Evans et al., 1982a,b; Jackson et al., 1993). An association between FP and the naturally produced tumour promoting compound okadaic acid (OA) was recently demonstrated (Landsberg et al., 1999). Okadaic acid is produced by a benthic dinoflagellate *Prorocentrum* sp. (Murakami et al., 1982; Yasumoto et al., 1987; Dickey et al., 1990; Morton et al., 1998) and in conjunction with an initiator causes tumour growth in mice by inhibition of the protein phosphatases pathways 1 and 2A (Fujiki and Suganuma, 1993). Landsberg et al. (1999) demonstrated that there was a greater abundance