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Phytostabilization of salt accumulated soil using plant and biofertilizers: Field application

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ABSTRACT

This field study aimed to evaluate the plant growth-promoting potential of biofertilizers. Plant growth-promoting rhizobacterium *Arthrobacter scleromae* SYE-3 were isolated from local plants grown in saline soil, and isolates were applied as biofertilizers to evaluate growth-promotion effects on lettuce, radish, and Chinese cabbage in cultivated plots (100 m^2) of salinized soil. Strain produced 89.15 ± 0.36 mg/L indole-3-acetic acid on day 3 of incubation, and 1-aminocyclopropane-1-carboxylate deaminase activity was 0.20 ± 0.06 OD at the end of the incubation period (after 72 h). After 102 days of cultivation, SYE-3 increased shoot lengths by 23.5%, 48.3%, and 19.5% in experimental lettuce, radish, and Chinese cabbage, respectively, compared with controls. Leaf number increased in lettuce only, by 45.1%. The viable cell count of SYE-3 was maintained during the vegetation restoration period. These results indicate that can serve as a promising microbial inoculant for improving plant growth and therefore enhancing yield in salinized environments, likely through raising plant growth promoting efficiency.

1. Introduction

As the desire for and consumption of environment-friendly agricultural products increase, greenhouse cultivation systems that enable year-round cultivation have been steadily increasing since the 1970s (Akimasa et al., 2003). In South Korea, the area of greenhouse cultivation sites (using facilities such as vinyl houses) increased about 20-fold from 4000 ha in the 1970s to 83 000 ha in 2002 (Oh et al., 2010). Because greenhouse cultivation raises land-use rate through year-long, intensive growing of crops that are isolated from the natural environment, material circulation is more limited than in outdoor culture, and the repeated cultivation can cause side effects (e.g., salt accumulation) (Cho et al., 2006). In addition, intensive cultivation increases the use of chemical fertilizers and compost; their accumulation then leads to higher salt concentration, reducing crop yields and soil biodegradation efficiency (Akimasa et al., 2003; Zhang et al., 2006).

Salts are highly soluble and therefore electrically conductive in water (Oh et al., 2010). The top soil layer of greenhouse cultivation sites tends to experience high salinity, as rainwater cannot wash away leftover fertilizer salts that dissolve and accumulate in any remaining irrigation water (Blanco and Folegatti, 2002; Kwak et al., 2003). Additionally, high indoor temperatures cause water on the soil surface to evaporate, and capillary action then results in soil water moving upward, leaving behind salt to accumulate around plant roots (Blanco and Folegatti, 2002). Because rainwater is actively blocked, salt leaching is also extremely rare even during the rainy season (Kim et al., 1997).

As soil becomes more saline, its electrical conductivity (EC) increases (Taki and Okino, 1991). The topsoil and subsoil EC values of greenhouse cultivation sites in South Korea are 3.5 dS m^{-1} and 2.2 dS m^{-1}, respectively, indicating that more salt accumulates in the top layer than in the subsoil; moreover, 59% of their topsoil exceeds the hazard threshold of 2.0 dS m^{-1} (Oh et al., 2010). Osmotic effects from excess accumulation of certain nutrient salts (e.g., Ca^{2+}, Mg^{2+}, Na^{+}, SO_4^{2-} and Cl^{-}) reduces crop water-use rates, while also hampering growth through ion toxicity and obstructing the absorption of other beneficial ions (Bernstein, 1975). Methods of relieving salt accumulation in greenhouse cultivation sites include watering, subsoil plowing, subsoil breaking, soil addition and the removal of topsoil, the cultivation of cleaning crops, the installation of perforated drain pipes, controlling fertilizer amount, as well as administering microbial formulations (Ok et al., 2005). However, reduction by watering has shortcomings because the water consumption involved is high: it can be only be applied to soils in which salt accumulation is not severe, and it may hinder

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plant growth through that the excess water may cause some waterlogging within the vicinity of the roots (i.e. in the rhizosphere). (Petersen, 1996; Oh et al., 2010). The disadvantages of subsoil plowing include its short-term effects and the need to re-salinize topsoil, whereas subsoil breaking is uneconomical and increases the risk of groundwater pollution (Tanji, 1990). Furthermore, cleaning-crop cultivation is unaffordable for many farmers (Hasanuzzaman et al., 2014), while topsoil removal and soil addition can reduce effective soil without relieving the fundamental cause of the problem (Qadir et al., 2000). Finally, the effectiveness of microbial formulations in the field has been questioned, because microorganisms frequently do not adapt to new environments and thus die as a result of the high salinity (Kim et al., 2006). Therefore, watering (high water content, use with only soils that aren't severely saline, etc.) is the least problematic of existing techniques. However, current watering methods are problematic because even though the fresh water treatment moves salt down from the topsoil to the subsoil, capillary action causes salts to move back up and accumulate again in the topsoil (Kim, 1996).

This study aimed to resolve these diverse difficulties by providing low-cost and high-efficiency desalination method that also increases plant productivity. We developed an environmentally friendly biological formulation through mixing ion exchange resins (Hong and Lee, 2014, 2016; Hong et al., 2016) capable of removing salts and salt-tolerant, plant growth-promoting rhizospheric (PGPR) bacteria *Arthrobacter scleromae* SYE-3 (Hong et al., 2016). The selected ion exchange resins contained strongly basic anions and strongly acidic cations (2:1, w/w). The SYE-3 strain exhibits both IAA productivity (89.15 ± 0.36 mg/L) and ACC deaminase activity (0.20 ± 0.06 at 72 h), indicating that it is a PGPB (Hong et al., 2016). This formulation has already been tested in laboratory experiments (Hong et al., 2016), but not yet in the greenhouse. Therefore, here we tested the formulation in a greenhouse cultivation site with severe salt accumulation, analyzing the resultant effects on soil properties and plant growth.

2. Materials and methods

2.1. Microbial formulation and ion-exchange resins for field application

For field application, formulations of *Arthrobacter scleromae* SYE-3 were made with skim milk, peptone, glucose, and glycerine (the most commonly used cryoprotectants). Bacterial survival rates were evaluated after culturing at 30 °C and 180 rpm. A 10% (v/v) suspension was mixed with a 5% (w/v) preservative into the microbial culture medium. Cultured SYE-3 was centrifuged, then added to pre-sterilized and lyophilized distilled water supplemented with 5% skim milk. Before lyophilization, 1 mL of suspended SYE-3 was taken, then was smeared and cultured through serial dilutions to obtain the initial viable cell count. After lyophilization, SYE-3 survival rate was calculated (survival rate = [post-lyophilization colony-forming units (CFU)/pre-lyophilization CFU] × 100) to evaluate lyophilization efficiency.

Substrate preference was tested to improve SYE-3 survival rate and identify optimal formulation conditions. Substrate selection was performed by optical density (OD) value with a GP plate (BIOLOG Co., USA). Skim milk (5%) and lipids (1%) were added to various substrates (1%) for lyophilization and improvements in survival rates were analyzed. Specifically, CFU was measured pre- and post-lyophilization.

2.2. Effects of SYE-3 formulation and ion-exchange resins on plant growth in the field

Salt-removal and vegetation-stabilization field experiments were conducted in a farmhouse (390-m^2 vinyl house) located in Paltan-myeon, Hwaseong-si, Gyeonggi-do, South Korea. On September 11, 2015, the vinyl house was divided into a control plot and an experimental plot (Fig. 1). Lettuce, Chinese cabbage, and radish seedlings (post-germination average 5 cm or less) were planted. The control plot was covered with a 5-cm soil layer and a 0.2-cm layer of a common fertilizer available in the market instead of a soil conditioner to fix the plants. Instead of fertilizer, lyophilized SYE-3 formulations containing 5% skim milk mixed with ion exchange resins (Hong et al., 2013; Park et al., 2014) were added to the experimental plot. During the cultivation period, soil moisture was maintained via water sprayed from a hose installed in the center of the vinyl house, and the temperature was maintained at 15–20 °C. Plant lengths and leaf number were measured five times throughout the experiment, spanning 102 days: on September 11, September 22, October 8, November 5, and December 22. At the end of the experiments, plants were collected to determine biomass differences between the control and experimental groups, as a measure of formulation efficacy. In addition to the existing control group, we created three more controls per plant lettuce: control-1: conventional chemical fertilizer only; control-2: ion-exchange resin only; and control-3: SYE-3 inoculation only. Control cultivation and measurements were conducted following the same method as above.

2.3. Soil analysis

Soil samples were air-dried and sieved (2 mm mesh) before measuring pH, EC, cation exchange capacities, nitrogen content, available phosphoric acid, as well as exchangeable potassium, calcium, magnesium, and sodium content. Sterilized water (5 mL; w:v) was mixed with soil (1 g) via stirring for 1 h and left to stand for 30 min, before analysis with a pH meter (Orion mode 921A, USA) and an EC meter (Orion model 50, USA). Total nitrogen content was measured with a Vario Max CN (Elementar Analyzesysteme GmbH, Germany), using a dry chemical method. Available phosphoric-acid concentration was measured with a UV-spectrometer (Varian Carry4000, Australia) using the Lancaster method (NIAST, 2000). Exchangeable potassium, calcium, magnesium, and sodium content were measured with an atomic absorption spectrometer (AA280FS, USA) after leaching the substances with a 1 N CH_3COONH_4 (pH 7.0) buffer solution (Hong et al., 2015). Cation exchange capacity was measured with inductivity coupled plasma (ICP) emission spectroscopy (ICP-OES, GBC Integra XL Dual, Australia) by leaching with 1 M-NH_4OA_c (pH 7.0).

2.4. Assessment of abundance after inoculation in the field

Arthrobacter scleromae SYE-3 was inoculated in the field on salinized soil, and microbial abundance was monitored using real time PCR (RT-PCR). To design the TaqMan probe and primers, SYE-3 16S rDNA was PCR-amplified using the primers 27f (AGA GTT TGA TCM TGG CTC AG; M = C:A) and 1492r (TAC GGY TAC CTT GTT ACG ACTT; Y = C:T) (Martin-Laurent et al., 2001). The thermocycling conditions were as follows: initial denaturation step at 95 °C for 5 min; 28 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s; final extension step at 72 °C for 5 min (Hong and Lee, 2014). The amplified 16S rDNA was purified with a kit (Qiagen, Germany) and then sequenced. Sequence

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