Mechanism by Which Bacillus-Derived 2-Aminobenzoic Acid Inhibits the Growth of Arabidopsis thaliana Roots

Lam Hoang1, Kyung Sik Song1, In Koo Rhee1, Jeong Hoe Kim2, and Sangman Lee1*1

1School of Applied Bioscience, College of Agricultural and Life Sciences, Kyungpook National University, Daegu 702-701, Korea
2Department of Biology, Kyungpook National University, Daegu 702-701, Korea

To analyze the growth inhibitory mechanism of a 2-aminobenzoic acid (2-AA) derived from Bacillus cereus EJ-121, we treated Arabidopsis thaliana plants with 2-AA, 2-AA analogs, auxin (NAA), a known auxin transport inhibitor [2,3,5-triiodobenzoic acid (TIBA)], and an ethylene action inhibitor [silver thiosulfate (Ag)]. Root development was significantly inhibited by 50 μM 2-AA, whereas the growth of bacteria and yeast was undeterred. The application of two 2-AA analogs -- 3-aminobenzoic acid (3-AA) and 4-aminobenzoic acid (4-AA) -- did not impair Arabidopsis root growth at concentrations below 100 μM. These results suggest that the effect of 2-AA is not due to its chemical structure, but because of its conversion to another metabolite, IAA. To confirm this, we supplemented TIBA in the growth medium, and found that the degree of inhibition was significantly reduced. Similarly, when plants were co-treated with 100 μM Ag, the negative effect of 50 μM 2-AA was greatly diminished. All of these observations support the proposal that this inhibition results from the conversion of 2-AA to IAA. Furthermore, the increased auxin level leads to a rise in ethylene synthesis, which then blocks root growth and, ultimately, retards overall plant development.

Keywords: 2-aminobenzoic acid, auxin, ethylene, plant growth inhibitor

The effect of rhizobacteria on plant growth can be positive, negative, or neutral (Nehl et al., 1996). For example, allelopathic rhizobacteria can inhibit development by secreting allelochemicals, either directly or indirectly, into the rhizosphere (Barazani and Friedman, 1999). Because of this property, some organic compounds produced by rhizobacteria can serve as weed-killers (Bernart and Cerwick, 1990).

Many reports have described how microbe-originated compounds inhibit plant growth. For example, hydroxysoy mothrin and sulochrin isolated from a fungus negatively affect the development of tea pollen tubes (Shimada et al., 2001), while penicillic acid, also from a fungus, prohibits root elongation in rice (Sasa et al., 1971). In fact, phosphonothricin and bialaphos have been used as commercial herbicides in agriculture (Barazani and Friedman 1999).

We previously isolated a 2-aminobenzoic acid (2-AA) from Bacillus cereus EJ-121, and confirmed its inhibitory effect on the growth of lettuce seedlings (Hoang et al., 2005). Here, we have focused on analyzing the mechanism of 2-AA as a growth inhibitor in Arabidopsis thaliana.

MATERIALS AND METHODS

Plant Materials and Growing Conditions

We germinated 20 seeds of wild-type A. thaliana (L.) Heynh., (ecotype Columbia) and grew them in 100 × 100 × 15 mm square plates containing a full-strength Murashige and Skoog (MS; Murashige and Skoog, 1962) agar medium (pH 5.8) supplemented with 2% (w/v) sucrose. These plates were maintained in a growth chamber at 23°C, under a 12-h photoperiod provided by cool-white fluorescent tubes (photon flux density approx. 80 μE m⁻² s⁻¹) (Kim and Lee, 2007).

Growth of Bacteria and Yeast

Escherichia coli and Saccharomyces cerevisiae were grown overnight in liquid LB and liquid YPD (yeast extract, peptone, and dextrose), respectively. They were then inoculated to fresh media containing various concentrations of 2-AA and incubated for 24 h further before their ODs were measured spectrophotometrically at 600 nm.

RESULTS AND DISCUSSION

We have now confirmed the inhibitory influence of 2-AA in Arabidopsis (Fig. 1). When 50 μM 2-AA was applied to the media from the beginning of seed germination, root elongation was significantly blocked (Fig. 1A). This effect was also observed during later growing stages (Fig. 1B). A concentration of 45 μM 2-AA was required to inhibit 50% (IC₅₀) of this Arabidopsis development. However, the growth of both E. coli and S. cerevisiae was not inhibited by treatment with 50 μM of 2-AA (Fig. 2). These microorganisms showed tolerance up to a concentration of 5 mM. Therefore, this deleterious effect seems to be specific to plants.

To determine whether this phenomenon was due to either the chemical structure of 2-AA itself, or its conversion to another metabolite, we tested two analogs of 2-AA -- 3-aminobenzoic acid (3-AA) and 4-aminobenzoic acid (4-AA). Applications of either did not inhibit Arabidopsis root growth at concentrations lower than 100 μM (Fig. 3). Therefore, this suggests that 2-AA is effective because of its conversion.

Normanly et al. (1993) have shown that applying 2-AA...
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To Arabidopsis plants causes an increase in their level of indole-3-acetic acid (IAA) via the tryptophan-independent biosynthetic pathway. Thus the effect of 2-AA on plant growth again seems to result from its conversion to IAA. Here, we also treated seedlings with alpha-naphthalene-acetic acid (NAA) to confirm that auxin inhibits root growth under our experimental conditions (Fig. 4). The negative influence of NAA was slightly increased when it was applied in conjunction with 50 µM 2-AA. This suggests that the 2-AA is converted to IAA, such that the auxin effect is further enhanced.

It is thought that plants take up exogenous 2-AA from the roots and transport it to the leaves, where it is converted into IAA. This newly synthesized IAA is then moved to the roots, where elongation is inhibited. In a separate experiment, we added an auxin transport inhibitor, 2,3,5-triiodobenzoic acid (TIBA), to the growth medium at the beginning of seed germination, and found that the inhibitory effect of 2-AA was significantly reduced (Fig. 5A). This also occurred...