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ANTIMICROBIAL ACTIVITY OF AN EGYTPTIAN MARINE CYANOBACTERIUM *LYNGBYA MAJUSCULA* GOMONT.

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Abstract

The cyanobacterium *Lyngbya majuscula* was found to form mats on the submerged pneumatophores and leaves of Mangrove plants in the red sea. Aqueous and methanolic extracts of *L. majuscula* mats were bioassayed for their bioactivity against mice, *Artemia salina*, fungi and bacteria. The results of this study revealed that both aqueous and methanolic extracts were not toxic to mice at a concentration of 0.5g/ml. The aqueous extract did not have any effect either on *Artemia* or bacteria. The methanolic extract of this cyanobacterium exhibited a toxicity against *A. salina* with LC_{50} value of 0.3 g/ml. Also, the methanolic extract inhibited the growth of *Bacillus subtilis*, *E. coli* and *Pseudomonas aeruginosa* with minimum inhibition concentration (MIC) of 0.3, 0.2, 0.45 g/ml, respectively. Both aqueous and methanolic extracts with MIC of 0.13 g/ml had antifungal activity against all tested fungi, except *Botryodiplodia* which has been affected only by methanolic extract. In conclusion, the data point out that the methanolic extract of *L. majuscula* has antibacterial and antifungal activities, while aqueous extract has antifungal activity only. This finding indicates that the marine cyanobacterium *L. majuscula* could contain more than one bioactive substance.

Introduction

The search for bioactive secondary metabolites from prokaryotic and eukaryotic algae will continually provide novel useful and structurally specific compounds having a bright future in scientific research and human affairs (Skulberg, 2000). Cyanobacteria (blue-green algae) are exciting source of novel bioactive natural products (Gerwick *et al.* 1994, Harrigan *et al.* 1998, Orjala *et al.* 1995a). Of all the marine cyanobacteria, the pantropical *Lyngbya majuscula* Gomont (Oscillatoriaceae) has been the richest source, yielding more than 100 different secondary metabolites, nearly half of which are of a lipopeptide nature (Singh *et al.* 1999). These metabolites are of interest to natural products researchers, mainly because of their potential application.

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Many of these metabolites possess the following interesting biological activities:

1. Anticancer such as dolastatin G (Harrigan *et al.* 1998), lyngbyastatin 2 and norlyngbyastatin 2 (Luesch *et al.* 1999).
2. Antifeeding such as ypaoamide (Nagle *et al.* 1996).
3. Antifungal such as tanikolide (Singh *et al.* 1999), lyngbyabellin B (Milligan *et al.* 2000) and laxaphycin B (Bonnard *et al.* 1997).
4. Antimitotic such as curacin C (Marquez *et al.*, 1998, Tan et al. 2000).
5. Antineuroblastoma such as hermitamides A & B (Tan *et al.* 2000).
6. Ichthyotoxic such as malyngamide H (Orjala *et al.* 1995a), antillatoxin (Orjala *et al.* 1995b), isomalyngamides A & B (Kan *et al.*, 2000) and hermitamide A (Tan *et al.*, 2000).
7. Irritant such as lyngbyatoxins (Aimi *et al* 1990).
8. Mollscicidal such as barbamide (Orjala & Gerwick 1996) and tanikolide (Singh *et al.*, 1999).

Since *L. majuscula* is a worldwide pronounced cyanobacterium which produces many structurally and bioactively diverse natural products, the abundant occurrence of this cyanobacterium in mangrove regions in Egypt has drawn our attention for the possibility of its biological activities. Thereby, it is, to our knowledge, the first study to report an Egyptian isolate of *L. majuscula* as a source of bioactive metabolites.

Materials and Methods

Collection:

The marine cyanobacterium *L. majuscula* mats were collected by hand from submerged pneumatophores and leaves of mangrove plants in the Red Sea, close to Safaga city, Egypt. The cyanobacterial mats were washed several times with sterilized sea water. The mats were investigated under microscope to ensure the removal of all contaminants. The cyanobacterial mats were stored at –20 °C until workup.

Extraction of bioactive compounds:

Aqueous extract was prepared by homogenizing 15 g dry cyanobacterial material in 100 ml dist. water, then centrifuged at 10,000g. The pellet was re-extracted in 100 ml dist. water. The supernatants were combined together and kept in the freezer until workup. The methanolic extract of *L. majsucula* mats was prepared by the same procedure as in aqueous extraction, except using 100% methanol as a solvent instead of dist. water, and the supernatants were air-dried to evaporate organic solvent.

Mouse bioassay:

The toxicity of both aqueous and methanolic extracts of *L. majuscula* to mice, was evaluated by injecting mice with different concentrations of these extracts.

Brine shrimp toxicity bioassay:

Evaluation of the crude extracts for the brine shrimp (*Artemia salina*) toxicity was performed according to the modified method used by Singh *et al.* (1999).

Antimicrobial Assay and Determination of MIC

Antimicrobial activity of both aqueous and methanolic extracts of *L. majuscula*, was preliminary tested against bacteria and fungi using agar diffusion method according to Falch *et al.* (1995). The bacterial strains used in this assay were *Bacillus subtilis, E. coli and Pseudomonas aeruginosa.* Whereas the fungal species were *Asperigillus flavus*, and 4 species of higher freshwater fungi, viz. *Botryodiplodia theobromae, Sictosporium cocophilum, Westerdykella dispersa* and unknown species of Basidiomycetes.

Minimal inhibition concentration (MIC) of these extracts was estimated for each bacterial and fungal species with different concentrations of these extracts in the media. Bacteria were grown in nutrient broth, and the optical density was obtained every 12 hours interval for 96 hours. While fungi were grown in Cezapek's medium for one week, and the growth was measured as dry weight/ 50 ml medium.

Results and Discussion

The cyanobacterium *L. majuscula* was found to form mats on submerged pneumatophores of mangrove plants in the red sea , Egypt. Previous researchers have demonstrated that *L. majuscula* occurs in shallow water (Orjala and Gerwick, 1997, Milligan *et al.* 2000) and on reefs (Luesch *et al.* 1999, Tan *et al.* 2000) Thus, to our knowledge, the present study is the first to record *L. majuscula* as an epiphyte on mangrove plants.

The results of biological assays for aqueous and methanolic extracts revealed that both extracts were not toxic to mice at a concentration of 0.5 g/ ml. No mortality was observed in tested mice within 3 days.

Methanolic extract of this cyanobacterium was toxic to brine shrimp (*A. salina*) with an LC_{50} value of 0.3 g/ml. While the aqueous did not exhibit any toxicity to *A. salina* until a concentration of 0.5 g/ml. These results are in accordance with those obtained by

Tan *et al* (2000) who found that hermitamides A and B show toxicity to *A. salina* at LD_{50} values of 5 μM and 18 μM, respectively.

Antibacterial assay showed that aqueous extract did not have any effect on the growth of test bacteria, while methanolic extract inhibited the growth of test bacteria at different minimial inhibition concentrations (MICs) (Table 1 & Fig. 1). Although, no study has reported the antibacterial activity of *L. majuscula*, the antibacterial activity of other freshwater cyanobacteria has been estimated. Ostensvik *et al.* (1998) revealed that *Anabaena lemmermannii, Aphanizomenon flos-aquae, Cylindrospermopsis raciborskii, Microcystis aeruginosa* and *Tychonema bourrellyi* showed antibacterial properties that are not associated with the effects of the toxins of these species.

Table 1. Minimal inhibition concentrations (MICs) (g/ml) of aqueous and methanolic extracts of *L. majuscula* for tetsed bacteria and fungi.

Species	Aqueous	Methanolic
Bacteria		
Bacillus subtilis	NA*	0.3±0.02
E. coli	NA	0.2±0.03
Pseudomonas aeruginosa	NA	0.45±0.02
Fungi		
Asperigillus flavus	0.13±0.01	0.13±0.02
Botryodiplodia theobromae	NA	0.13±0.01
Dictyosporium cocophilum	0.13±0.01	0.13±0.03
Unknown Basidiomycete	0.13±0.02	0.13±0.02
Westerdykella dispersa	0.13±0.01	0.13±0.01

NA* = not active

Antifungal assay indicated that both aqueous and methanolic extracts inhibited the growth of four of the five test fungi with similar MICs, while the growth of the fifth fungus *Botryodiplodia theobromae* has been only inhibited by methanolic extract (Table 1 & Fig. 2).

The cyanobacterium *L. majuscula* mats found on the submerged pneumatophores of mangrove plants in the Red Sea, Egypt, is a promising source of antibacterial, antifungal, and other bioactive metabolites. Thus, further work is needed to identify the bioactive compounds produced by this cyanobacterium.

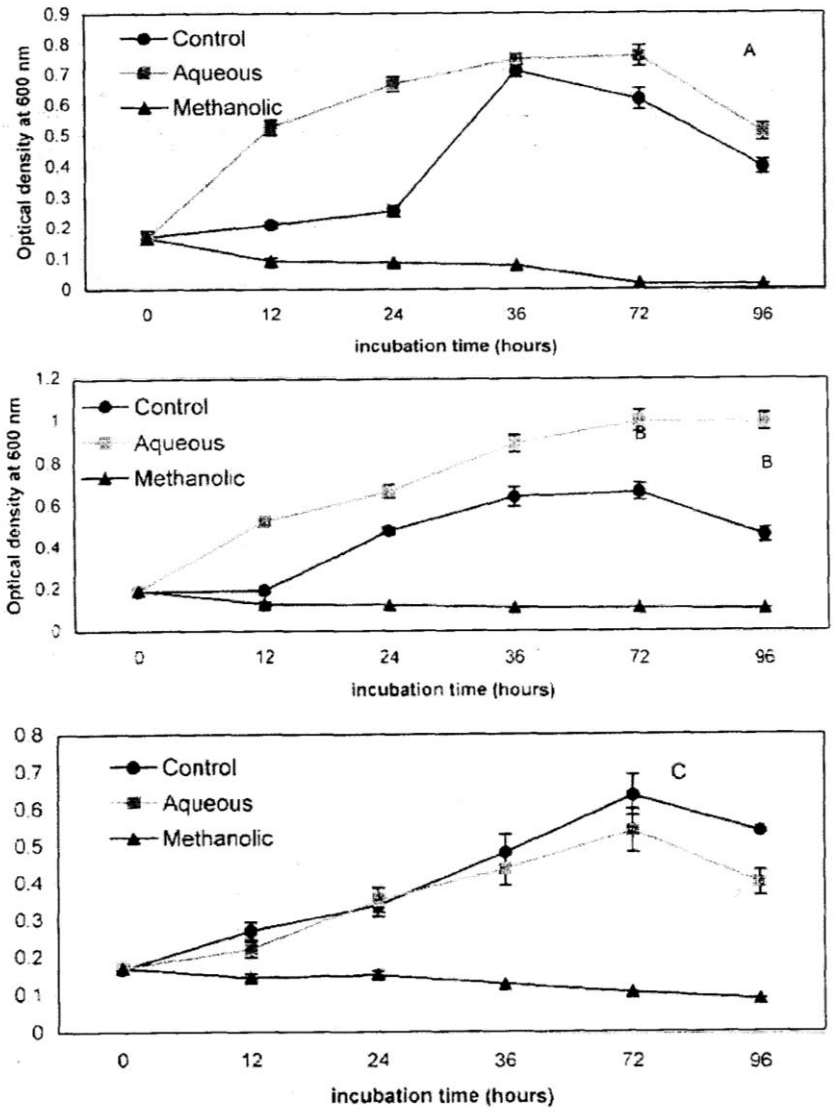

Fig. 1. Effect of methanolic extract of *L. majuscula* at MICs on the growth of (A) *Bacillus subtilis*, (B) *E. coli*, and (C) *Pseudomonas aeruginosa*

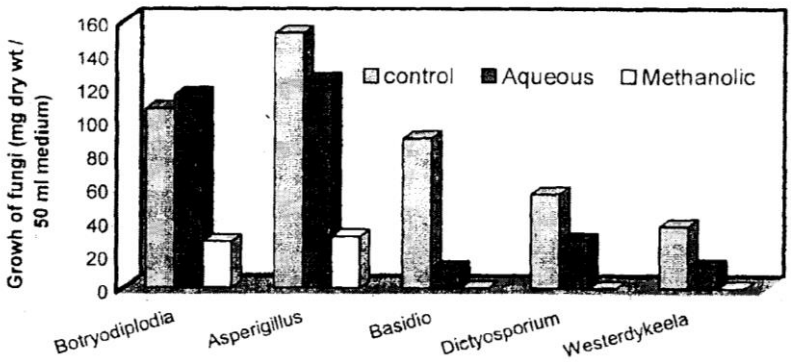

Fig. 2. Effect of Aqueous and methanolic extract at MICs on the growth of tested species of fungi.

References

Aimi, N., H. Odaka, S. Sakai, H. Fujiki, M. Suganuma, R.E. Moore, and G.M. Patterson. 1990. Lyngbyatoxins B and C, two new irritants from *Lyngbya majuscula*. J, Nat. Prod. **53:** 1593-1596.

Bonnard, I., M. Rolland, C. Francisco, and B. Banaigs. 1997. Total structure and biological properties of laxaphycins A and b, cyclic lipopeptides from the marine cyanobacterium *Lyngbya majuscula*. Lett. Peptide Sci. **4:** 1-4.

Falch, B. S., G.M. kong, A.D. Wright, O. Sticher, C.K. Angerhofer, J.M. Pezzuto, and H. Bachmann.1995. Biological activities of cyanobacteria: evaluation of extracts and pure compounds. Planta Medica. **61:** 321-328.

Gerwick, W. H., P. J. Proteau, D. C. Nagle, E. Hamel, and A. Blokhin. 1994. Structure of curacin A, a novel antimitotic, antiproliferative and brine shrimp toxic natural product from the marine cyanobacterium *Lyngbya majuscula*. J. Org. Chem. **59:** 1243-1245.

Harrigan, G.G., W.Y. Yoshida, R.E. Moore, D.G. Nagle, P.U. Park, J. Biggs, V.J Paul, S.L. Mooberry, T.H. Corbett, and F.A. Valeriote. 1998. Isolation, structure determination and biological activity of dolastatin 12 and lyngbyastatin 1 from *Lyngbya majuscula/ Schizothrix calcicola* cyanobacterial assemlages. J. Nat. Prod. **61:** 1221-1225.

Kan, Y., B. Sakamoto, T. Fujitta, and H. Nagai. 2000. New malyngamides from the Hawaiian cyanobacterium *Lyngbya majuscula*. J. Nat. Prod. **63:** 1599-1602.

Luesch, H., W.Y. Yoshida, R. E. Moore, and V.J. Paul. 1999. Lyngbyastatin 2 and norlyngbyastatin 2, analogues of dolastatin G and nordolastatin G from the marine cyanobacterium *Lyngbya majuscula*. J. Nat. Prod. **62:** 1702-1706.

Marquez, B., P. Verdier-Pinard, E. Hamel, and W.H. Gerwick. 1998. Curacin D, an antimitotic agent from the marine cyanobacterium *Lyngbya majuscula*. Phytochemistry. **49:** 2387-2389.

Milligan, K. E., B.L. Marquez, R. T. Williamson, and W.H. Gerwick. 2000. Lyngbyabellin B, a toxic and antifungal secondary metabolite from the marine cyanobacterium *Lyngbya majuscula*. J. Nat. Prod. **63:** 1440-1443.

Nagle, D.G., V.j. Paul, and M. A. Roberts. 1996. Ypaoamide, a new broadly acting antifeedent metabolite from the cyanobacterium *Lyngbya majuscula*. Tetrahedron Lett. **37:** 6263-6266.

Orjala, J. and W. H. Gerwick. 1996. Barbamide, an chlorinated metabolite with molluscicidal activity from the Caribbean cyanobacterium *Lyngbya majuscula*. J. Nat. Prod. **59:** 427-430.

Orjala, J. and W. H. Gerwick. 1997. Two quinoline alkaloids from the caribbean cyanobacterium *Lyngbya majuscula*. Phytochemistry. **45:** 1087-1090.

Orjala, J., D. Nagle, and W.H. Gerwick. 1995a. Malyngamide H, an ichthyotoxic amide possessing a new carbon skeleton from the Caribbean *Lyngbya majuscula*. J. Nat. prod. **58:** 764-768.

Orjala, J., D. Nagle, V.L. Hsu, and W. H. Gerwick. 1995b. Antillatoxin: an exceptionally ichthyotoxic cyclic lipopeptide from the tropical marine cyanobacterium *Lyngbya majuscula*. J. Am. Chem. Soc. **117:** 8281-8282.

Ostensvik, O., O.M. Skulbrg, B. Underdal and V. Hormazabal. 1998. Antibacterial properties of extracts from selected planktonic freshwater cyanobacteria- a comparative study of bacterial bioassays. J. Appl. Microbiol. **84:** 1117-1124.

Skulberg, O. M. 2000. Microalgae as a source of bioactive molecules- experience from cyanophyte research. J. Appl. Phycol. **12:** 341-348.

Singh, I. P., K. E. Milligan, and W. H. Gerwick. 1999. Tanikolide, a toxic and antifungal lactone from the marine cyanobacterium *Lyngbya majuscula*. J. Nat. Prod. **62:** 1333-1335.

Tan, L.T., T. Okino, and W. H. Gerwick. 2000. Hermitamides A and B, toxic malyngamide-type natural products from the marine cyanobacterium *Lyngbya majuscula*. J. Nat. Prod. **63:** 952-955.

الملخص العربى

نشاط التضاد الميكروبي للسيانوباكتريم لينبيا ماجوساكيولا المعزول من بيئة المالحة المصرية

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خلال الزيارات المتكررة لمناطق المانجروف بالبحر الأحمر، لوحظ تواجد حصائر كثيفة من خيوط السيانوباكتيريم لينبيا ماجوساكيولا علي الأفرع والأوراق المغمورة لنباتات المانجروف. اختبر النشاط الحيوي لكل من المستخلص المائي و الميثانولي لهذا الكائن ضد الفئران و الارتيميا و الفطريات و البكتريا.

أوضحت النتائج أن المستخلصات المائية و الميثانولية ليس لها أي تأثير سام علي الفئران حتى تركيز ٠,٥ جم م مل. لم يمتلك المستخلص المائي أيضا أي تأثير مثبط علي أي من الارتيميا أو البكتريا، بينما اظهر المستخلص الميثانولي تأثيرا مثبطا ضد الارتيميا عند تركيز ٠,٣ جم/ مل. كما ثبط المستخلص الميثانولي نمو سلالات البكتريا باسيلس سائلس، أشيرشيا كولاي و سيدوموناس اريجينوزا عند القيم الدنيا للتركيزات المثبطة ٠,٣، ٠,٢ ، ٠,٤٥ جم؟ مل علي الترتيب. أظهرت المستخلصات المائية و الميثانولية تأثيرا مثبطا ضد الفطريات المستخدمة في هذه الدراسة عند ادني تركيز مثبط ٠,١٣ جم؟مل فيما عدا فطر البوتريوديبلوديا الذي تأثر فقط بالمستخلص الميثانولي دون المستخلص المائي.

في الخلاصة، تشير نتائج هذه الدراسة إلي أن المستخلص الميثانولي للسيانوباكتيريم لينبيا ماجوساكيولا له نشاط مضاد لكل من الفطريات و البكتريا، بينما المستخلص المائي كان له تأثيرا مثبطا فقط ضد الفطريات دون البكتريا. لذلك تتوقع الدراسة أن هذا النوع من السيانوباكتريا يمكن أن يحتوي علي اكثر من مادة ذات تأثير مثبط للميكروبات.