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Inhibition of *Candida albicans* and *Staphylococcus aureus* by phenolic compounds from the terrestrial cyanobacterium *Nostoc muscorum*

Mónica M.S. de Cano, M. Cristina Z. de Mulé, Gloria Z. de Caire & Delia R. de Halperin
Departmento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (UBA). Intendente Güiraldes 2620 (1428) Buenos Aires, Argentina

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Abstract

Phenolic compounds were determined in methanolic extract from the algal mass of a *Nostoc muscorum* culture. Bioassays with two human pathogens, *Candida albicans* and *Staphylococcus aureus* indicated that algal phenolic compounds evoked significant growth inhibition for both species (89.1% and 88.2%, respectively). It is suggested that this strong inhibitory effect is of potential medicinal value.

Introduction

Microalgae, including cyanobacteria, produce a variety of compounds with biological activity, some of which are of potential medicinal value. The purpose of the present study was to isolate algal substances with an inhibitory effect on the growth of two human pathogens, *Staphylococcus aureus* and *Candida albicans*, and to determine the chemical nature of these substances.

Nostoc muscorum has previously been shown to have compounds with an inhibitory effect on those microorganisms and others of economic importance (Metting & Pyne, 1986; Cano *et al.* 1986; Caire *et al.* 1987).

Material and methods

An axenic exponential culture of *Nostoc muscorum* Ag. No. 79a growing in Watanabe medium (Halperin *et al.*, 1979) [O. D.620 nm 0.345 and 1.2 μg Chl a ml^{-1} culture, methanol K_{665}: 74.5 (Mackinney 1941)] with a final pH 6.7 was used for the extract preparation. The dry weight of the algal mass (24–26 h, 105 °C) was 1.04 mg ml^{-1} of culture. A methanolic extract from the algal mass was prepared with boiling 80% methanol in the ratio of solvent to fresh algal mass of 10 mg g^{-1} (Ribéreau-Gayon, 1972). Previously dried extract was resuspended in sterile destilled water in a ratio water-methanol = 1, and filtered through a nitrocellulose sterilizing membrane (0.22 μm porosity). The final concentration was 4 mg dry wt ml^{-1}.

In order to establish if the methanolic extract contained phenolic compounds, we used a chromatographic procedure followed by detection by fluorescence and colour development. Samples equivalent to 0.5 g of fresh wt (23.5 mg d. wt) were spotted on Whatman No. 3 MM paper and analyzed by two-dimensional descend-

Table 1. Effect of methanolic extract and phenolic eluted compounds on *Candida albicans* and *Staphylococcus aureus* growth. Mean values of optical density (O.D.).

	O.D. (620 nm)			Student *t*-test	
	MeOH extract	Phenolic compounds	Control	MeOH extract	Phenolic compounds
C. albicans	0.876[1]	0.079	0.725	5.39[2]	56.16[2]
S. aureus	0.475	0.057	0.490	0.33	11.69[2]

[1] Dilutions were made in order to obtain O.D. values.
[2] Highly significant.

ing chromatography (Ribéreau-Gayon 1972). The solvent systems employed were:

(1) n-butyl alcohol-glacial acetic acid-water (6 : 1 : 2), and
(2) acetic acid (2%).

Phenols were detected under U.V. light (366 nm) before and after fuming the papers with ammonia and characterized by their Rf values.

For determination of phenolic compounds, the papers were sprayed with the following reagents: (a) 1% aqueous $FeCl_3$; (b) Folin-Ciocaltue reagent in water (1 : 5) MK. Fluorescent spots were cut from non-sprayed papers, eluted with 80% methanol, transferred to distilled water, and sterilized through nitrocellulose membrane (0.22 μm porosity).

The effect on growth of *Staphylococcus aureus* and *Candida albicans* was determined by bioassayas. In each bioassay, 21 Erlenmeyer flasks (50 ml with 2.5 ml of Sabouraud or tryptone-soya (Oxoid) medium, respectively, were used. Seven replicates were used for each treatment:

1st treatment: methanolic extract 2.5 ml into each flask.
2nd treatment: phenolic compounds 2.5 ml into each flask.

For each assay seven control flasks were filled with 5 ml sterile distilled water. Each flask was inoculated with 0.5 ml *S. aureus* or *C. albicans* suspension (O.D.: 0.29 and 0.51, respectively) and shaken continuously at 22–26 °C. Growth was quantified by optical density 18 h after inoculation (Table 1). A Student *t*-test was applied to the results.

Results and conclusion

The colours of the spots detected under U.V. light before and after fuming the papers with ammonia (light and bright blue, respectively) and those obtained with the reagents ferric chloride (brown) and Folin-Ciocalteu (blue after ammonia) and the Rf 0.25 0.21 0.05 and 0.73 0.92 0.80 in systems 1 and 2 indicated the phenolic nature of these substances. These results agree with those of Metting and Pyne (1986).

Table 1 shows the effect of methanolic extract and phenolic cluted compounds on *S. aureus* and *C. albicans* growth. Algal methanolic extract had no significant effect on growth of *S. aureus*, while phenolic compounds exerted a bacterial inhibition of 88.2%. The promotion and inhibition percentages are shown in Table2. The inhibitory effect of phenolic compounds is neutralized in methanolic extract, probably by the presence of other substances, among which there may be a strong growth promotor.

We must consider the possibility of phenolic

Table 2. Promotion (+) and inhibition (−) of growth (%).

	MeOH extract	Phenolic compounds
C. albicans	+ 20.83	− 89.10
S. aureus	− 3.06	− 88.26

substanced being more active in the eluted compounds because of a change in their chemical configuration after chromatography, and there is also the possibility of them being in a higher concentration.

Previous studies have already established that *N. muscorum* produces substances which affect the growth of human phytopathogens: *S. aureus, Bacillus subtilis, C. albicans, Sclerotinia sclerotiorum* (Cano *et al.*, 1986; Cannell *et al.*, 1988). Other cyanobacteria also have an inhibitory effect on some of these pathogens (Caire *et al.*, 1974; Mulé *et al.*, 1976). Extracts of *N. muscorum* and other species also display activity on P388 lymphocytic mouse leukaemia (Mynderse *et al.*, 1977; Barchi *et al.*, 1983) and filtrates and extracts of *Nostoc* sp. inhibit α-glucosidase (Cannell *et al.*, 1987).

Our data demonstrate that the compounds eluted which show a strong inhibitory effect on the growth of the pathogens studied are phenolic and may have potential medicinal value.

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