Note

Identification of Jasmonic Acid from *Euglena gracilis* Z as a Plant Growth Regulator

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A green algae, *Euglena* is classified into the *Euglenophyta* division, and widely distributed in freshwater pools, often in such abundance as to form water blooms. *Euglena* has chloroplasts containing chlorophyll a and b as well as carotenes and xanthophylls.¹⁾ Moreover, because of the presence of a β -1:3-linked glucan called paramylon, *Euglena* has recently been considered to be a new type of food resource.²⁾

Though the growth and development of algae appears to be regulated by growth regulators like plant hormones in higher plants,³⁾ it has not been clear what kind of growth regulator is involved in the growth and development of *Euglena gracilis* Z. In this paper we report the successful identification of jasmonic acid in *Euglena gracilis* Z, which is recognized as a very important growth regulator of higher plants.

Euglena gracilis Z was cultured in the light of 4,500 lux at 30°C in modified Koren–Hutner's medium, as shown in Table I. At the end of the culture 4–5 days after inoculation, *Euglena gracilis* Z cells were collected by centrifugation, washed with water, and then lyophilized. Lyophilized materials were stored at 5°C before use. This material was extracted with 9 l of 70% aqueous ethanol two times at 4°C. Alcoholic extracts were combined and concentrated *in vacuo* to give an aqueous residue followed by partitioning with ethyl acetate. After partitioning with ethyl acetate at pH 7, the aqueous phase was adjusted to pH 3 with HCl and then partitioned with ethyl acetate in the usual way, giving an ethyl acetate soluble-active material of 3.82 g that showed the growth inhibitory effects in a bioassay that was done using the water culture method of Tan-ginbozu rice seedlings. This active material was dissolved in a small amount of *n*-hexane, put onto a silicic acid partition column (Wako gel C-100, 150 g; 2.5 × 35 cm)

impregnated with 0.5 M aqueous formic acid (94 ml), and then eluted with a mixed solvent of *n*-hexane–ethyl acetate saturated with 0.5 M formic acid. The growth inhibitory effect was eluted with the 1% ethyl acetate fraction (1.57 g). About a tenth of this eluate (150 mg) was further purified with preparative thin-layer chromatography (silica gel PF₂₅₄ Merck & Co., Inc., 0.25 mm thick) developed with *n*-hexane–ethyl acetate–acetic acid (10:2:1, v/v/v). The zone of *R_f* 0.27–0.38 causing slight growth inhibition of rice seedlings was scraped off, eluted with ethyl acetate, and then dried over anhydrous Na₂SO₄ to give an active material. From the results of its chromatographic behavior and biological activity, it was thought to be jasmonic acid. After methylation using ethereal diazomethane, this material was studied by GC/SIM (JEOL DX-300 mass spectrometer combined with a gas chromatograph, 160°C isothermal, 70 eV, 300 μ A). A DB-1 fused silica capillary column (0.254 mm × 30 m) was used. The carrier gas was He, and the split ratio was 12:1. Two prominent fragments, *m/z* 151 and 224, which are characteristic of methyl jasmonate, were monitored. These fragments were observed at 7.53 min, which is the same retention time as that of an authentic methyl jasmonate. The peak height ratio was also the same as that of an authentic sample (Fig. 1). On the basis of the above evidence, jasmonic acid (1) was identified as an active material in this fraction. The absolute configuration of jasmonic acid from *E. gracilis* in this study is not clear. It has been known, however, that naturally occurring jasmonic acid has the *cis* configuration, and about 90% of the *cis* form has been easily converted to the *trans* form during the isolation process.⁴⁾ The content

Table I. THE CULTURE MEDIUM OF *Euglena gracilis* Z USED IN THIS STUDY

Compound	Concentration
(Macro nutrients)	(g/l)
Glucose	10.0
(NH ₄) ₂ SO ₄	2.0
KH ₂ PO ₄	0.25
MgCO ₃	0.6
CaCO ₃	0.12
(Micro nutrients)	(mg/l)
EDTA 2Na	50.0
FeSO ₄ (NH ₄) ₂ SO ₄ ·6H ₂ O	50.0
MnSO ₄ ·H ₂ O	18.0
ZnSO ₄ ·7H ₂ O	25.0
(NH ₄)MoO ₇ O ₂₄ ·4H ₂ O	4.0
CuSO ₄	1.2
NH ₄ VO ₃	0.5
CoSO ₄ ·7H ₂ O	0.5
H ₃ BO ₃	0.6
NiSO ₄ ·6H ₂ O	0.5
Vitamin B ₁	2.5
Vitamin B ₁₂	0.005

The pH of the medium is adjusted to 4.0.

Fig. 1. GC/SIM Profile of an Authentic Sample of Methyl Jasmonate (Upper) and Methyl Ester Form of an Unknown Material in Jasmonic Acid Fraction of *Euglena gracilis* Z. (Lower).

of jasmonic acid was estimated to be 1.46 ng/g dry weight. Jasmonic acid at the concentration of 1 μ M was effective in inhibiting the growth of rice seedlings to about 87%. Therefore, the inhibitory effect of this fraction was partially due to that of jasmonic acid.

Jasmonic acid was identified in the green algae of *Euglena gracilis* Z in this study. This is the first report confirming the occurrence of jasmonic acid in green algae using physicochemical instruments. On the other hand, methyl jasmonate (**2**) which is the methyl ester form of jasmonic acid was not found in the ethyl acetate soluble neutral fraction of *Euglena gracilis* Z even using GC/SIM analysis. Recently, Vick and Zimmerman have reported that the enzyme systems in relation to the biosynthetic pathway of jasmonic acid from linolenic acid could be detected in *Chlorella pyrenoidosa* cells, but jasmonic acid and its related compounds were not in *Chlorella pyrenoidosa*.⁵⁾

In our preliminary studies, however, we could detect jasmonic acid and methyl jasmonate in an identified *Chlorella* sp., and in which the level of these compounds were almost equivalent to that of *Euglena gracilis* Z. Furthermore, jasmonic acid, but not methyl jasmonate, was also found in *Spirulina maxima* (a procaryote) cultured in the open air (unpublished data).

Interestingly, it has been reported that ABA has not been detected in liverworts and algae, and that the dihydrohydrangeic acid termed lunularic acid is contained in all liverworts, and all fresh-water and marine algae.⁶⁾ In this study, we could not detect ABA or lunularic acid both in *Euglena gracilis* Z cells and in its culture medium using the Tan-ginbozu bioassay system.

Since jasmonic acid is really found as a plant growth regulator in *Euglena gracilis* Z, this compound may be important for the growth and development of algae as well as higher plants. Though green algae are distantly removed from higher plants in the evolutionary scale, the biosynthetic pathway of jasmonic acid is well conserved. Considering the results of this and other studies,⁷⁾ jasmonic acid and its related compounds are of universal occurrence in the plant kingdom.

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