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NOTE

A New Alkalophilic Bacterium Producing Ethylene

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A new isolate, *Bacillus* sp. ALK-7 can synthesize ethylene from 1-aminocyclopropane-1-carboxylic acid (ACC) as well as from methionine. The ACC has only been recognized as a key intermediate found in the metabolic pathway leading to ethylene formation in various plants. The efficiency of ethylene formation from the ACC by *Bacillus* sp. ALK-7 was about 2 times as high as that from the methionine. The reaction from ACC to ethylene formation was also shown to be mediated by the cell-free extracts of *Bacillus* sp. ALK-7.

Ethylene is a plant hormone involved in physiological processes such as fruit ripening, breaking of dormancy, etc. (6, 8). The ethylene is synthesized not only by plants but also by many microorganisms. The microbial production of ethylene has gradually been paid much more attention with its agronomic and petrochemical significances. Two groups of ethylene-forming bacteria are known. *Escherichia coli* synthesizes ethylene from methionine (10, 11, 12), while *Pseudomonas syringae* pv. *phaseolicola* uses α-ketoglutarate as a precursor for the ethylene formation (1, 3, 4). Recently, we found another group of alkalophilic bacterium which can produce ethylene from 1-aminocyclopropane-1-carboxylic acid (ACC) as shown by many plants. One alkalophilic bacterium, ALK-7 was isolated from compost, which forms ethylene with the highest effiency among the isolates examined. The ALK-7 is gram-positive, spore-forming and motile rod. It also shows catalase-positive. It grows well at pH range between 9 to 10.5 but did not show any growth at pH below 7. Thus, the strain ALK-7 was alkalophilic, and tentatively identified to belong to the genus of *Bacillus* (data not shown).

A loopful of alkalophilic *Bacillus* sp. ALK-7 was inoculated into alkaline medium I containing 1% glucose, 0.5% polypeptone, 0.5% yeast extract, 0.1% KH_2PO_4, 0.02% $MgSO_4 \cdot 7H_2O$ and 1% Na_2CO_3 (pH 10.3), and cultured at 30°C overnight. Then, aliquots of above seed culture was inoculated into alkaline medium II in 30 ml-vial for ethylene-production assay. The alkaline medium II has the same composition as the medium I except that only glucose of the medium I was replaced by 1% soluble starch. After inoculation the vial was sealed with rubber aluminum cap, and cultured at 30°C for a given period. Gas samples of the head space were withdrawn from the vials by a gas-tight syringe and analyzed by gas chromatography (Shimadzu GC-9A) equipped with a hydrogen flame-ionization detector and a column of Porapak Q: the column temperature was 80°C and the carrier gas was nitrogen with flow rate of 40 ml/min. Gas samples from alkalophilic *Bacillus* sp. ALK-7 culture had been proven ethylene by GC-Mass chromatography (Fig. 1).

The cell-free extracts were prepared from the cells at the maximal rate of ethylene production. The culture was centrifuged at 2,700 g for 20 min and the cells were suspended in the 10 mM glycine-NaOH buffer (pH 10.3). Sonication was done with a Braun-sonic 1510 at 350 W for 6 min. The sonicated preparation was centrifuged at 4,300 g for 20 min at 4°C and used as the cell-free extracts.

Recently, using in vitro assays with *Pseudomonas syringae* pv. *phaseolicola* (1, 5) and *Pencillium digitatum* (2), oxygen was reported to be one of the essential factors in ethylene formation. In this study the ethylene formation increased slightly when the vials were stirred on the shaker followed by supplying a small amount of sterile-air using a gas-tight syringe (data not shown). The oxygen effect should be reexamined further to investigate the quantitative effects of dissolved oxygen and the growth rate for the ethylene production by the alkalophilic organism.

The intact cells of *Bacillus* sp. ALK-7 effectively produced ethylene from ACC as well as from methionine (Fig. 2). During the ethylene formation by *Cryptococcus albidus*, however, ethylene was shown to form chemically in the presence of Fe^{2+}, EDTA, O_2 and 2-keto-4-methiol-butyrate (KMB) (9). In this work a small amount of ethylene also formed in the alkaline medium containing polypeptone and yeast extract altogether without the bacteria inoculated or without the cell-free

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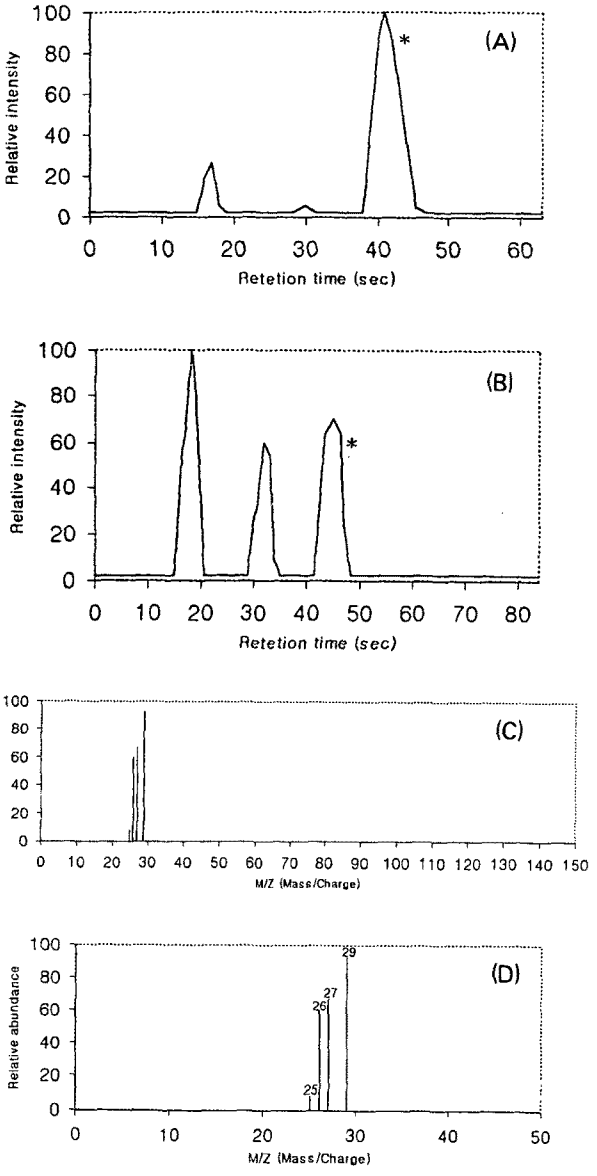

Fig. 1. Gas (A, B)-Mass (C, D) chromatograms of pure ethylene (A, C) and gas samples from *Bacillus* sp. ALK-7 (B, D).
*'s in A and B indicate peaks by ethylene.

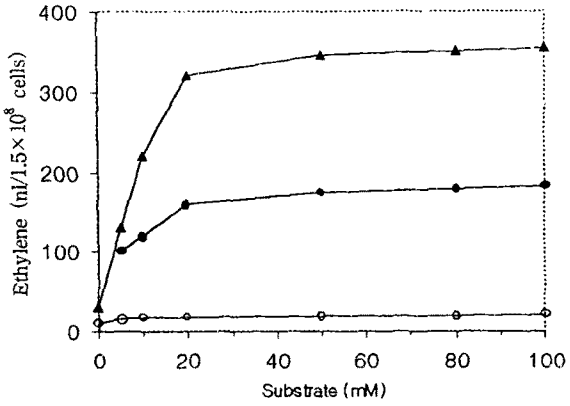

Fig. 2. Effect of concentration of L-methionine (●) and ACC (▲) as precursors for ethylene production by the intact cells of *Bacillus* sp. ALK-7.
Alkaline medium II 4.2 ml and bacteria 0.3 ml were mixed with 0.5 ml of 50 mM substrate (5 mM), 0.5 ml of 100 mM substrate (10 mM), and 0.5 ml of 500 mM substrate (50 mM) etc., respectively in 35 ml-vials. Vials were incubated at 30°C for 48 h. Open circle indicates endogenous ethylene evolution in a medium containing polypeptone or peptone without cells.

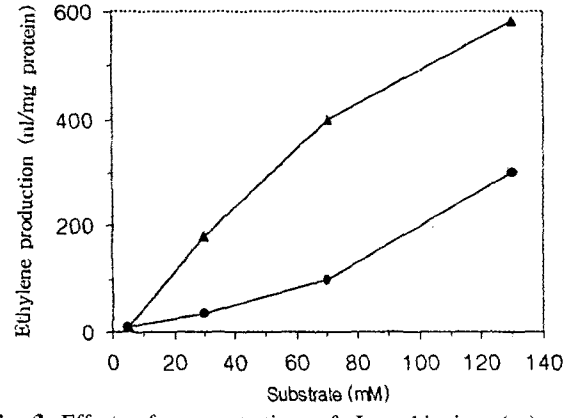

Fig. 3. Effect of concentration of L-methionine (●) and ACC (▲) as precursors for ethylene production by the cell-free extracts of *Bacillus* sp. ALK-7.
Reaction mixture contained each substrate at the concentrations between 5 and 130 mM, 5 mM dithiothreitol and the cell-free extracts (2 ml) harbouring the equal amount of cellular proteins determined by the Lowry method (7). The mixtures were put in 15 ml vial, sealed with rubber aluminum cap, and shaken at 30°C for 48 h.

extracts (Fig. 2). The ethylene production was also possible by the cell-free extracts of *Bacillus* sp. ALK-7 (Fig. 3). Thus, the *Bacillus* sp. ALK-7 uses ACC for ethylene biosynthesis in addition to the methionine (Fig. 2). But it can not synthesize ethylene from α-ketoglutarate under the same conditions (data not shown). This indicates that the *Bacillus* sp. ALK-7 produces ethylene from methionine through ACC, not through KMB from α-ketoglutarate, as an intermediate. The effiency of ACC as a substrate to support ethylene biosynthesis is superior to that shown by methionine at the same concentrations. From these results we assume that ACC is derived from methionine by ACC synthase (8) of the *Bacillus* sp. ALK-7 as shown by many plants.

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