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Article

Ubiquitous Presence and Activity of Thiosulfate Oxidizing Bacteria in Rhizosphere of Economically Important Crop Plants of Korea

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The presence of thiosulfate oxidizing bacteria was examined in rhizosphere soils of 19 economically important plant species belonging to 10 different families. The results showed that the thiosulfate oxidizing bacteria were present in all the tested rhizosphere soils, and the total 32 thiosulfate oxidizing bacteria were recovered. Furthermore, the biochemical characterization revealed that 56% and 44% of the isolates belonged to the obligate chemolithoautotrophs and facultative heterotrophs, respectively. The isolates ATSR15P utilized 19.17 mM of thiosulfate and accumulated 11.65 mM of sulfate in the medium. Concurrently, the decrease in pH of the medium was observed. This study comprehensively demonstrates that the active sulfur oxidation is a ubiquitous phenomenon in the rhizosphere of crop plants in Korea.

Key words: Facultative heterotrophs, Obligate chemolithoautotrophs, Rhizosphere, Thiosulfate oxidizing bacteria

Introduction

Biological oxidation of hydrogen sulfide or sulfur is abundant in soil and water, and is the major reaction in volcanic and other environments. The oxidation reactions in these ecosystems are performed by prokaryotes of the domains archaea and bacteria. The sulfur oxidizing bacteria are phylogenetically diverse, and belong to several genera (Friedrich et al., 2001, 2005).

The rhizosphere, a complex habitat defined as the fraction of soil adhering to plant roots, is characterized by high microbial densities (Hiltner, 1904). Microbiological investigations pertaining to rhizosphere are largely carried out from the perspectives of biological nitrogen fixation and other nutritional facets of plant growth promotion or bio-control, but this microhabitat was seldom viewed as a potential seat of microbial sulfur oxidation by aerobic chemolithotrophs and or photolithotrophs (Ghosh and Roy, 2006b). However, a few studies have reported the existence of sulfur oxidizing bacteria in the rhizosphere of different plant species (Ghosh and Roy, 2006a, 2006b, 2007; Ghosh et al., 2005, 2006).

Since the functional genes of sulfur oxidation pathways are not conserved and have only recently started to become subject for molecular analysis (Friedrich et al., 2001, 2005), culture independent approach is not yet available for diversity analysis of sulfur oxidizing bacteria. Therefore, the traditional methods of enrichment and isolation in pure culture remain the main approach to study the sulfur oxidizing bacteria in various environments (Sorokin et al., 2006).

The purpose of the present study was to investigate the occurrence of thiosulfate oxidizing bacteria in the rhizosphere of economically important crop plants of Korea and to study their thiosulfate oxidation ability under *in vitro* conditions.

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Materials and Methods

Rhizosphere soil sampling and enrichment isolation
Twenty one rhizosphere soil samples from 19 economically important crop plants belonging to 10 different families were collected from the cultivated fields in Jungha- dong, Kimhae (E 128° 49′ - 128° 56′ , N 35° 10′ - 35° 17′), Kyungnam Province, Republic of Korea (Table 1 & 2). Five plants of each plant species at their flowering stage were selected randomly from a single row at 3 m intervals, then removed from the soil with a trowel, placed in a sterile bag. The collected samples were immediately returned to laboratory and processed. The plants were shaken gently to separate the soil not tightly adhering to the roots. The rhizosphere soils (attached to the roots after gentle shaking) and fine roots

Table 1. Physico-chemical properties of the soil used for isolation of thiosulfate oxidizing bacteria.

Rhizosphere soil sample	Physical properties of the soil						Chemical properties of the soil			
	pH	EC	Soil texture (%)			Texture classification	Organic matter	Total Nitrogen	Total phosphorous	Total SO_4-S
			Sand	Silt	Clay					
		dS m^{-1}					%	%	mg kg^{-1}	mg kg^{-1}
Paddy c.v. Nampyeong	6.0	327	21.7	32.4	45.9	Clay	3.4	0.24	132.2	58.7
Paddy c.v. Unbong	6.4	136	20.6	34.8	44.6	Clay	3.0	0.18	157.6	15.8
Paddy c.v. Danjing	6.9	265	30.8	32.0	37.2	Clay loam	5.0	0.33	163.2	67.0
Maize	8.0	133	73.4	13.6	13.0	Sandy loam	1.8	0.09	295.7	2.04
Onion	6.2	381	74.0	11.6	14.4	Sandy loam	2.0	0.18	1031.3	5.79
Garlic	7.8	108	75.0	12.0	13.0	Sandy loam	0.64	0.05	236.5	2.88
Lettuce	7.4	175	42.9	48.0	9.10	Loam	1.91	0.11	168.8	24.1
Sunflower	6.7	41	63.0	16.4	20.6	Sandy clay loam	1.79	0.13	602.9	0.79
Egg plant	8.0	167	75.3	11.6	13.1	Sandy loam	1.09	0.10	166.0	3.29
Tobacco	8.2	57	73.0	14.8	12.3	Sandy loam	0.82	0.06	306.9	5.79
Red pepper	6.4	184	26.9	33.2	39.9	Clay loam	3.97	0.28	698.7	33.3
Potato	6.9	313	22.4	31.6	46.0	Clay	4.88	0.30	126.6	77.5
Chinese cabbage	6.7	817	29.9	28.0	42.1	Clay	5.56	0.36	1138.4	335.0
Radish	6.9	78	68.4	15.6	16.0	Sandy loam	1.53	0.11	656.4	1.63
Red bean	7.4	522	28.8	22.4	48.8	Clay	3.31	0.20	1025.6	207.5
Peanut	7.4	45	31.3	17.6	21.2	Sandy clay loam	2.06	0.16	543.7	1.21
Soybean	6.2	83	21.3	33.2	45.5	Clay	2.05	0.15	106.8	40.0
Sesame	6.1	311	40.8	26.0	33.2	Clay loam	5.34	0.32	160.4	111.2
Sweet Potato	7.2	66	82.1	12.0	5.90	Loamy sand	1.76	0.12	329.5	1.21
Ginseng	7.4	15	75.3	12.8	12.0	Sandy loam	0.89	0.07	301.3	32.0
Carrot	7.2	202	30.6	29.6	39.8	Clay loam	3.95	0.28	129.4	67.5

Values in each column are the mean of four replications.

(approximately 1 cm length) were collected from 5 plants and combined into one composite sample and then homogenized thoroughly by hand. Likewise, 4 replicated samples, in total, were prepared from each plant species, and those samples were used for subsequent enrichment isolation experiments.

For the isolation of thiosulfate oxidizing bacteria, 10 g each rhizosphere soil sample was added into 100 mL of liquid mineral salts thiosulfate (MST) medium containing (g l^{-1}) NH_4Cl, 1.0; K_2HPO_4, 4.0; KH_2PO_4, 1.5; $MgSO_4 \cdot 7H_2O$, 0.5; $Na_2S_2O_3 \cdot 5H_2O$, 5.0; yeast extract, 0.05; bromocresol purple, 0.002; trace element solution 5 ml; pH 6.5 (Mukhopadhyaya et al., 2000) and incubated at 30℃ on a rotary shaker in the dark to avoid the growth of phototrophic bacteria until the color of the bromocresol purple was changed to yellow. For the isolation of pure cultures, 10 fold dilutions (10^2 - 10^6) were made in the sterile deionized water and 0.2 mL of the aliquots were spread with sterile glass rod over the surface of MST agar plates. The colonies that developed yellow halo against purple background, which indicates the production of sulfuric acid by the oxidation of thiosulfate, were picked up and streaked on solid MST medium until uniform colony morphology was observed. Colonies were transferred atleast 6 times to make it sure that they can be considered as pure. In addition, the purity of the strains was checked microscopically. The pure bacterial strains were maintained on MST agar plates, subcultured every week and subjected to further studies.

Soil physical and chemical analysis Soil samples were dried, sieved (<2 mm mesh sieve), and used for analysis. Grain-size analysis was performed after the destruction of organic matter by wet sieving on a 63 μm sieve. After drying, the coarse residue was separated by sieving. The fine fractions (silt and clay) were determined by the pipette method. Soil pH and EC was measured in water (1:5) after a equilibration time of 15 min. Total carbon (Walkley- Black) and total nitrogen (Kjeldahl) were according to Black et al. (1965). Total-P was measured by the Lancaster method as outlined by Cox (2001). Sulfate sulfur of the soil was extracted with ammonium acetate and determined turbidometrically (Pramer and Schmidt, 1964).

Table 2. Ubiquitous presence of thiosulfate oxidizing bacteria in the rhizosphere soils of crop plants of Korea.

Details of rhizosphere soil samples			Presence of thiosulfate oxidizing bacteria	Isolates recovered
Family	Common name/ cultivar	Botanical name		
Poaceae				
	Paddy c.v. Nampyeong	*Oryza sativa* ssp. *japonica*	+	7CP, 7DP, 7D2P, 4P, 5EP, 7P, 7PH1, 7PH2
	Paddy c.v. Unbong	*Oryza sativa* ssp. *japonica*	+	8BP
	Paddy c.v. Danjing	*Oryza sativa* ssp. *japonica*	+	ATSR6
	Maize	*Zea mays*	+	1BP, ATSR13, 3D1P
Alliaceae				
	Onion	*Allium cepa*	+	ATSR15P
	Garlic	*Allium sativum*	+	6P
Astraceae				
	Lettuce	*Lactuca sativa*	+	ATSR24P
	Sunflower	*Helianthus annus*	+	3HP
Solanaceae				
	Egg plant	*Solanum melongena*	+	ATSR30P
	Tobacco	*Nicotiana tabacum*	+	5D2P
	Red pepper	*Capsicum annuum*	+	ATSR17
	Potato	*Solanum tuberosum*	+	ATSS4
Brassicaceae				
	Chinese cabbage	*Brassica rapa*	+	4EP
	Radish	*Raphanus sativus*	+	ATSR22
Fabaceae				
	Red bean	*Vigna angularis*	+	ATSR29P
	Peanut	*Arachis hypogaea*	+	22P
	Soybean	*Glycine max*	+	ATSS2, 11D1P
Pedaliaceae				
	Sesame	*Sesamum indicum*	+	7B
Convolvulaceae				
	Sweet Potato	*Ipomea batatas*	+	ATSRP1, ATSSP1
Araliaceae				
	Ginseng	*Panax ginseng*	+	ATSR11
Apiaceae				
	Carrot	*Daucus carota*	+	ATSR32

Morphological and physiological characterization of thiosulfate oxidizing bacteria Physiological and biochemical characteristics of thiosulfate oxidizing bacterial strains were examined according to Bergey's manual of determinative bacteriology (Holt et al., 1994). Gram staining was performed with a Gram stain kit (Difo Laboratories, Detroit, Michigan, USA). Qualitative test for production of oxidase and catalase was performed with Difco strips as per the manufacturer recommendation. Growth under heterotrophic conditions was monitored for 10 d at 30℃ in mineral salts medium without thiosulfate amended with several organic compounds: sodium succinate, sodium acetate, sodium citrate, D- glucose, D- fructose, ***α***-lactose, D- mannose, sucrose, xylose, glycerol, mannitol, L- glutamic acid, sorbitol, malic acid, L- glutamine, L- cysteine, yeast extract, methanol, and starch. Each compound was added at a concentration of 0.2% (w/v) for carbohydrates and 0.1% (w/v) for organic acids, sugar alcohols and yeast extract. In addition, mixotrophic growth of bacteria was examined in mixotrophic medium as described by Ghosh et al. (2005).

Growth experiments and assay of inorganic sulfur compounds The batch culture experiment was performed in order to examine thiosulfate consumption and sulfate accumulation by thiosulfate oxidizing bacteria. The bacterial strains were grown in 250 ml of Erlenmeyer

flasks containing 100 ml of MST medium at 30℃ on shaking incubator at 120 rpm for 4 d. Thiosulfate consumption was assayed spectrophotometrically by the cyanolytic method (Kelly et al., 1969; Kelly and Wood, 1994). The sulfate content of the medium was determined as per the method of Kolmert et al. (2000).

Results and Discussion

Thiosulfate is used by the most of the sulfur oxidizing bacteria including chemolithotrophic and chemoorganotrophic bacteria. Thiosulfate is a stable sulfur compound of intermediate oxidation state and fulfills an important role in natural sulfur cycle (Podogoresk and Imhoff, 1999), thus they are most suitable for the investigation of sulfur lithotrophic process (Mukhopadhayaya et al., 2000). Therefore, in this study thiosulfate was used as model sulfur compounds for isolation and cultivation of aerobic sulfur oxidizing bacteria from rhizosphere soils of crop plants.

Physico-chemical properties of the soils collected from the rhizospheres are given in Table 1. Soil pH ranged from 6.01 (paddy field) to 8.20 (tobacco field). Organic matter content ranged from 0.82% to 5.56%, whereas the total sulfate-sulfur content ranged from 0.79 to 335 mg kg-1. Generally the major mineral fraction in soils was sand. However, paddy, red pepper, potato, Chinese cabbage, red bean, and soybean fields showed high significant high clay. On the other hand, lettuce field was abundant in silt. In the present study, the thiosulfate oxidizing bacteria were isolated from all the tested rhizosphere soils (Table 2). In total, 32 thiosulfate oxidizing bacterial strains were isolated, of which 8 isolates were recovered from paddy cv. Nampyeong followed by 3 isolates from maize.

With the exception of the isolates 8BP, ATSSP1 and ATSS2 all the other isolated bacteria were Gram Negative rods (Table 3). In addition, all the tested isolates could grow chemolithoautotrophically with thiosulfate. All the isolates were positive for aerobic nitrate reduction, oxidase (except 4EP & ATSS2) and negative for starch hydrolysis (Table 3). In the previous studies, sulfur oxidizing bacteria, such as *Thiobacillus, Bosea thiooxidans, Pseudaminobacter salicylatoxidans, Paracoccus bengalensis, Mesorhizobium thiogangeticum, Tetrathiobacter kashmirensis, Paracoccus pantotrophus,* and *Paracoccus thiocyanatus* have been isolated from the rhizosphere and bulk soils of agricultural fields of India (Rupela and Tauro, 1973; Das et al., 1996; Deb et al., 2004; Ghosh and Roy, 2006a, 2006b, 2007; Ghosh et al., 2005, 2006). The presence of *Thiobacillus thioparus* was reported in Canadian and Scottish agricultural soils (Germida et al., 1985; Chapman, 1990). Also the thiosulfate oxidizing bacteria such as *Xanthobacter tagetidis* and *Methylobacterium thiocyanatum* were isolated from rhizosphere of marigold and Persian onion, respectively (Padden et al., 1997; Wood et al., 1998).

Root exudates fluctuate in response to changes in plant growth and physiology (Jaeger et al., 1999), and the composition of the exudates has been shown to exert selective effects on bacterial groups (Marilley and Arango, 1999; Smit et al., 2001). Such selective effects are some times evident from the cultivar level (Dalmastri et al., 1999; Kinkel et al., 2000; Marschner et al., 2001). Similarly, remarkable variety differences in root associated diazotrophic communities of lowland rice were reported (Knauth et al., 2005). However, in the present study, there was no apparent correlation between the isolated thiosulfate oxidizing bacteria and soil type, plant species, or their families.

The plant-associated habitat is a dynamic environment in which many factors may affect the structure and species composition of the bacterial communities that colonize the rhizosphere. Some of these factors exhibit seasonal variations depending on plant species, cultivar and soil type (Kuklinsky-Sobral et al., 2004). Various physio-chemical properties of soil (e.g., water content, particle size, organic matter content, soil nutrients and applied fertilizers) interact each other in a complex manner, and this in turn determine the spatial patterns of microorganisms diversity around the roots (Unno et al., 2005).

Thiosulfate oxidation and chemolithotrophic growth by Gram positive bacteria is an unusual phenomenon in general, however, our results clearly showed that Gram positive strains (8BP, ATSSP1 and ATSS2) could grow chemolithoautotrophically with thiosulfate. Hudson et al. (1988) documented the autotrophic growth of Gram positive *Bacillus schlegelii* with thiosulfate. The isolates could grow autotrophically with thiosulfate also grow in the medium without thiosulfate in the presence of organic compounds. Hence, these isolates are facultative heterotrophs.

In this study, 56% and 44% of isolated bacteria were obligate autotrophs and facultative heterotrophs,

Table 3. Morphological and physiological characterization of thiosulfate oxidizing bacteria isolated from rhizosphere of crop plants.

Strain Name	Colony morphology	Cell shape	Gram's reaction	Oxidase	Catalase	Denitrification*	Starch hydrolysis	Mixotrophic growth**	Autotrophic growth
1BP	a	rod	- Ve	+	-	+	-	+	+
7CP	a	rod	- Ve	+	-	+	-	+	+
7DP	a	rod	- Ve	+	-	+	-	+	+
7D2P	a	rod	- Ve	+	-	+	-	+	+
ATSR15P	a	rod	- Ve	+	-	+	-	+	+
ATSR24P	a	rod	- Ve	+	-	+	-	+	+
ATSR29P	a	rod	- Ve	+	-	+	-	+	+
ATSR30P	a	rod	- Ve	+	-	+	-	+	+
3D1P	a	rod	- Ve	+	-	+	-	+	+
3HP	b	rod	- Ve	+	-	+	+	+	+
4P	a	rod	- Ve	+	-	+	-	+	+
4EP	a	rod	- Ve	-	-	+	-	+	+
5D2P	b	rod	- Ve	+	+	+	-	+	+
5EP	a	rod	- Ve	+	-	+	-	+	+
6P	a	rod	- Ve	+	-	+	-	+	+
7B	b	rod	- Ve	+	-	+	-	+	+
7P	a	rod	- Ve	+	-	+	-	+	+
7PH1	a	rod	- Ve	+	-	+	-	+	+
7PH2	a	rod	- Ve	+	-	+	-	+	+
8BP	c	rod	+ Ve	+	+	+	-	+	+
11D1P	a	rod	- Ve	+	-	+	-	+	+
22P	d	rod	- Ve	+	+	+	-	+	+
ATSRP1	a	rod	- Ve	+	+	+	-	+	+
ATSSP1	c	rod	+ Ve	+	-	+	-	+	+
ATSR6	e	rod	- Ve	+	+	+	-	+	+
ATSR11	e	rod	- Ve	+	+	+	-	+	+
ATSR13	e	rod	- Ve	+	+	+	-	+	+
ATSR17	e	rod	- Ve	+	+	+	-	+	+
ATSR22	e	rod	- Ve	+	+	+	-	+	+
ATSR32	e	rod	- Ve	+	+	+	-	+	+
ATSS 2	f	rod	+ Ve	-	+	+	-	+	+
ATSS4	e	rod	- Ve	+	+	+	-	+	+

a- Small, white yellowish smooth entire colonies in which sulfur is deposited; b- Smooth circular, brown entire colonies; c- White colonies of 2 day old culture turn to pale brown, glossy smooth entire; d- Color less, smooth, circular; e- Shiny entire raised creamy white head; f - Circular, slightly, convex with entire margins shiny, moist and the pigment of colonies was translucent and became lemon yellow in old cultures. * Denitrification was assayed in mineral salt medium with 20mM of thiosulfate as electron donor and 25 mM of potassium nitrate in aerobic condition ; ** Mixotrophic growth was assessed in mineral salt medium with 20 mM of thiosulfate and 18.5mM of sodium succinate. + Presence of growth or trait; - Absence of growth or trait.

respectively (Table 4). The isolates could grow autotrophically, implying their ability to switch their metabolism to chemoorganotrophy. This might help these organisms to sustain their activity. In addition, mixotrophic life might be the preferred metabolic traits of these organisms. Since low concentrations of sulfur compounds potentially limit the growth, utilizing organic carbon or even co-oxidation of sulfur compounds together with organic substrates for biomass synthesis can assure better survival and growth of sulfur oxidizing bacteria in the rhizosphere (Graff and Stubner, 2003).

The isolate ATSR15P consumed approximately 96% of the supplied thiosulfate and accumulated about 11.65 mM of sulfate in the medium (Table 5). Accordingly, concurrent decrease in the pH of the medium was observed. Sulfur is an essential plant nutrient and taken up by the plants in the form of sulfate (Scherer, 2001). Sulfur oxidizers enhance the rate of sulfur oxidation and the production of sulfates thus making them available to plants at their critical stages, consequently resulting in increased yield (Wainwright, 1984; Anandham et al., 2007). Thiosulfate oxidizing bacteria isolated in this study can contribute to the enhancement of sulfur

Table 4. Utilization of different carbon source by thiosulfate oxidizing bacteria isolated from rhizosphere of crop plants of Korea.

Bacterial strains	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1BP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7CP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7DP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7D2P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ATSR15P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ATSR24P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ATSR29P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ATSR30P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3D1P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3HP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4EP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5D2P	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5EP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7PH1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7PH2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8BP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11D1P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22P	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ATSRP1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ATSSP1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ATSR6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ATSR11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ATSR13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ATSR17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ATSR22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ATSR32	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ATSS 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ATSS4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

1. Succinate; 2. Acetate; 3.Citrate; 4.D- Glucose; 5.D- Fructose; 6.α-Lactose; 7.D-Mannose; 8. Sucrose; 9.Xylose; 10.Glycerol; 11.Mannitol; 12.L-Glutamic acid; 13.D-Sorbitol; 14.D-Malic acid; 15.L-Glutamine; 16.Yeast extract; 17. Methanol;8. L-Cysteine; 19.Starch; Carbohydrates 2 g / L; Organic acid 1 g/ L; Glycerol and methanol 0.5% and yeast extract 5 g/ L were supplemented in mineral salts medium without thiosulfate. All cases 50 mg of yeast extract was added as growth factor. + Presence of growth; - Absence of growth.

availability in a similar way.

Phosphorous (P) is one of the most important plant nutrients and a large proportion of inorganic phosphates applied to soil as fertilizer are rapidly immobilized after application and becomes unavailable to plants (Nautiyal, 1999). Final end product of sulfur oxidation is sulfuric acid and it may solubilize the fixed P in the soil (Stamford et al., 2007).

Our observations confirmed that active sulfur oxidation is a ubiquitous phenomenon in the rhizosphere of different crop plants growing in Korean soils. To best of our knowledge, this is the first comprehensive report on universal existence of thiosulfate oxidizing bacteria. Because plants as well as the soil-dwelling microorganisms obtain sulfur necessary for the synthesis of sulfur-containing amino acids through the assimilatory sulfate reduction, it is likely that plants tend to develop close rhizospheric association with aerobic species of chemolithoautotrophic sulfur oxidizing microorganisms.

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Table 5. Sulfate production and Thiosulfate consumption of thiosulfate oxidizing bacteria isolated from crop plant of Korea.

Isolates	Sulfate production (mM)	Reduction in pH†	Thiosulfate consumption (mM)‡
1BP	9.23	3.61	18.87
7CP	5.19	4.42	18.65
7DP	7.65	5.70	17.37
7D2P	10.86	3.27	19.09
ATSR15P	11.65	3.10	19.17
ATSR24P	8.41	3.18	18.90
ATSR29P	10.86	3.21	19.06
ATSR30P	11.14	3.30	19.04
3D1P	6.63	3.17	15.93
3HP	5.92	3.43	16.19
4P	6.47	4.61	13.91
4EP	5.98	4.51	14.66
5D2P	8.17	4.61	12.82
5EP	8.57	2.95	16.26
6P	8.34	3.13	15.97
7B	8.34	3.10	16.02
7P	6.90	5.83	10.67
7PH1	4.78	3.02	15.55
7PH2	9.35	2.94	15.98
8BP	9.37	5.50	12.13
11D1P	3.58	6.00	10.51
22P	7.78	2.98	17.05
ATSRP1	9.37	5.50	12.13
ATSSP1	3.58	6.00	10.51
ATSR6	13.69	5.76	16.56
ATSR11	15.47	6.00	16.25
ATSR13	13.69	6.00	16.97
ATSR17	19.02	5.73	17.32
ATSR22	14.13	5.61	17.18
ATSR32	21.69	5.71	16.30
ATSS 2	21.69	6.46	15.99
ATSS4	19.91	5.73	17.29

† Initial pH of the medium 6.50;

‡ Initial amount of thiosulfate supplemented in medium 20 mM and assay was carried out end of the day 4. Values in each column mean of three replications of two experiments.

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국내 작물 근권에 서식하는 황산화세균의 분포와 합성

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식물에 필수영양소인 황은 대부분 sulfate의 형태로 식물이 흡수하며, thiosulfate 형태로는 영양소로서 흡수하지 못한다. 황산화세균은 이러한 thiosulfate를 산화시켜 sulfate로 만들어 준다. 국내 토양에서 황산화세균의 분포를 조사하기 위하여 경제적으로 중요성을 갖는 19가지 작물의 근권에서 토양을 채취하였다. 항산화세균은 조사한 모든 작물의 근권에서 존재하였으며, 황산화능이 우수한 32가지의 황산화세균을 분리하였다. 또한 분리 균주의 생화학적 특징을 검토한 결과 32종 중 56%가 필수 화학합성자가생물이었으며, 44%가 기생 종속영양생물이었다. 분리 균주 ATSR15P는 배양과정에서 19.2 mM의 thiosulfate를 사용하였고, 11.7 mM의 sulfate를 축적하였다. 또한 ATSR15P 배양 과정 중 배지의 pH가 6.5에서 3.1로 감소하였다. 본 연구에서는 황산화세균에 의한 황의 산화가 국내 작물의 근권에서 포괄적으로 나타나는 현상이라는 것을 증명하고 있다.