Mycobiology 38(3) : 176-179 (2010)
DOI:10.4489/MYCO.2010.38.3.176


# Plant Hormones Promote Growth in Lichen-Forming Fungi

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(Received May 4, 2010. Accepted May 11, 2010)

**The effect of plant hormones on the growth of lichen-forming fungi (LFF) was evaluated. The use of 2,3,5-triiodobenzoic acid and indole-3-butyric acid resulted in a 99% and 57% increase in dry weight of the lichen-forming fungus *Nephromopsis ornata*. The results suggest that some plant hormones can be used as inducers or stimulators of LFF growth for large-scale culture.**

**KEYWORDS : Fungal growth, Indole-3-butyric acid, Lichen-forming fungi, *Nephromopsis ornata*, 2,3,5-Triiodobenzoic acid**

Lichens are symbionts of fungi (mycobiont) and algae (photobiont). They comprise various unrelated groups of fungi, mostly ascomycetes, that share a dependence on green algae and/or cyanobacteria [1]. Lichens produce characteristic secondary metabolites, called lichen substances, which seldom occur in other organisms. Lichen substances have therapeutic effects for several spectrums of biological activity [2, 3], and lichen-forming fungi (LFF) are a potential resource for several secondary metabolites [4]. But, LFF are neglected by mycologists and overlooked by the pharmaceutical industry because of their slow growth in nature and difficulties of artificial cultivation. However, large-scale LFF culture can overcome the disadvantage of natural lichen extracts to industrialize their metabolites due to much faster growth and larger metabolite production than natural thalli [5, 6]. But even under optimum culture conditions, the LFF growth rate is still much slower than other filamentous fungi. Therefore, there is a strong need to understand LFF growth promoting or stimulating agents in culture systems. Plant hormones are involved in different stages of plant growth and development. However, earlier reports regarding the effect of plant hormones on microbial growth are conflicting [7]. Moreover, only a few reports are available regarding the relationship between plant hormones and lichens [6, 8, 9], but the production of secondary fungal metabolites could be affected by plant hormones [10]. However, the effect of plant hormones on LFF growth to stimulate their growth in aposymbiotic culture has seldom been studied. In this study, we evaluated the influence of several plant hormones on LFF growth.

## Materials and Methods

Three LFF, including *Nephromopsis ornata* (041359, fast growing), *Myelochroa irrugans* (040608, moderately growing), and *Usnea longissima* (CH050154, slow growing) were obtained from the Korean Lichen and Allied Bioresource Bank (http://www.lichen.re.kr). LFF isolation and confirmation were described previously [11]. The fungi were cultured on malt-yeast extract (MY) medium at 15°C for 1 mon before the experiment.

Four major classes of plant hormones including abscisic acid, auxin, cytokinin, and gibberellin were examined [12]. Fourteen plant hormones were selected from each class to screen for enhanced LFF growth (Table 1). For preliminary hormone screening, the LFF were cultured on MY solid medium for 80 days at 15°C after adding the hormones at 1 μM/L. Four hormones including indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), jasmonic acid (JA), and 2,3,5-triiodobenzoic acid (TIBA) enhanced fungal growth and were used for further examinations.

*N. ornata* fungal masses (500 mg DW) were aseptically ground and suspended in 15 mL of distilled water. The suspension (0.1 mL) was inoculated into 100 mL of MY liquid media adjusted with 1, 2, 5, 10 and 50 μM/L of IAA, IBA, JA, and TIBA. The cultures were incubated in a shaking incubator at 150 rpm for 80 days at 15°C, and the dried weights of fungal biomass were measured. Five replicates were used for the experiment.

## Results

No clear relationship was observed between the fungal growth response and plant hormone class (Table 1). Among the 14 hormones tested, only IAA, IBA, JA, and TIBA promoted fungal growth of the LFF at 1 μM/L. IBA, JA, and TIBA stimulated fungal growth of the fast growing *N. ornata*. IBA induced fungal growth in all three LFF. IAA only induced growth in *M. irrugans.* In contrast, 2,4,5-trichlorophenoxyacetic acid inhibited the

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**Table 1.** Effects of several plant hormones on lichen-forming fungal growth in malt-yeast extract (MY) solid medium

| Hormone classes | Hormone names | Lichen-forming fungi | | |
|---|---|---|---|---|
| | | *Nephromopsis ornata* | *Myelochroa irrugans* | *Usnea longissima* |
| Auxin | Indole-3-acetic acid | N | + | N |
| | 1-Naphthaleneacetic acid | N | N | N |
| | Indole-3-butyric acid | + | + | + |
| | 2,4-Dichlorophenoxyacetic acid | N | N | N |
| | 2,4,5-Trichlorophenoxyacetic acid | – | – | – |
| Abscisic acid | Abscisic acid | N | N | N |
| | Jasmonic acid | + | N | N |
| | Chlorocholine chloride | N | N | N |
| | 2,3,5-Triiodobenzoic acid | + | N | N |
| | Indole-3-propionic acid | N | N | N |
| | 4-Chlorophenoxyacetic acid | N | N | N |
| Cytokine | Kinetin | N | – | – |
| | Zeatin | N | N | – |
| Gibberellin | Gibberellic acid | N | N | N |

+, growth promotion; –, growth inhibition; N, no effect on fungal growth.
The lichen-forming fungi were cultured on MY solid medium containing each hormone (1 μM/L) for 80 days at 15°C.

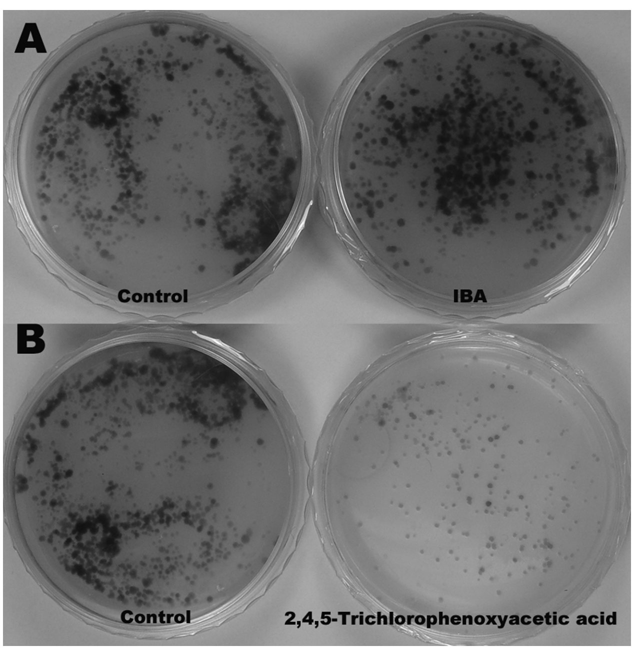


**Fig. 1.** Effects of plant hormones on lichen-forming fungal growth of *Nephromopsis ornata* grown in malt-yeast extract solid medium. The fungus was cultured in the dark at 15°C for 80 days. A, Growth promoting effect of indole-3-butyric acid (IBA, 1 μM/L); B, Growth inhibiting effect of 2,4,5-trichlorophenoxyacetic acid (1 μM/L).

fungal growth of all three LFF (Fig. 1).

For further clarification of the hormone effect, the four hormones were tested at different concentrations with *N. ornata* in liquid medium (Table 2). It was evident that TIBA induced the most stimulating effect on fungal growth of *N. ornata* at 2 μM/L, similar to the preliminary screening test; the dry weight increased almost two times that of the control at that concentration. Furthermore, a much smaller and finer spherical form of the fungal mass and much darker yellow color of the culture suspension developed in the tissue treated with hormones than the control (Fig. 2). This finding clearly demonstrated that TIBA influenced not only fungal growth rate but also morphological development and metabolite production in the LFF. However, the *N. ornata* growth rate decreased with an increase in TIBA concentration. The fungus showed a similar growth response to IBA as that of TIBA. Although a higher fungal growth rate was found for the 1 μM/L JA treatment, as in the preliminary results, no consistent response of fungal growth to the hormone at the tested concentrations was observed. The growth response of the fungus to IAA seemed somewhat different from that of the other hormones; IAA severely inhibited fungal growth, resulting in a fatal effect at concentrations of 10 and 50 μM/L.

## Discussion

Because lichens are very slow-growing symbiotic associations, there are probably delicate signals and mechanisms controlling reciprocal growth of the symbiotic partners. Endogenous auxin production has been reported in *Ramalina duriaei* lichen [9]. However, the authors could not distinguish whether the hormones were synthesized by the symbiotic algae or the fungal symbiont. Some studies have reported auxin production in algae [13, 14] and fungi [15, 16]. Therefore, it is critical to identify the source of plant hormone production in lichens for a better understanding of the growth regulating activity of plant hormones on aposymbiotically grown LFF. Recently, Ayhan

**Table 2.** Effects of plant hormones on lichen-forming fungal growth of *Nephromopsis ornata* in malt-yeast extract liquid medium

| Hormones | Dry weight (mg) | | | | |
|---|---|---|---|---|---|
| | 1 μM/L | 2 μM/L | 5 μM/L | 10 μM/L | 50 μM/L |
| IAA | 16.0 ± 1.2 | 14.2 ± 1.4 | 10.4 ± 1.5 | Died | Died |
| IBA | 15.6 ± 3.4 | 21.5 ± 3.6 | 13.8 ± 1.4 | 15.6 ± 2.7 | 10.0 ± 2.7 |
| JA | 17.5 ± 1.4 | 11.4 ± 2.5 | 13.5 ± 3.3 | 17.3 ± 2.3 | 15.0 ± 3.5 |
| TIBA | 23.4 ± 4.3 | 27.3 ± 2.2 | 19.4 ± 2.1 | 16.5 ± 1.5 | 12.0 ± 3.2 |
| Control | | | 13.7 ± 4.6 | | |

Data are means ± standard deviation of five replicates.
IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; JA, jasmonic acid; TIBA, 2,3,5-triiodobenzoic acid.

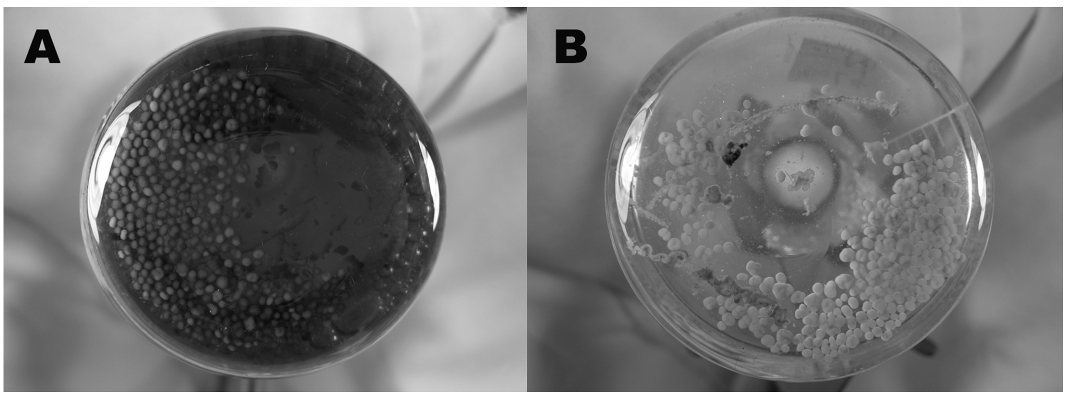


**Fig. 2.** Effects of 2,3,5-triiodobenzoic acid (TIBA) on the fungal growth and morphology of *Nephromopsis ornata* in malt-yeast extract liquid medium. The fungus was cultured in a shaking incubator (150 rpm) in the dark at 15°C for 80 days. A, *N. ornata* growing in TIBA-containing medium (2 μM/L); B, *N. ornata* growing in hormone-free medium (control). TIBA stimulated fungal growth and also induced a smaller and spherical form of the fungal mass and a much darker yellow pigment in the liquid culture than the control.

[8] found that abscisic acid (ABA) is a signal responsible for slow growth in lichens, and that ABA levels change with algal density. Because cell turnover in a photobiont is strictly controlled by the mycobiont [17], it might be possible that the mycobionts produces ABA, which controls symbiotic algal growth during differentiation of the lichen thallus.

Stimulatory as well as inhibitory effects of plant growth hormones on microbial growth have been reported [18-22]. Auxin stimulates microbial growth [18, 22] and might control fungal cell elongation, as in higher plants [23, 24]. Consistent with earlier reports, IBA showed a growth promoting effect in the LFF of the present study. The growth inhibiting effect of IAA on the LFF was probably due to the high concentrations used in this study, as shown by Gryndler *et al.* [25] who demonstrated a perceptible decrease in the proliferation of hyphae from the arbuscular mycorrhizal fungus *Glomus fistulosum* at 3 μM IAA.

TIBA induced the most stimulating effect on *N. ornata* fungal growth in this study. As proposed by Ayhan [8], ABA might act as a suppressive regulator to control high algal density in the symbiotic association. If this is true, particular levels of TIBA might act on the fungus as a stimulating regulator to promote fungal growth, as we found in the present study.

Yamamoto *et al*. [6] reported that 1-naphthaleneacetic acid, IAA, ABA, gibberellic acid cinnamic acid, and 2-(p-chlorophenoxy) isobutyric acid, did not promote aposymbiotic growth or usnic acid production of LFF in *Usnea rubescens* at $10^{-3}$ to $10^{-9}$ M. In contrast, IBA and TIBA tested in the present study stimulated *N. ornata* growth to different extents depending on the concentrations used. The beneficial effect of the two hormones on fungal growth was only detectable at concentrations less than 2 μM/L. Furthermore, a much dark yellow color developed in the fungal mass that received the 2 μM/L TIBA treatment. Because this concentration is lower than the concentration common in plant tissue *in vivo* [25] and intact lichen thalli [9], the stimulation may be biologically significant. It is concluded that some plant hormones enhance biomass production and possibly influence secondary metabolites production of aposymbiotically grown LFF. It will be of biological interest to identify which symbiotic partner synthesizes the plant hormones and how the plant hormones regulate aposymbiotic growth of LFF in relation to lichen substance production at the molecular level.

## Acknowledgements

This work was supported by a Korea Research Foundation Grant (MOEHRD, Basic Research Promotion Fund) (KRF-2007-313-C00669) and also by the Korea National Research Resource Center Program (Grant 2010-0000660), Korea.

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