Antiviral Activity of the Exopolysaccharide Produced by *Serratia* sp. Strain Gsm01 Against Cucumber Mosaic Virus

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The potential of the exopolysaccharide (EPS) from a *Serratia* sp. strain Gsm01 as an antiviral agent against a yellow strain of Cucumber mosaic virus (CMV-Y) was evaluated in tobacco plants (*Nicotiana tabacum* cv. Xanthi-nc). The spray treatment of plants using an EPS preparation, 72 h before CMV-Y inoculation, protected them against symptom appearance. Fifteen days after challenge inoculation with CMV-Y, 33.33% of plants showed mosaic symptoms in EPS-treated plants compared with 100% in the control plants. The EPS-treated plants, which showed mosaic symptoms, appeared three days later than the controls. The enzyme-linked immunosorbent assay (ELISA) and reverse transcriptase polymerase chain reaction (RT-PCR) analyses of the leaves of the protected plants revealed that the EPS treatment affected virus accumulation in those plants. Analysis of phenylalanine ammonia lyase, peroxidase, and phenols in protected plants revealed enhanced accumulation of these substances. The pathogenesis-related (PR) genes expression represented by PR-*1b* was increased in EPS-treated plants. This is the first report of a systemic induction of protection triggered by EPS produced by *Serratia* sp. against CMV-Y.

Keywords: Exopolysaccharide (EPS), Cucumber mosaic virus (CMV-Y), *Serratia*

Viruses are one of the most destructive plant pathogens, affecting many economically important crops in the world. Conventional breeding procedures along with transgenic plants containing genes to resist viruses have been developed and employed to control these diseases.

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Most of the plants possess defense mechanisms against pathogen attack, which when triggered by a stimulus prior to the pathogen attack, reduces the disease [13, 16, 34]. The stimulus can increase the concentration of existing defense compounds that the plant possesses or induce production of new defensive structures and chemicals. Some of the stimuli-inducing protection in plants includes some plant extracts, synthetic chemicals, and rhizobacteria. Spinach and rhubarb leaf extracts are known to induce protection against *Colletotrichum lagenarium* in cucumber [8]. Several chemicals like salicylic acid and its chemical analog, benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), have been known to induce protection against CMV infection in tomato [9] and *Arabidopsis* [30, 31].

Nonpathogenic rhizobacteria can induce protection against a wide variety of pathogens, known as induced systemic resistance (ISR). *Pseudomonas aerugenosa* 7NSK2 strain is known to elicit induced protection against TMV [7]. *P. fluorescens* CHA0 was shown to induce resistance against Tobacco necrotic virus (TNV) in tobacco [20].

Among *Serratia* species, *Serratia marcescens* is an important bacterium that is reported to induce systemic resistance to various pathogens. The *S. marcescens* B2 is known to induce resistance in rice against *Rhizoctonia solani* AG-1 IA [33]. *S. marcescens* NBRI1213 protects betel vine against foot and root rot caused by *Phytopthora nicotianae* [15]. The strain *S. marcescens* 90-166 is known to protect plants against fungal pathogens, *Fusarium oxysporum* f. sp. *cucumerinum* [19], *C. orbiculare* [33], bacterial pathogens, *P. syringae* pv. *lachrymans* [19], and Cucumber mosaic virus [29, 30].

ISR in plants against various pathogens is induced by lipopolysaccharide (LPS), salicylic acid, siderophores, and exopolysaccharides [11, 17, 28]. Bacterial outer membrane LPS from *P. fluorescence* WC417 induced resistance against *Fusarium* wilt in carnations [17]. Crude EPS from

Xanthomonas campestris is known to induce resistance in coffee [11] and turnip [27]. The LPS from *S. marcescens* is known to possess inducer activity in tobacco against *P. solanacearum* [10].

The induced resistance leads to the enhanced PR gene transcript and defense-related substances. Many of these substances are known to be involved in defense reaction against plant pathogens. These include oxidative enzymes such as peroxidase (PO), which are implicated in the formation of phenols contributing to the synthesis of defense barriers for the cells [1]. Enzymes such as phenylalanine ammonia lyase (PAL) mediate phenolic compound biosynthesis. These enzymes have been correlated with defense against pathogens in several plants [3].

We have previously shown that the culture filtrate from *Serratia* sp. strain Gsm01 controls CMV infection in tobacco [12]. The aim of this study was to test the role of EPS from *Serratia* sp. strain Gsm01 in induced systemic protection in tobacco against CMV-Y under greenhouse conditions, and investigate its effect on plant defense-related genes and compounds.

MATERIALS AND METHODS

Isolation of EPS

The *Serratia* sp. strain Gsm01 was originally isolated from the ginseng rhizosphere in a field at Hongcheon, Kangwon Province, Republic of Korea [12]. The strain was maintained on mannitol glutamate yeast extract (MGY) agar media [14].

The EPS was isolated from *Serratia* sp. strain Gsm01 culture broth (2 l), as described previously [22], with some modifications. Briefly, the strain was cultured for 48 h on a rotary shaker at 28°C. The culture supernatant was obtained by centrifugation at 12,000 rpm for 10 min at 4°C and concentrated to approximately $1/10^{th}$ volume by freeze drying. The exopolysaccharide was precipitated by adding a double volume of ethanol. The mixture was kept at 4°C for 12 h and centrifuged (12,000 rpm, 10 min). The pellet was collected and air dried at room temperature and suspended into 10 mM phosphate buffer, pH 7.0. It was re-precipitated using a double volume of ethanol and redissolved in 10 mM phosphate buffer. The procedure was repeated twice. The pellet was then treated with DNase (2 U/ml), RNase (10 µg/ml), and Protease (20 µg/ml), and dialyzed against 10 mM phosphate buffer (MWCO 12,000) for 24 h, followed by lyophilization.

Maintenance of Virus Inoculum

CMV-Y was originally obtained from the virus collection of the College of Forestry Sciences, Kangwon National University, and used in all the experiments. The virus was maintained in tobacco plants (*Nicotiana tabacum* cv. Xanthi-nc). The CMV inoculum used throughout the experiments consisted of infected tobacco leaf tissue ground in 0.01 M sodium phosphate buffer, pH 7.0, containing 0.002 M EDTA (1 g tissue: 50 ml sodium phosphate buffer). All inoculation materials were chilled at 4°C prior to inoculation and maintained on ice during inoculation. Plants were inoculated at 4 weeks after planting by rub inoculation onto the oldest leaf.

Treatment of EPS

The tobacco plants (*N. tabacum* cv. Xanthi-nc) were grown in the pots containing artificial soil. They were maintained in a greenhouse at 20–25°C, 70% humidity, and light/dark cycle of 15/9 h. Experiments were performed on tobacco plants, each having four to five leaves.

A fresh solution of EPS at a final concentration of 200 ppm in distilled water was applied as spray to the leaves of tobacco plants. Control plants were sprayed with distilled water. Seventy-two hours after EPS treatment, plants were inoculated with CMV-Y, using sterile cotton buds soaked in virus preparation containing carborundum-600 mesh powder, on the abaxial surface of a lower leaf. Plants were placed in the greenhouse and observed for symptom appearance. The experiment also included plants treated with EPS (200 ppm) and nontreated plants, which were mock-inoculated with 0.01 M sodium phosphate buffer, pH 7.0, containing 0.002 M EDTA, in the same manner as plants inoculated with CMV-Y.

Up to 15 DPI (days post inoculation), disease incidences were observed every day as the number of plants showing yellow mosaic symptoms on at least one leaf. Disease incidence was expressed as the percentage of plants that developed symptoms among the number of plants inoculated with CMV-Y on the 15th DPI.

Enzyme-linked Immunosorbent Assay (ELISA)

Sandwich enzyme-linked immunosorbent assay (ELISA) as described previously [5] was used to determine CMV-Y concentration. The uppermost expanded leaves of tobacco plants were collected at 6 and 15 DPI, and sap was expressed using phosphate buffer saline containing 0.05% Tween-20 (PBS-T) at a ratio of 1:10 (w/v). Falcon flat-bottom plates were coated with anti-CMV IgG obtained from Agdia Inc. Indiana (U.S.A.) and diluted at 1:200 in phosphate buffer. Plates were incubated for 4 h at room temperature (RT). Antigen samples were incubated in the coated plate at RT for 2 h before adding alkaline phosphatase-conjugated IgG diluted at 1:200 in PBS-T. After a 2-h incubation at RT, substrate (*p*-nitrophenylphosphate at 1 mg/ml in diethanolamine, pH 9.8) was added and incubated at room temperature for 1 h. Absorbance values were determined at 405 nm.

Total RNA Preparation

Total RNA from the upper three leaves of tobacco plants under treatment were isolated using TRIzol reagent (Invitrogen Co., Carlsbad, CA, U.S.A.) according to the manufacturer's recommendations and treated with RNase-free DNase (Promega, U.S.A.) at 37°C for 10 min. It was extracted with saturated phenol, followed by phenol chloroform iso-amyl alcohol (25:24:1, vol/vol/vol), and chloroform iso-amyl alcohol (24:1, vol/vol). The RNA was precipitated by adding a double volume of 95% ethanol, followed by incubation at −20°C for 2 h. It was then centrifugation at 12,000 ×g for 20 min at 4°C, and quantified with a spectrophotometer (GeneQuant II, PharmaciaBiotech, U.K.).

CMV-Y Detection by RT-PCR

A reverse transcriptase (RT) reaction was performed on 1 µg of total RNA with 200 units of RNasin, Moloney murine leukemia virus (M-MLV) RTase (Promega Co. Madison, WI, U.S.A.), 100 mM dithiothreitol, 20 pmol P1 primer, developed from the RNA3 region of CMV (1,109–1,129 region, 5'-TAG TTT TGA GGT TCA ATT CC-3'), and 10 mM dNTPs, in a final volume

of 20 μl. The reaction was allowed at 42°C for 45 min, followed by 94°C for 3 min. The PCR reaction using cDNA thus obtained was performed with P1 and P2 (2,036–2,054 region, 5'GAC TGA CCA TTT TAG CCG3') primers designed from the RNA3 region of CMV, 20 pmol each. It was amplified for 35 cycles of 94°C for 1.5 min, 48°C for 30 s, and 72°C for 45 s, with a final extension at 72°C for 10 min. The RT-PCR fragments were separated by electrophoresis in 1% agarose gel and analyzed under UV light by comparing signal intensity.

PR-*1b* Gene Expression Analysis

Semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed to assess the gene expression, as described by Nei *et al.* [26], with some modifications. Total RNA (1 μg) in a 2.5-μl volume was mixed with 1 μl (1 μg) of hexanucleotides (Roche Diagnostics), incubated at 68°C for 8 min, and cooled on ice for 3 min. A total 6.5 μl of the RT mixture was added to obtain a final concentration of 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 10 mM DTT, 2.5 mM MgCl₂, 1.0 mM of dNTP (dATP, dTTP, dGTP, and dCTP), 5 units of RNasin ribonuclease inhibitor (Promega, U.S.A.), and 100 units of Moloney murine leukemia virus (M-MLV) RTase (Promega, U.S.A.). The samples were incubated at 42°C for 1 h and at 95°C for 2 min. Then, 40 μl of dH₂O was added to the cDNA mix, and the resulting cDNA solution was used for semiquantitative PCR analysis.

The PCR reactions were carried out as previously described [26] with some modifications. Two PCR reactions, one amplifying the cytochrome *c* oxidase subunit 1 (*COX1*) as a control gene, and the other amplifying the PR-*1b* gene, were performed. The primers for *COX1* (Accession No. BA000042), 5'CTAATTCTGGGCTAGA-CATTGC3' and 5'CTTCACATAGCTTTTCGTCTCC3', amplified a 500 bp fragment, and the primers for PR-*1b* (Accession No. X66942), 5'TAATAACGGTTACGTGATCA3' and 5'TGACTCA-TCACCTTGTTCACT3', amplified a fragment of 244 bp. The amplification was conducted in a volume of 25 μl containing 4-μl aliquots of cDNA mixture, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 100 mM of each dNTP, 0.1 μg of each sense and antisense primers, and 0.625 units of *Taq* polymerase (Biotools, Spain). Samples were amplified for 25, 30, and 35 cycles using a Thermocycler (Bio-Rad, U.S.A.) with DNA denaturing at 92°C, annealing at 60°C, and extension at 72°C, each for 45 s. A final elongation at 72°C for 10 min was also included. A total of 10 μl of amplification product was fractionated by electrophoresis on 1% agarose gel containing 0.5 μg/ml ethidium bromide, photographed under UV illumination, and analyzed based on the intensity of the signals. One-kb DNA marker (Promega, U.S.A.) was used to determine the size of the amplified product.

Phenylalanine Ammonia Assay

The enzyme assay for phenylalanine ammonia lyase (PAL) was determined as previously described [15]. Tobacco leaves were homogenized with liquid nitrogen using a sterile mortar and pestle. Homogenized slurry was added with the same volume of 10 mM sodium phosphate buffer (pH 6.0) at 4°C and centrifuged at 10,000 ×g for 20 min at 4°C. The supernatant was transferred to 1.5-ml eppendorf tubes and stored at -80°C. Bradford assay was used to test the protein concentration in the leaf extracts. The enzyme activity was measured in the leaves after 6 and 15 days in all treatments.

To determine PAL activity, 100 μl of leaf extracts was mixed with 900 μl of 6 μM L-phenylalanine in 0.5 M Tris-HCl buffer solution. The mixture was incubated at 37°C for 70 min. Absorbance was determined spectrophotometrically using a UVmini-1240 spectrophotometer (Shimadzu Corp. Japan) at 290 nm wavelength. The results were expressed as units PAL activity/mg protein.

Peroxidase Assay

The peroxidase (PO) assay was carried out as described by Shetty *et al.* [32] and modified by Nie *et al.* [25]. Leaf tissue was homogenized in ice-cold extraction buffer (50 mM potassium acetate, pH 5.2, 100 mM KCl, 1 M NaCl, 1 mM CaCl₂, and 1 mM ascorbic acid) using a mortar and pestle (0.1 g leaf tissue/ml). It was centrifuged at 12,000 ×g for 12 min at 4°C. The supernatant was transferred into a 1.5-ml microtube and stored. Total protein content was determined using the Bradford assay. The peroxidase activity was recorded spectrophotometrically at room temperature using the UVmini-1240 spectrophotometer (Shimadzu Corp. Japan). To 1 ml of substrate solution containing 100 mM sodium acetate, pH 5.5, 360 μM 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 0.63 mM H₂O₂, and 10 μl leaf extract were added. The absorbance at 405 nm (A_{405}) was recorded every 10 s up to 4 min. The change of A_{405} versus time was plotted, and the linear portion of the graph was used to calculate the enzyme activity, which was expressed as units/mg protein.

Phenol Content Assay

Phenol contents in the leaves were analyzed using Folin Ciocalteu's phenol reagent as described by Zieslin and Ben-Zaken [37]. The leaves were homogenized in 80% methanol at the rate of 10 mg/ml and kept at 70°C for 15 min with frequent agitation. A 200 μl extract was combined with 7 ml of distilled water and 250 μl of Folin Ciocalteu's phenol. The mixture was incubated at 25°C for 30 min. Later, 1 ml of a saturated solution of Na₂CO₃ and 1 ml of distilled water were added and the reaction mixture was allowed to stand for 1 h at 25°C. The absorbance A_{725} was recorded using the UVmini-1240 spectrophotometer (Shimadzu Corp. Japan) and total soluble phenols were calculated according to a standard curve obtained from a catechol solution, used as the standard. The phenol content was expressed as phenol equivalent mg/g of fresh leaf tissue.

RESULTS

Effect of EPS Treatment on Disease Incidence of CMV-Y in *N. tabacum* cv. Xanthi-nc

Numerous reports have suggested the use of nonpathogenic bacterial strains and their metabolites as resistance inducers to efficiently manage diseases caused by fungi, bacteria, and viruses. Most of these strains usually produce salicylic acid (SA), siderophores, and lipopolysaccharides (LPS) as primary determinants of their inducer function [17, 21]. Some strains of *Serratia*, when applied as an individual strain or along with other inducer strains, were reported to show activity in a number of crop plants against a wide range of pathogens including viruses [15, 18, 23, 24, 29, 30, 35, 36].

Table 1. Induction of systemic resistance in tobacco plants against CMV-Y.

Disease incidence (%) 15 DPI[b]				
		Trials		
Treatments[a]	1	2	3	Mean[c]
EPS treatment	30	40	30	33.33
Inoculated untreated	100	100	100	100

[a]Each treatment consisted of ten plants under each trial.
[b]Disease incidences were visually assessed on the 15th day and calculated as number percent of plants showing yellow mosaic symptoms under each treatment.
[c]Mean percentage of disease incidences in three trials.

The experiments were performed to evaluate the effect of EPS from *Serratia* sp. strain Gsm01 on tobacco plants, which included time of symptom appearance, disease incidences per number of plants treated, and the amount of CMV accumulated in EPS-treated and nontreated plants.

Treatment of 200 ppm of EPS as a spray to the leaves of tobacco plants prevented the appearance of disease symptoms caused by CMV-Y. Fifteen days after challenge inoculation by CMV-Y, the average number of plants showing disease symptoms in plants treated with EPS was 33.33% compared with a 100% disease incidence in the nontreated control population (Table 1). The onset of yellow mosaic symptoms in EPS-treated plants was delayed by three days compared with the nontreated plants in CMV-Y inoculated plants (data not shown).

Effect of EPS Treatment on CMV-Y Accumulation

CMV-Y accumulation in EPS-treated and nontreated plants was evaluated using ELISA and RT-PCR analyses. The results of ELISA represent the mean value for 10 samples in each treatment. When the mean ELISA absorbance value for those plants infected with CMV-Y at 6 DPI was compared, EPS treatment showed lower values in symptomatic plants. The nonsymptomatic leaves showed no viral symptoms. At 15 DPI, the symptomatic leaves under EPS treatment showed significantly lower virus content compared with
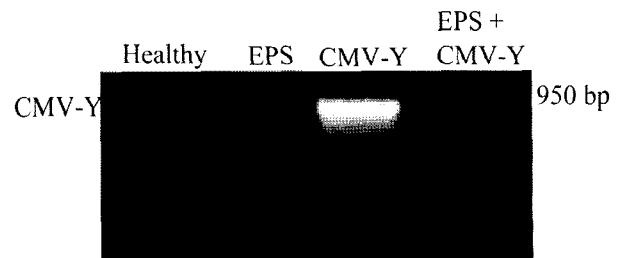
Fig. 1. Effect of EPS on accumulation of Cucumber mosaic virus Y in tobacco plants (*N. tabacum* cv. Xanthi-nc).
RNAs from the upper three leaves emerging above the treated and CMV-Y inoculated leaves along with nontreated CMV-Y-inoculated, EPS-treated, and healthy plants were used to analyze CMV-Y transcript accumulation. Reverse transcriptase polymerase chain reaction (RT-PCR) using the primer pairs targeting the RNA3 region of CMV-Y was performed at 35 cycles. A 10-μl aliquot of the PCR product was loaded on 1% agarose gel and band intensity was compared.

inoculated untreated plants. The nonsymptomatic leaves of tobacco, however, showed slightly elevated levels of CMV-Y compared with the controls. EPS treatment significantly reduced virus accumulation compared with the nontreated plants. The plants that showed yellow mosaic symptoms in EPS treatment had less concentration of virus compared with that of the control population showing mosaic symptoms (Table 2).

The genomic RNA that accumulated in the case of EPS-treated plants was determined by RT-PCR at 6 DPI. The band intensity of the RT-PCR products from EPS-treated, CMV-Y-inoculated plants were compared with that of the CMV-Y-inoculated plants on 1% agarose gel. It was found that the RNA accumulation was lower in EPS-treated plants compared with the controls (Fig. 1).

Effect of EPS Treatment on Phenylalanine Ammonia Lyase, Peroxidase, and Phenolics Level in Tobacco Plants

To reveal the possible involvement of plant defense enzymes in EPS-induced protection against CMV-Y in tobacco, the activities of phenylalanine ammonia lyase (PAL) and peroxidases (PO) along with phenol contents were monitored in all the treatments at 6 DPI.

Table 2. Enzyme-linked immunosorbent assay (ELISA) values of tobacco plants (*N. tabacum* cv. Xanthi-nc) treated with exopolysaccharide (EPS) and challenge-inoculated with Cucumber mosaic virus (CMV-Y).

	ELISA[b]			
	6 DPI		15 DPI	
Treatments[a]	S[c]	N	S	N
Uninoculated controls	–	0.153±0.003	–	0.211±0.009
EPS treatment	0.887±0.011	0.152±0.003	1.254±0.015	0.262±0.005
Inoculated untreated	0.925±0.013	0.157±0.003	1.625±0.003	–

[a]EPS treatment consisted of 200 ppm of exopolysaccharide prepared in distilled water and applied as spray to leaves of tobacco plants followed by CMV-Y inoculation after 72 h. Uninoculated controls were healthy tobacco plants of same age without any treatment.
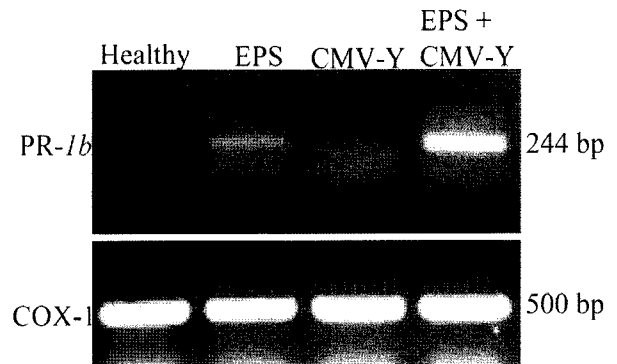[b]Enzyme-linked immunosorbant assay (ELISA) results are represented as absorbance values (405 nm) of means of an upper expanded leaf of each plant under observation.
[c]S=Symptomatic plants; N=nonsymptomatic plants.
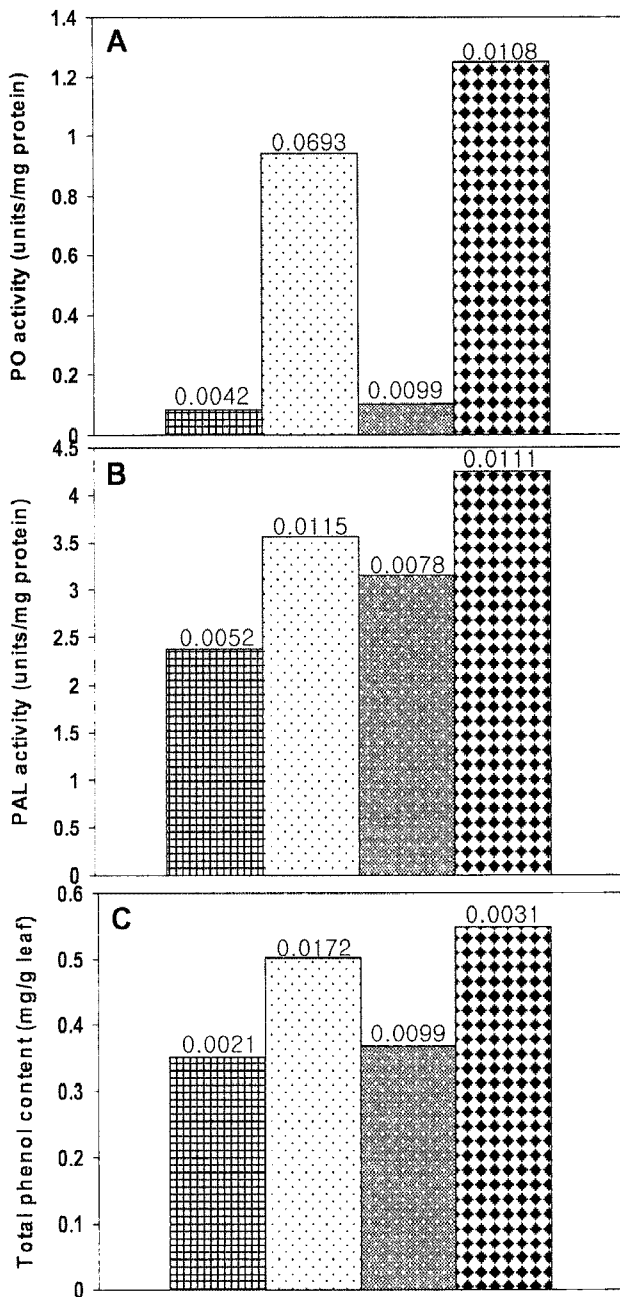
Fig. 2. Activities of plant defense-related enzymes [**A**. Peroxidase (PO); **B**. Phenylalanine lyase (PAL); and **C**. Phenol levels], after 6 and 15 DPI, in healthy (▦), EPS-treated (▤), CMV-Y-inoculated (▨), and EPS-treated followed by CMV-Y-inoculated (♦♦) leaves. The value on the top of each column indicates the standard deviation.

Fig. 3. Effect of EPS on accumulation of PR-*1b* genes in tobacco plants.

RNAs from three leaves emerging above the treated and CMV-Y-inoculated leaves along with nontreated CMV-Y-inoculated, EPS-treated and healthy plants were used to analyze PR-*1b* gene transcript accumulation. Semiconservative reverse transcriptase polymerase chain reactions (RT-PCR), including the primer pairs targeting the cytochrome *c* oxidase subunit 1 (COX-1) as a positive control and PR-*1b*, were performed for 25, 30, and 35 cycles. After 35 cycles, 10 µl of PCR product was loaded and analyzed on 1% agarose gel.

EPS-treated and EPS-treated, CMV-Y-inoculated plants was elevated compared with the healthy plants. The CMV-Y inoculated plants did not show any significant difference in phenol contents compared with the healthy plants (Fig. 2).

Effect of EPS Treatment on Pathogenicity related Gene Expression in Tobacco Plants

The expression of PR-*1b* genes was studied at 6 DPI. The expression of the gene was observed in the case of EPS-treated and EPS-treated plus CMV-Y-inoculated leaves, however, based on the band intensity of the expressed gene products, it was observed that the expression of the PR-*1b* gene was more in EPS-treated, CMV-Y-inoculated plants compared with EPS treatment alone. The expression was not observed in the case of healthy noninoculated and CMV-Y-inoculated leaves of tobacco plants (Fig. 3).

DISCUSSION

The EPS treatment on young *N. tabacum* cv. Xanthi-nc tobacco plants protected them against CMV. It was observed that the induction of resistance in tobacco plants triggered by EPS treatment resulted in delayed symptom appearance. Similar delay in symptom appearance was observed by Bergstrom *et al.* [2] in the case of induced cucumber plants when challenged with CMV. The EPS treatment in tobacco plants reduced disease incidences up to 70% as compared with the nontreated control plants. These data were in accordance with the two previous studies [29, 9], where induction of the cucumber and tomato plants reduced CMV disease incidences.

Peroxidase activity was increased by 11-fold in EPS-treated plants and 14-fold in EPS-treated, CMV-Y-inoculated tobacco plants. There was no significant difference between healthy controls and CMV-Y-inoculated plants. PAL activity increased in the EPS-treated plants compared with the healthy plants. It was higher in EPS-treated, CMV-Y-inoculated tobacco plants. A slight increase in PAL activity was observed in the CMV-Y-inoculated plants compared with the healthy plants. The total phenol contents in both

The data clearly indicate that EPS treatment has affected virus replication and movement, since the majority of plants did not show viral coat protein in systemically protected plants at 6 DPI. Some plants that showed mosaic symptoms even after treatment with EPS had lower viral coat protein contents than nontreated plants. This information indicates that the systemic induction by EPS treatment may have activated certain plant defense-related genes, which resulted in reduced viral replication and movement.

During microbial invasion or mechanical damage to the plants, physiologic changes occur at the cellular level activating plant-defense enzymes. PAL and PO are the key enzymes in the plant-defense mechanism, which exert their effect through different pathways. Phenols are produced through the PAL pathway in cucumber [6]. Peroxidases are known to catalyze the biosynthesis of lignin and hydrogen peroxide [4]. In the present study, we found an increase in these enzyme activities in EPS-treated leaves of tobacco plants, showing their systemic induction. EPS treatment enhanced enzyme activity, which became more pronounced in the presence of the CMV-Y. This demonstrates that EPS from *Serratia* sp. strain Gsm01 induced these enzymes in plants in response to the CMV-Y challenge. To our knowledge, this is the first report of a systemic induction of plant-defense enzymes in tobacco by EPS from *Serratia* sp. strain Gsm01.

The *S. marcescens* strain 90-166, when inoculated in the rhizosphere, is known to induce protection against CMV *via* a jasmonic-acid-dependent ISR mechanism and not *via* the SA pathway [30]. We found that the induction by EPS from *Serratia* sp. strain Gsm01 involves the PR-*1b* gene. It is presumed that there is a possibility of more than one pathway or some new uncharacterized pathways responsible for the antiviral activity.

In conclusion, EPS protects tobacco plants systemically against CMV-Y. However, further research is needed to characterize the pathway(s) involved in the systemic resistance induced by EPS from *Serratia* sp. strain Gsm01, to a significant scale. Similar studies also need to be undertaken in other hosts of CMV to clearly understand the mechanism involved in antiviral activity.

Acknowledgment

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