IJSM International Journal of Secondary Metabolite

Int. J. Sec. Metabolite, Vol. 4: 3 (2017) pp. 16-19

Special Issue 1: Research note

ISSN: 2148-6905 online Journal homepage: http://www.ijate.net/index.php/ijsm

Effect of Salt and pH Stress of Bioactive Metabolite Production in *Geitlerinema carotinosum*

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Received: 02 May 2017 - Revised: 05 July 2017 - Accepted: 15 July 2017

Abstract: Cyanobacterial metabolites are natural products that have an important features in pharmaceutical and medicinal industries. In this study, the presence of the secondary metabolite norharmane in the indole structure was determined in *Geitlerinema carotinosum* (Geitler) Anagnostidis isolated from Tokat Yesilirmak River and its production in salt stress and pH stress was investigated. In salt stress experiments, cyanobacterium was cultured for two weeks by adding NaCl to BG11 medium in erlenmayers of 0.5, 1.0, 3.0, 5.0 M. pH stress was executed at 5 and 9. Norharmane amount was determined by HPLC using C18 reverse phase column at a temperature of 40 °C and a flow rate of 1 ml / min. The amount of norharman metabolite (µg/g) was calculated according to the Gauss method by drawing a calibration curve over the absorbance value of the standard 247 nm wavelength. According to the analysis results, metabolite production was 0.612, 1.299, 0.011 at 0.5 M, 1.0 M, 3.0 M respectively. At 5 M, there was no norharmane production. The norharmane production is higher at pH 5 (1.293 µg /g) than that of the pH 9 (0.448 µg /g).

Keywords: *Geitlerinema carotinosum*, stress conditions, norharmane, HPLC

1. INTRODUCTION

Cyanobacteria produce many important secondary metabolites which are promising compounds for drug discovery and development process. Among the cyanobacteria, *Spirulina* Turpin ex Gomont, *Anabaena* Bory ex Bornet & Flahault, *Nostoc* Vaucher ex Bornet & Flahault and *Oscillatoria* Vaucher ex Gomont contain a large variety of substantial secondary metabolites [1] which reveal the toxins, antitumor, antifungal, antiinflammatory, siderophores, phytohormones, photoprotective effects and protease inhibitors [2]. Some of these secondary metabolites have been produced from cyanobacterial biomass extraction with solvents (intracellular). In addition, cyanobacteria are able to secrete various organic compounds in their environment as exo-metabolites (extracellular) [3]. Norharmane (9H-pyrido (3,4-b) indole) (Figure 1) is an exo-metabolite that can be produced in some cyanobacteria species and is released into the growth environment [4]. Due to the importance of norharmane for

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ISSN: 2148-6905 online /© 2017 DOI: 10.21448/ijsm.356247

pharmaceutically and biologically, we investigated the production of norharmane in salt and pH stress conditions on *G. carotinosum*.

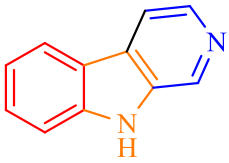

Figure 1. Chemical structure of norharmane.

2. MATERIAL and METHODS

2.1. Collection and growth conditions of *G. carotinosum*

Cyanobacterium was collected from Yesilirmak river, Tokat, Turkey (49° 17.40' 19" N, 36° 23' 4.69" E). *G. carotinosum* was isolated by micro piped and micro injector under the inverted microscope. Then it was streaked onto agarised Blue Green Algae (BG-11) medium *G. carotinosum* was grown under controlled conditions at 26 ± 2 °C with white fluorescent lamp of 2465 lux [5].

2.2. Salt and pH experiments

In salt stress experiments, cyanobacterium was cultured for two weeks by adding NaCl to BG11 medium in erlenmayers the concentration of 0.5, 1.0, 3.0, 5.0 M. pH stress was executed at 5 and 9. The control cultures were kept in the BG11 medium without sodium chloride (pH 7). Each inoculation was carried out by adding from 25 ml of stock culture at logarithmic phase of growth to 250 ml erlenmayer [6].

2.3. Biomass

Culture samples (14 ml) were centrifuged at 4000 g for 15 min. The pellets were washed twice with distilled water (pH 4), dried at 60 °C for 6 h, and weighed with a precision balance [7].

2.4. Statistical analysis

The statistical analysis were analysed by ANOVA and using the SPSS software (SPSS Inc., version 20). Tests of significance were carried out using Duncan's multiple range tests.

3. RESULTS and DISCUSSIONS

3.1. Morphological analyses

G. carotinosum was identified under the light microscope with a micrometer. The trichomes are smooth or slightly wrapped. Fill it up towards the ends. The cells were arranged in bundles in the form of fascicles. Cells are 1.5-3 µm wide; It is 3-9 µm long [9, 10].

3.2. Effects of salt stress on the growth and production of norharmane

When the stress conditions were applied to *G. carotinosum*, the control cell biomass was 0.260 g/l. While the salt concentrations increased, biomass of cells decreased. Therefore, this cyanobacterium was not halotolerant. In 1.0 M salt stress, the amount of norharmane was observed to have been 1.299 µg / g, which was more than the control. On the other hand, norharmane production decreased to 0.011 µg/g at 3.0 M concentration. Norharmane secretion was not took place at 5.0 M. As a consequence, the optimum norharmane production was observed at 1.0 M concentration (Table 1).

Table 1. Biomass and norharmane production under salt stress

Salt (M)	Biomass (g l^{-1})	Norharmane (µg g^{-1})
Control	0.260 ± 0.000^{e}	0.830 ± 0.000^{d}
0.5 M	0.248 ± 0.003^{d}	0.612 ± 0.007^{c}
1.0 M	0.106 ± 0.005^{c}	1.299 ± 0.005^{e}
3.0 M	0.052 ± 0.005^{b}	0.011 ± 0.004^{d}
5.0 M	0.023 ± 0.002^{a}	0.000 ± 0.000^{a}

Values are means ± Standard deviation (n= 3)
Means followed by different letters are significantly different at $p < 0.001$

3.3. Effects of pH stress on the growth and production of norharmane

At pH 5 and 9, the biomass of cells was lower than that of the control cells. It was found out that the acidic and basic media were not suitable for growth. The Natural medium (pH 7) has been determined the best growth condition of *G. carotinosum*. Hovewer, the production of norharmane at pH 5 was 1.293 µg/g, which is better than that of pH 7 and 9 (Table 2).

Table 2. Biomass and norharmane production under pH stres

pH	**Biomass (g l^{-1})**	**Total norharmane (µg g^{-1})**
5	0.068 ± 0.002^{a}	1.293 ± 0.006^{c}
9	0.115 ± 0.007^{b}	0.448 ± 0.002^{a}
Control	0.260 ± 0.000^{c}	0.830 ± 0.00^{b}

Values are means ± Standard deviation (n= 3)
Means followed by different letters are significantly different at $p < 0.001$

The needs for the survive of cyanobacteria are mainly water, light, carbon dioxide and simple inorganic compounds [11]. In addition, cyanobacteria can grow rapidly under certain environmental conditions [12]. Cyanobacteria can adapt to a wide range of environmental factors. For example, *Synechococcus* sp., *Microcystis* sp., *Arthrospira* sp. are thermotolerant, alkalitolerant, halotorant respectively [13].

Cyanobacteria contain numerous pharmacologically important secondary metabolites. Metabolites retained within the cell may not be released unless cell integrity is impaired. However, they become free under stress conditions or when the cells are disintegrated [14].

The secretion of significant compounds by algae caused by the protection of algal cells against stressful conditions like ultraviolet radiation, temperature change, fluctuation in nutrient and salinity level [15]. In this study, cyanobacterium *G. carotinosum* was exposed to salt and pH stress, resulted in higher norhamane production than controls.

4. CONCLUSION

Cyanobacteria contain numerous pharmacologically important secondary metabolites. *G.carotinosum* could be an important source of norharmane which is valuabe compound for pharmaceutical.

Conflict of Interests

Authors declare that there is no conflict of interests.

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