Plant Biotechnology 25, 489–492 (2008)

Short Communication

Growth acceleration of plants and mushroom by erythritol

Kouichi Kuroda[1], Shuji Hirakawa[1], Masayuki Suzuki[2], Kazunori Shinji[2], Kazuo Ogasa[2], Tatsuya Uraji[2], Teruo Amachi[1,2], Mitsuyoshi Ueda[1,*]

[1] Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan;
[2] Nikken Fine Chemicals Co., Ltd., Chita, Aichi 478-0046, Japan
* E-mail: miueda@kais.kyoto-u.ac.jp Tel: +81-75-753-6110 Fax: +81-75-753-6112

Received April 28, 2008; accepted June 6, 2008 (Edited by K. Yoshida)

Abstract The effects of erythritol, a safe and nontoxic sweetener, on organisms other than mammals have not been clarified. We investigated the effects of erythritol on plants and microorganisms. The supplementation of erythritol to white radish (*Raphanus sativus* L.), garlic (*Allium sativum*), and arabidopsis (*Arabidopsis thaliana*) enhanced the growth of their seedlings and roots, and shortened germination time. The supplementation of erythritol to a mushroom (*Lentinula edodes*) markedly enhanced the development of fruiting bodies. These results would suggest that erythritol has the attractive potential as a safe and useful growth accelerator for plants and microorganisms.

Key words: Erythritol, *Arabidopsis thaliana*, mushroom.

Erythritol is a four-carbon sugar alcohol that exists naturally in bacteria, mushrooms, fruits, and wine among others (Bernt et al. 1996; Cock 1999). Although other sugar alcohols have been produced by chemical reduction in the presence of nickel catalysts, erythritol has been produced by yeast fermentation using glucose (Ishizuka et al. 1989). Erythritol has many interesting physicochemical characteristics; for example, it is approximately 70% as sweet as sucrose, it is not metabolized by *Streptococcus mutans*, which plays a major role in tooth decay, and it has very low energy (≒0 kcal/g), high endothermy, and high thermal storage (Bernt et al. 1996; Cock 1999). Over 90% of ingested erythritol is absorbed by the small intestine, but most of the absorbed erythritol is not metabolized and is instead excreted into urine (Noda and Oku 1992; Hiele et al. 1993). In addition, because absorbed erythritol reduces serum glucose, insulin, and lipid peroxidation levels, erythritol attenuates the complications of diabetes (Noda et al. 1994; Ishikawa et al. 1996; Yokozawa et al. 2002). Furthermore, the safety of erythritol as a food and drug additive has already been established. However, the effect of erythritol on living organisms other than humans has not been investigated. In this study, the effects of erythritol on the growth of plants and mushroom were investigated, and we proposed a new excellent practical use of erythritol.

The effect of erythritol on the model plant *A. thaliana* (ecotype Columbia) was investigated, because the utilization of genomic information is desirable for further analyses. Arabidopsis seedlings were cultivated on Murashige and Skoog medium containing 2% (w/v) sucrose, 0.3% (w/v) gellan gum and additional 0.68% (w/v) (20 mM) sucrose or 0.24% (w/v) (20 mM) erythritol (Nikken Fine Chemicals Co., Ltd., Aichi, Japan). After the cultivation at 4°C in the dark for 3 d, Arabidopsis was grown in a growth chamber at 22°C and a light intensity of approximately 27 μmol m^{-2} s^{-1} with a 16-h-light/8-h-dark photoperiod. Primary root length was slightly increased and the number of lateral roots was increased by 0.24% (20 mM) erythritol supplementation (Figure 1, Table 1). Furthermore, the elongation of root hair was also enhanced (data not shown). However, no growth enhancement of aerial parts by erythritol supplementation was observed. Mannitol is a sugar alcohol similar to erythritol and an osmotic agent. Although the supplementation of mannitol at more than 0.91% (50 mM) markedly inhibited the growth of plant shoots, that of erythritol at more than 0.61% (50 mM) hardly inhibited shoot growth.

The effect of erythritol on another crop, garlic (*A. sativum*) was also investigated. Garlic is a popular bulb vegetable worldwide and is used as a spice and a revitalizer among others. Garlic seedlings were grown at various erythritol concentrations (0.01% (w/v), 0.05%, and 0.5%). Garlic bulbs were sown on potting compost

Abbreviations: cAMP, cyclic adenosine monophosphate; ERT, erythritol; GA, gibberellin; Glu, glucose

This paper is dedicated to late Dr. Kazuya Yoshida.

This article can be found at http://www.jspcmb.jp/

and grown at 25°C and a light intensity of approximately 4 μmol m^{-2} s^{-1} with an 8-h-light/16-h-dark photoperiod. The number of days required for garlic bulbs to germinate in the presence of 0.05% erythritol was 6.1 days, whereas that without erythritol supplementation was 8.4 days. This observation showed that the number of days required for the germination of garlic bulbs was decreased by about 2 days in the presence of 0.05% erythritol (Figure 2A). On the other hand, garlic bulbs cultivated in the presence of 0.5% erythritol germinated on day 9.1. The germination of bulbs was delayed for about 1 day in the presence of 0.5% erythritol (Figure 2A). We also found that 0.01% or 0.05% erythritol supplementation enhanced the growth of garlic seedlings, although 0.5% erythritol supplementation inhibited growth, and 5% erythritol supplementation completely inhibited germination (Figure 2B, C). The

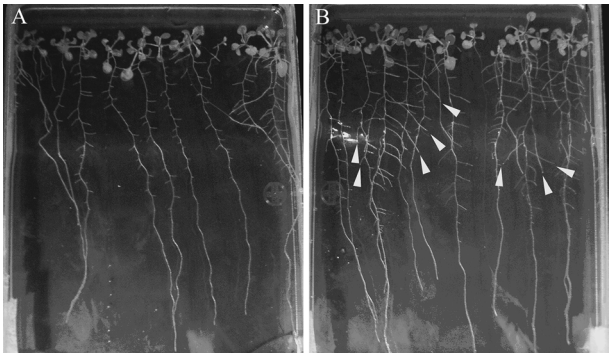

Figure 1. Photograph of roots of *A. thaliana* cultivated with erythritol. Arabidopsis was cultivated on MS medium containing 2% (w/v) sucrose and additional 0.68% (20 mM) sucrose (A) or 0.24% (20 mM) erythritol (B). Photographs were taken 11 days after germination. Arrowheads indicate well-grown lateral roots.

Table 1. Enhancement of root growth by erythritol supply

	MS+Suc (0.68%)	MS+ERT (0.24%)
Primary root length (cm)	8.0±0.22	8.8±0.16
(%)	(100)	(110)
Number of lateral root	17±0.94	21±0.92
(%)	(100)	(130)

Values are means±SE. MS+Suc (0.68%): n=91; MS+ERT (0.24%): n=102. MS: Murashige and Skoog plant salt mixture containing 2% sucrose, Suc: Sucrose, ERT: Erythritol

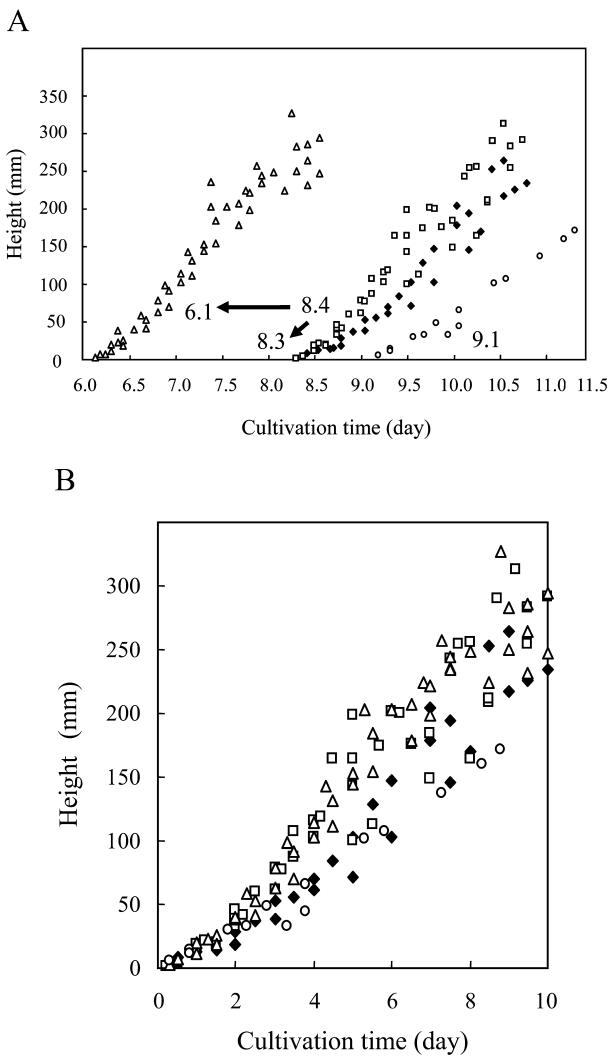

Figure 2. Effect of erythritol on growth of garlic (*A. sativum*). (A) Growth rate of garlic cultivated on potting compost. Closed diamonds, plants cultivated without erythritol; open squares, 0.01% (w/v) erythritol; open triangles, 0.05% erythritol; open circles, 0.5% erythritol. The numbers in the graph indicate the numbers of days to germination. (B) Replotted graph in the case that the times for germination at each condition are set to 0. Symbols are the same as those shown in (A). (C) Garlic cultivated without erythritol (left), 0.05% erythritol (middle), and 5% erythritol (right). Photographs were taken at 17 days after germination on soil.

supply of 0.01% or 0.05% erythritol also enhanced root growth and increased root weights (data not shown), but 0.5% erythritol supply inhibited root growth, and 5% erythritol supply completely inhibited it (Figure 2C). In the case of 0.05% erythritol supply, the time required for growth to the maximum size of seedlings was shortened by about 1 month (data not shown).

The effect of erythritol on the mushroom *L. edodes* was also investigated. The mushroom was grown on a sawdust-based medium (Mori Sangyo Co., Ltd., Gunma, Japan) with an 8-h-light/16-h-dark photoperiod. During the light period, the temperature was set at 25°C, and during the dark period, the temperature was set at 4°C. The various concentrations of erythritol were firstly supplied to the sawdust cultivation bed by soaking in water containing erythritol, and then distilled water was sprayed once a day. The supply of 0.5% (w/v) and 5% erythritol markedly accelerated the growth of fruiting bodies (Figure 3). The weights and diameters of fruiting bodies of the mushroom grown with erythritol supplementation were much greater than those of control mushroom grown without erythritol supplementation (data not shown). The supply of 0.5% erythritol shortened the time for developing to a certain size of fruiting bodies approximately by 30%. Although the inner structure of fruiting bodies was observed under a stereomicroscope, there was no decrease in the tissue density of the fruiting body of the mushroom grown with erythritol supply (data not shown).

Erythritol has valuable characteristics and physiological functions in mammals (Noda and Oku 1990; Noda and Oku 1992; Yokozawa et al. 2002). However, the effect of erythritol on plants and microbes has not been clarified. In this study, plants and mushroom growths were accelerated, and in particular, plant roots and root hair also grew well in the presence of erythritol. The optimal concentrations of erythritol to exert its maximal effect ranged approximately from 0.05% to 0.2% and a high concentration of erythritol inhibited plant and root growths. As it is known that plant root hair is essential for the absorption of water and nutrient elements (Gilroy and Jones 2000), there is a possibility that erythritol supply may induce the enhancement of absorption of various elements necessary for plant growth. The observation that a high concentration of erythritol inhibits plant growth, germination, and root growth also might suggest that the mechanism underlying the enhancement of growth by erythritol is associated with osmotic pressure or water uptake. One of sugar alcohols, mannitol inhibits ethylene production by osmotic shock (Imaseki and Watanabe 1978). Auxins and ethylene are involved in root hair growth and lateral root formation (Pitts et al. 1998; Santeria et al. 2005). Thus, there is a possibility that erythritol affects the levels of auxins, ethylene or some phytohormones in plants. Further analysis would be required for the clarification of the mechanism underlying root development. The number of days required for the germination of *A. sativum* was decreased by 0.05% erythritol supply. The phytohormones abscisic acid (ABA) and gibberellin acid (GA) regulate seed germination. ABA establishes and maintains the dormancy of seeds and bulbs, whereas GA has the opposite effect, that is, breaking dormancy and inducing germination (Steber et al. 1998; Yamazaki et al. 1999; Koornneef et al. 2002; Price et al. 2003). To confirm this

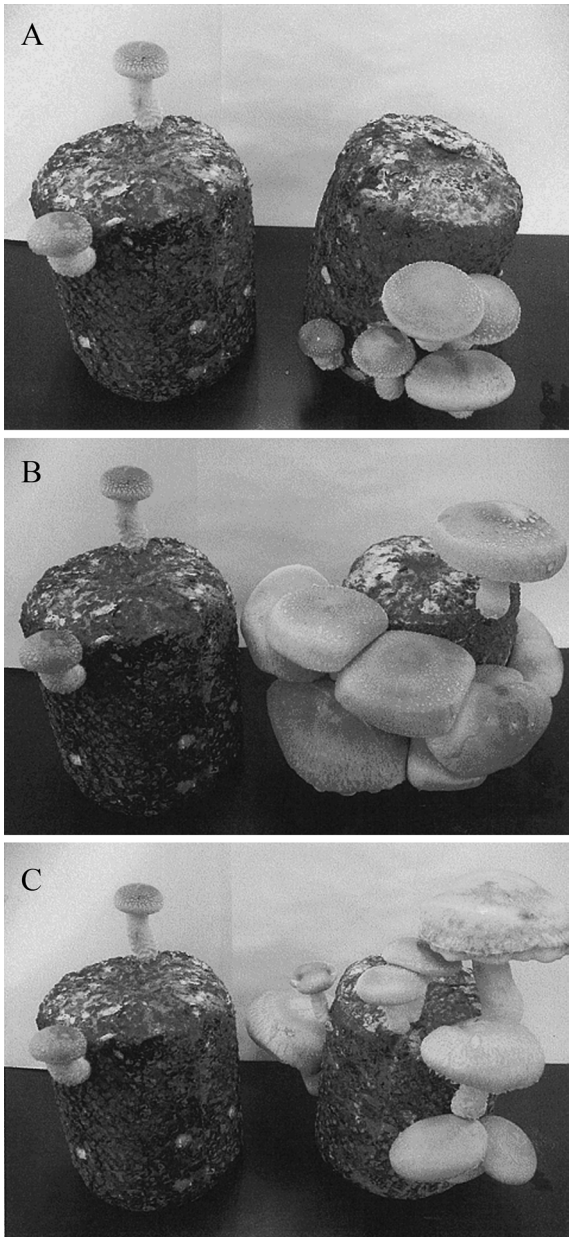

Figure 3. Effect of erythritol on growth of mushroom (*L. edodes*). Fruiting bodies of mushroom without erythritol (left) or with erythritol (right) in each photo. H_2O was used as treatment control. (A) 0.05% (w/v) erythritol; (B) 0.5% erythritol; (C) 5% erythritol supply. Photographs were taken at 11 days after the first soak of a cultivating bed in water with or without erythritol.

hypothesis, endogenous phytohormones, auxin, ethylene, ABA, or GA would be further quantitated.

The growth of *L. edodes* was also enhanced by erythritol supply, but the supplementation of erythritol at more than 0.5% led to the rapid development of fruiting bodies, which is different from the effect on *A. sativum* (Figure 3). The fruiting bodies were larger in the presence of erythritol, but the tissue density did not decrease. These observations suggest that the supply of erythritol leads to not an enlarged body, but growth enhancement. Although the mechanism underlying fruiting body development is not well understood, intracellular cAMP level is closely related to the onset of fruiting body development (Hori et al. 1991), and laccase activity is highest immediately before fruiting body initiation, and cellulase activity is highest during fruiting body development (Ohga et al. 1999). On the other hand, the C-N ratio is important for fruiting body induction (Madelin 1956). There is a possibility that erythritol would affect these parameters.

It has been previously established that erythritol is a safe, nontoxic sweetener (Bernt et al. 1996; Cock 1999). In light of these results, we suggest that erythritol has the attractive potential as a safe, inexpensive, useful growth accelerator for plants and microorganisms. In agriculture, the shortening of production cycle can contribute to the increase in food production. Thus, producers can maintain a stable supply of food by the supplementation of erythritol in crop production with ease and at a low cost. Further studies may reveal the mechanism underlying growth promotion by erythritol.

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