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Original Article

Characterizations of purple non-sulfur bacteria isolated from paddy fields, and identification of strains with potential for plant growth-promotion, greenhouse gas mitigation and heavy metal bioremediation

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Abstract

This study was aimed at selecting purple non-sulfur bacteria (PNSB) isolated from various paddy fields, including Cd- and Zn-contaminated paddy fields, based on their biofertilizer properties. Among 235 PNSB isolates, strain TN110 was most effective in plant growth-promoting substance (PGPS) production, releasing 3.2 mg/L of NH_4^+, 4.11 mg/L of 5-aminolevulinic acid (ALA) and 3.62 mg/L of indole-3-acetic acid (IAA), and reducing methane emission up to 80%. This strain had *nifH*, *vnfG* and *anfG*, which are the Mo, V and Fe nitrogenase genes encoded for key enzymes in nitrogen fixation under different conditions. This strain provided 84% and 55% removal of Cd and Zn, respectively. Another isolate, TN414, not only produced PGPS (1.30 mg/L of NH_4^+, 0.94 mg/L of ALA and 0.65 mg/L of IAA), but was also efficient in removing both Cd and Zn at 72% and 74%, respectively. Based on 16S rDNA sequencing, strain TN110 was identified as *Rhodopseudomonas palustris*, while strain TN414 was *Rubrivivax gelatinosus*. A combination of TN110 and TN414 could potentially provide a biofertilizer, which is a greener alternative to commercial/chemical fertilizers and an agent for bioremediation of heavy metals and greenhouse gas mitigation in paddy fields.

Keywords: 5-Aminolevulinic acid; Cadmium; Indole-3-acetic acid; Nitrogenase genes; Purple non-sulfur bacteria; Zinc

1. Introduction

The worldwide increase in human population has contributed to food shortage, including rice (*Oryza sativa* L.), which is consumed by half of the world population [1]. As a result, chemical fertilizers, particularly those with a nitrogen base, have been heavily used to increase rice yield. In addition to a higher cost of production associated with the fertilizers, their long term use has been reported to result in loss of soil quality and ground water contamination with nitrate [2]. In addition, contamination of heavy metals (HMs) such as Cd and Zn in agricultural land is a serious concern for safe food production. Contamination usually occurs through runoff from mining, industrial drainage discharge and phosphate fertilizer applications [3,4]. Zn ore naturally contains Cd at a ratio of 1:25–1:500 [5], while rock phosphate, a source of phosphate fertilizer, is often contaminated with Cd. Cd in a range of 2–100 mg/kg in ten types of rock phosphate was reported [6].

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This leads to a common problem of agricultural soils being contaminated with Cd via phosphate fertilizer [7]. Hence, both HMs have been detected in paddy fields in various countries [8–11]. Cd and Zn in rice grains harvested from contaminated paddy fields have been reported [9]. Cd safety levels in soil and rice are 1.4 mg/kg soil and 0.2 mg/kg grain, according to the agricultural soil pollution control standards of the Canadian Council of Ministers of the Environment and the food quality standards of the Codex Committee on Food Additives and Contaminants draft Maximum Permissible Level, respectively [12–14].

Recently, sustainable agriculture has been promoted, and plant growth-promoting bacteria and biofertilizers are among the substitutes for chemical fertilizers and have the potential to reduce HMs. Nitrogen is one of the major essential elements for plant growth. Biological nitrogen fixation by N_2-fixing microorganisms is an effective mechanism for converting N_2 into ammonium ions (NH_4^+) which can be used by plants [15]. A key enzyme for N_2 fixation is nitrogenase, which is found in all N_2-fixing bacteria. There are three nitrogenase isozymes based on the cofactor: molybdenum (Mo)-iron (Fe), vanadium (V)-Fe and Fe–Fe nitrogenases. Mo–Fe nitrogenase is a canonical form of the enzymes and is found in all N_2-fixing bacteria. Only some of the N_2-fixing bacteria have V–Fe and/or Fe–Fe nitrogenases, which are referred to as alternative nitrogenases [16]. Alternative nitrogenases are adapted from Mo–Fe nitrogenase under dizotrophic conditions with limited Mo [17]. Purple non-sulfur bacteria (PNSB) are among nitrogen-fixing bacteria and are candidates for applications in paddy fields due to their extraordinary metabolic versatility, i.e. photoautotrophic, photoorganotrophic, chemoautotrophic and chemoorganotrophic [18,19].

PNSB not only fix N_2, but also produce plant growth-promoting substances (PGPSs) such as indole-3-acetic acid (IAA) and 5-aminolevulinic acid (ALA). IAA, which is a phytohormone playing a role in growth activation of plants by inducing plant mineral uptake and root cell elongation [20]. ALA is used as a precursor in the synthesis of chlorophyll and induction of antioxidative enzymes under stress conditions such as catalase, glutathione reductase and superoxide dismutase [21,22]. Studies have found that PNSB strains mitigate methane emission by suppressing the growth of methanogens in paddy soil slurries [23]. Methane emissions from the soil slurries inoculated with *Rhodopseudomonas palustris* strains were significantly lower than those from slurries without the inoculants, up to 88% [24]. Other benefits of PNSB are reduction of plant stress from HMs by various mechanisms such as adsorption on extracellular polymeric substances (EPS) on the cell surface, accumulation inside the cells, transformation into less toxic derivatives by redox transformations and precipitation into carbonate or sulfide compounds [25,26].

Limited work has been conducted on potential use of PNSB as a plant growth promoter in HM contaminated environments, including paddy fields. Hence, this study aimed to isolate and select PNSB with capabilities as a plant growth promoter or biofertilizer for NH_4^+, ALA and IAA releases, a bioremediation agent for HMs (Cd and Zn) and a greenhouse gas (CH_4) emission reducer.

2. Materials and methods

2.1. Sample collection and heavy metal analysis

Soil or sediment and water samples were collected from Cd- and Zn-contaminated paddy fields in Thailand. The contaminated paddy fields were close to a zinc mine in the Maesot District, Tak Province and a gold mine in the Wangsaphung District, Loei Province (referred to as sites C1 and C2, respectively). The soil or sediment samples were collected at a depth of 0–5 cm from the surface of soil, while the water samples were collected at 0–50 cm from the surface of the water column. Each soil or sediment sample was air-dried and passed through a 2 mm sieve. Then the samples were extracted by acid digestion (HNO_3: HCl = 1: 3) at 70 °C with manual shaking every 15 min for 1 h. After digestion, the extracts were diluted using 6 mL of deionized water and filtered through Whatman filter paper no.1. For water samples, 10 mL of each sample was filtered through Whatman filter paper no.1 before HM analysis [27]. Cd and Zn in the samples were analyzed by an inductively coupled plasma-optical emission spectrometer (ICP-OES) (Perkin Elmer, Germany).

2.2. PNSB isolation and inoculum preparation

A glutamate–acetate (GA) medium consisting of 3.8 g sodium glutamate, 5.44 g sodium acetate monohydrate, 2.0 g yeast extract, 0.5 g KH_2PO_4, 0.5 g K_2HPO_4, 0.8 g $(NH_4)_2HPO_4$, 0.2 g $MgSO_4$ $7H_2O$, 53 mg $CaCl_2$ $2H_2O$, 1.2 mg $CoCl_2$ $6H_2O$, 1.2 mg $MnSO_4$ $5H_2O$, 0.01 mg biotin, 2.5 mg ferric citrate and 1 mg nicotinic acid in 1000 mL distilled water (adjusted to pH 7.0) was used to isolate PNSB from soil/sediment and water samples. One gram of each soil sample was transferred to 8 mL of GA medium, whereas 4.5 mL from each water sample were transferred to 4.5 mL of double-strength GA medium in a screw-cap test tube (10 mL volume). Based on the nature of PNSB under anaerobic photoautotrophic/photoheterotrophic growth, they are stimulated to outcompete other organisms [28]; therefore, the anaerobic light conditions were used for PNSB isolation. Sterile liquid paraffin was added on top of the growth medium to achieve anaerobic light conditions. Light at 3000 lux was continuously provided using tungsten bulbs for 5–7 days at room temperature. The incubation turned the culture broths from light yellow to pink, red or brown. The broths were streaked onto GA agar, which was then incubated under the same conditions for the liquid cultures to purify the PNSB. Streaking and incubation were repeated until a pure culture was obtained. The pure culture of each isolate was maintained as a stock culture by stabbing it in GA agar and storing at 4 °C, or re-suspending the colonies from the agar in 20% glycerol and storing at −80 °C until used.

Each stock culture was subcultured twice to obtain an active inoculum which was then inoculated into GA broth

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