Bot. Bull. Academia Sinica 25: 81-86 (1984)

ETHYLENE PRODUCTION BY BLUE-GREEN ALGAE[1]

TAN-CHI HUANG and TE-JIN CHOW

Institute of Botany, Academia Sinica
Nankang, Taipei, Taiwan, Republic of China

(Received September 15, 1983; Accepted November 28, 1983.)

Abstract

Axenic cultures of blue-green algae including the unicellular, filamentous and branched types were assayed for ethylene production. A branched form identified as strain of *Hapalosiphon* produced ethylene either cultivated on synthetic media or on the surface of soil. Properties of this blue-green alga and the environmental factors affecting the ethylene production were described.

Introduction

Ethylene has been known to be able to regulate plant growth and development (Abeles, 1973). Occurence of ethylene in anaerobic soils or in soil water at the concentration capable of causing appreciable damage to cereals has been reported (Smith and Russel, 1969; Smith and Robertson, 1971). Some soil-born bacteria and fungi have been demonstrated to produce ethylene (Dasilva *et al.*, 1974; Ilag and Curtis, 1968; Lynch, 1972; Primrose and Dilworth, 1976). Blue-green algae (BGA) could occur abundantly on the soil surface, especially when the soil was wetted or flooded. The significance of BGA to the agriculture was not fully understood. However, to produce growth factors for higher plants could be one of the important roles (Shukla and Gupta, 1967; Singh and Trehan, 1973; Venkataraman and Neelakanthan, 1967). In this report, axenic cultures of BGA including the unicellular, the filamentous, and the branched types were assayed for the ethylene production. The strain which could produce ethylene was then selected and characterized.

Materials and Methods

Source and Cultivation of the Blue-Green Algae

Axenic cultures of blue-green algae (BGA) tested in this study were isolated

[1] Paper No. 269 of the Scientific Journal Series, Institute of Botany, Academia Sinica, Taipei, Taiwan, Republic of China.

and purified in this laboratory. The unicellular BGA were isolated from coastal water, the others were from the local rice fields. For the assay of ethylene production, the unicellular BGA were cultivated on solid BG-11 medium (Allen, 1968); the filamentous and branched BGA were on the nitrogen-free SM medium (Huang, 1982). They were incubated under about 6,000 lux of continuous cool white fluorescent light at 26°C.

Identification of Ethylene

Ethylene was identified by gas chromatography and mercuric perchlorate aborption (Young *et al.*, 1951). For gas chromatography, a Shimadzu model GC-3BF equipped with FID was used. The range was set at 0.04 V and the sensitivity at 10^3 MΩ. Authentic ethylene from Merck was used as standard. For the experiment of ethylene absorption, a small glass tube (0.8×5.0 cm) containing 1 ml of mercuric perchlorate solution prepared by mixing 0.2 M HgO and 2 M $HClO_4$ was kept in a 50 ml flask. Ethylene-producing BGA was then inoculated in the flask. The flask was then sealed with serum rubber stopper and then incubated. A flask containing BGA but no mercuric perchlorate was used as control. After 24 hours of incubation, the ethylene concentrations presented in the gas phase of both flasks were assayed by gas chromatography. After the assay, the mercuric perchlorate solution was transferred to a test tube (2×11 cm) for testing the absorbed ethylene. The test tube was sealed with serum rubber stopper, and one ml of 6 N HCl was injected into the test tube with a syringe for the release of ethylene. The amount of ethylene released was then estimated by gas chromatography.

Quantitative Determination of Ethylene Production

A pre-weighed and sterilized filter paper (glass microfibre, 9.0 cm, Whatman) was put on SM agar plate. BGA were evenly spreaded on the filter paper and inoculated. After the growth of BGA, the algae along with the paper were transferred to the desired fresh medium contained in a specially designed petri dish. The cover and the bottom valves of the petri dish had the same diameter, with the cover having an opening located at the center. After the BGA was transferred to it, both valves were fitted together and then wrapped outside with plastic tape (810 magic transparent tape, Scotch), and the opening was sealed with serum rubber stopper. The culture was incubated and the gas samples were withdrawn at different intervals for the assay of ethylene content. After the assay, the paper along with the BGA were dried and re-weighed. The weight increased due to the presence of BGA was estimated and taken as the dry weight of BGA. The activity of ethylene production was expressed as nanomoles of ethylene produced per mg dry weight of BGA per day.

Chemicals

1-Aminocyclopropane-1-carboxylic acid (ACC) was bought from Sigma. We checked different orders of the commercial ACC, and found that the ACC might decomposed slowly to form ethylene and other unknown gaseous products under axenic condition sterilized by millipore filtration. So the stability of the commercial ACC was checked before used in each experiment.

Results and Discussion

Axenic cultures of blue-green algae (BGA), including three strains of *Synechococcus*, fourteen of *Anabaena*, ten of *Nostoc*, and one of *Cylindrospermum*, *Calothrix*, *Scytonema*, and *Hapalosiphon*, respectively, were assayed for the ethylene production. Among them, a true branched form identified as a strain of *Hapalosiphon* produced ethylene in a rate which could be easily detected by gas chromatograph. The ethylene-producing strain was assigned as *Hapalosiphon* 101 (Fig. 1). It has nitrogen-fixing activity. The alga was found to occur frequently on the wetted soil surface. Its cells arranged in one row and the heterocyst located intercalary. Some of the branches might extrude into the air, and some immersed inside

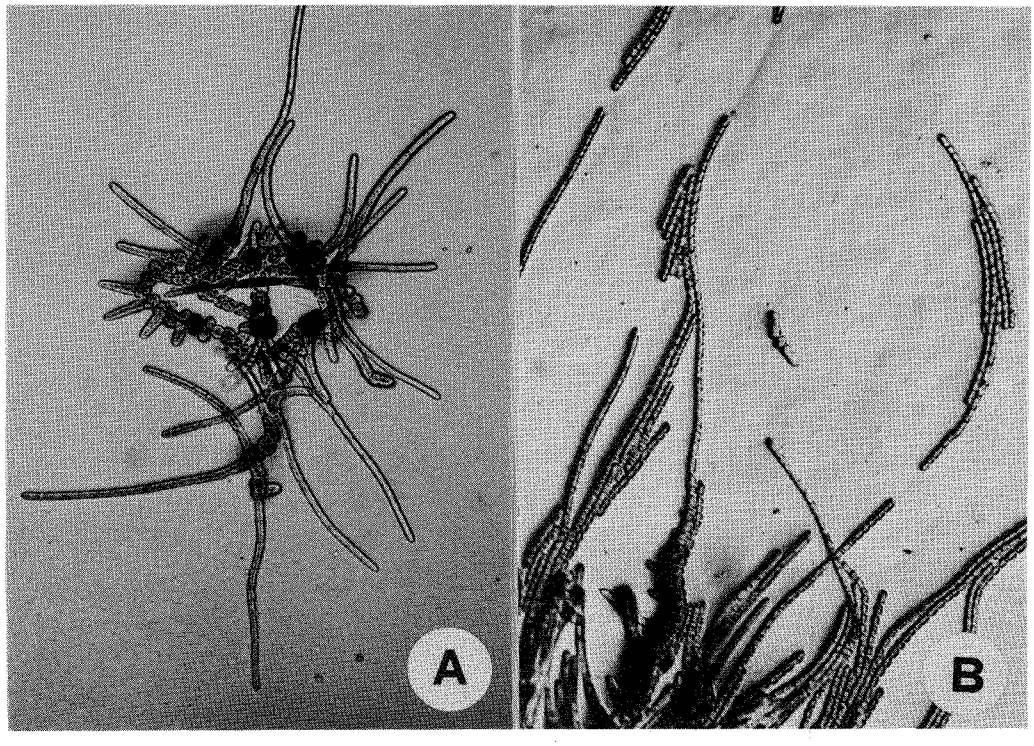

Fig. 1. Photograph of the *Hapalosiphon* 101. (A): At the early developing stage; (B): At the late stage. ×250

Table 1. *Ethylene production by* ***Hapalosiphon*** *101 cultivated on different media under about 6,000 lux of white fluorescent light at 26°C*

Media	Ethylene production (nmole/mg d. w./day)
Nitrogen-free basal medium (SM)	0.16
SM+Glucose (1%)	0.33
SM+Sodium acetate (0.1%)	0.21
SM+Sodium succinate (0.1%)	0.18
SM+Methionine (0.01%)	0.47
SM+Serine (0.01%)	0.16

the agar when cultivated on the solid medium. It formed hormogonia from the side branches as the thallus was well developed. The hormogonia could break into short fragments which glided very slowly on the agar.

Hapalosiphon 101 produced ethylene either growing on synthetic medium or on the surface of soil. It is evident that glucose and methionine are important substrates for the ethylene production of fungi and bacteria (Dasilva *et al.*, 1974; Lynch, 1972; Primrose and Dilworth, 1976). For the BGA, the activity of ethylene

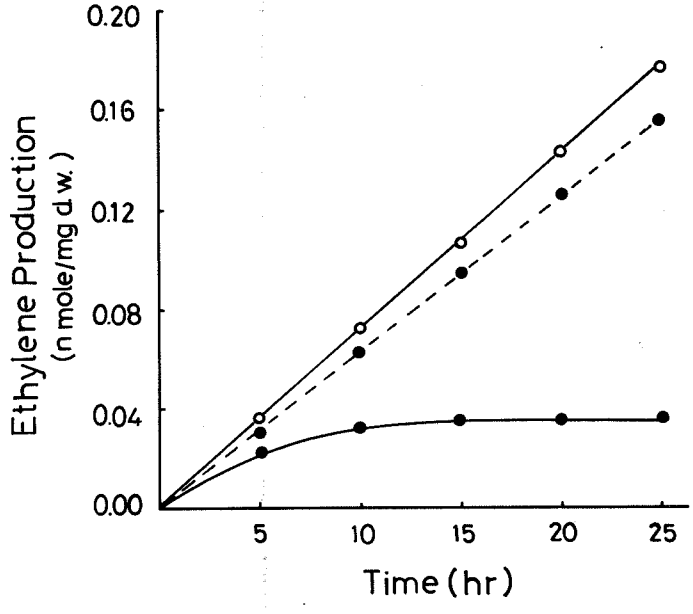

Fig. 2. Ethylene production of *Hapalosiphon* 101 grown under illuminating condition (○—○), in darkness (●—●), or under dark conditions supplemented with glucose (●----●).

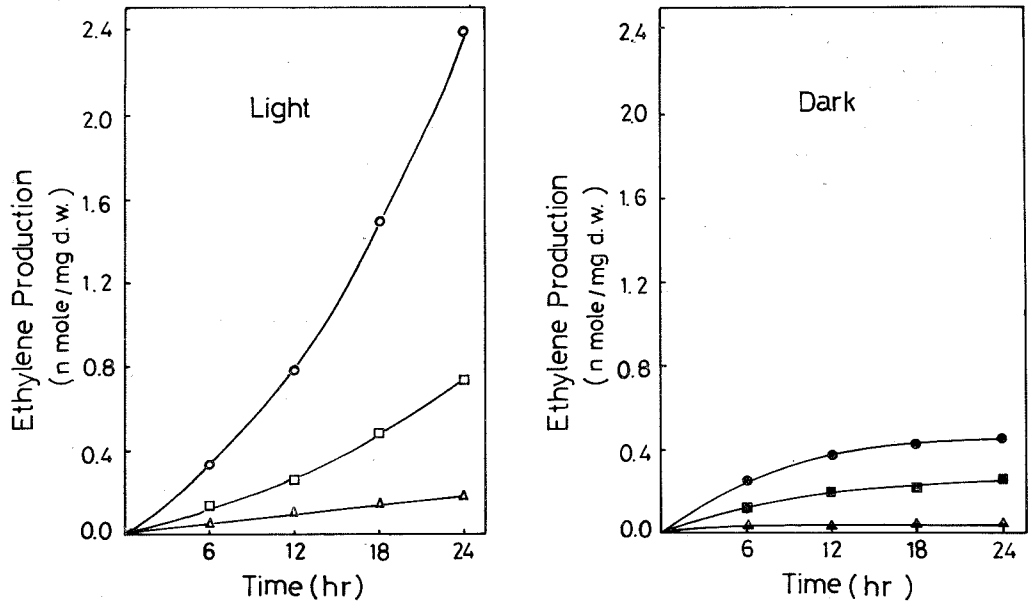

Fig. 3. Effect of ACC on the ethylene production of *Hapalosiphon* 101. The circles (○—○ or ●—●) indicate 1 mg/ml; the squares (□—□ or ■—■) represent 0.1 mg/ml; the triangles (△—△ or ▲—▲) indicate the control experiments.

production was enhanced to about two to three times in the presence of glucose or methionine (Table 1). This enhancing effect could not be replaced by serine, sodium acetate or sodium succinate. The rate of ethylene production remained constant under illuminating condition. The activity decreased gradually and then completely lost when inoculated under dark environment (Fig. 2). But in the presence of glucose, the BGA could produce ethylene continuously under dark condition.

There is no commonly accepted mechanism for the ethylene biosynthesis in microorganisms (Lynch, 1974). Methionine has been shown to be the precursor of ethylene in a number of higher plants (Yang, 1974), and 1-aminocyclopropane-1-carboxylic acid (ACC) has been identified as the intermediate in the conversion of methionine to ethylene (Adams and Yang, 1979). Since the ethylene production by *Hapalosiphon* 101 was enhanced in the presence of methionine (Table 1), the effect of ACC on ethylene production by BGA was tested. As shown in Fig. 3, the ethylene production by *Hapalosiphon* 101 increased significantly when the ACC was added. This result suggests that methionine can be used by the BGA as the precursor for the ethylene biosynthesis.

Literature Cited

Abeles, F.B. 1973. Ethylene in Plant Biology. Academic Press, New York, pp. 103-152.
Adams, D.O. and S.F. Yang. 1979. Ethylene biosynthesis: Identification of 1-aminocyclopropane-

1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc. Natl. Acad. Sci. USA **76**: 170-174.

Allen, M.M. 1968. Simple conditions for growth of unicellular blue-green algae on plates. J. Phycol. **4**: 1-3.

Dasilva, E.J., E. Henriksson, and L.E. Henriksson. 1974. Ethylene production by fungi. Plant Sci. Lett. **2**: 63-66.

Huang, T.C. and H.Y. Wu. 1982. Predation of amoebae on the filamentous blue-green algae. Bot. Bull. Academia Sinica **23**: 63-70.

Ilag, L. and W. Curtis. 1968. Production of ethylene by fungi. Science **159**: 1357-1358.

Lynch, J.M. 1972. Identification of substrates and isolation of microorganisms responsible for ethylene production in the soil. Nature **240**: 45-46.

Lynch, J.M. 1974. Mode of ethylene formation by ***Mucor heiemalis***. J. Gen. Microbiol. **83**: 407-411.

Primrose, S.B. and M.J. Dilworth. 1976. Ethylene production by bacteria. J. Gen. Microbiol. **93**: 177-181.

Shukla, A.C. and A.B. Gupta. 1967. Influence of algal growth promoting substances on growth, yield and protein content of rice plants. Nature **213**: 744.

Singh, V.P. and T. Trehan. 1973. Effects of extracellular products of ***Aulosira fertilissima*** on the growth of rice seedlings. Plant and Soil **38**: 457-464.

Smith, K.A. and R.S. Russel. 1969. Occurrence of ethylene, and its significance, in anaerobic soil. Nature **222**: 769.

Smith, K.A. and P.D. Robertson. 1971. Effect of ethylene on root extension of cereals. Nature **234**: 148-149.

Venkataraman, G.S. and S. Neelakanthan. 1967. Effect of cellular constituents on the nitrogen fixing blue-green alga ***Cylindrospermum musicola*** on the root growth of the rice seedlings. J. Gen. Appl. Microbiol. **13**: 53-61.

Yang, S.F. 1974. The biochemistry of ethylene: biogenesis and metabolism. ***In*** V.C. Runeckles, E. Soundheimer, and D.C. Walton (eds.), The Chemistry of Plant Hormones. Recent Advances in Phytochemistry, Vol. 7, Academic Press, New York, pp. 131-164.

Young, R.E., H.K. Pratt, and J.B. Biale. 1951. Identification of ethylene as a volatile product of the fungus ***Penicillium digitatum***. Plant Physiol. **26**: 304-310.

藍綠藻產生乙烯之探討

黃檀溪 周德珍

中央研究院植物研究所

此文在探討藍綠藻產生乙烯的能力，所用材料包括單胞、絲狀、以及分枝等種類，它們均已純化至無菌。實驗發現屬於分枝狀之軟管藻品系（*Hapalosiphon* 101），於培養在合成培養基或土壤上均能產生乙烯。文中並對有關此藻的形態，以及環境因子對產生乙烯的影響等加以討論。