Proteome analysis for identifying effect of the natural clay mineral illite on the enhanced growth of cherry tomato (*Lycopersicon esculentum*)

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Abstract
Illite is neutral in electrical charge and contains a variety of trace elements. Especially, potassium (K) is present in it; it can neutralize soil acidity and provide other nutrients to the soil. It can improve the soil quality and help the plant root extend. Also, it is well known that the illite may be used as a soil conditioner, can increase the contents of sugar in crop plants and crop productivity, and decrease the application amounts of pesticide used for the protection of plants from pests. It is frequently reported that the illite may function as a soil conditioner for the agricultural purposes. Cherry tomato (*Lycopersicon esculentum*), selected as the target crop vegetable in this study, is known to contain high amount of vitamins that play a role in retarding the senescence of skin and preventing cancer. It is also easy to cultivate and to eat and is rich in sugar. In this study, we tried to elucidate the enhanced effect of illite on the growth of cherry tomato through the use of proteomic analysis. The experimental results demonstrated that the size of cherry tomato was 11-23% greater due the application of illite relative to non application. Two dimensional electrophoresis(2-DE) and MALDI-TOF-MS were used to separate and identify the differentially expressed proteins extracted from the leaf of the cherry tomato and involved in the enhanced growth of cherry tomato induced by the particulate(PA) and powder(PW) forms of illite, respectively. From this study, compared to non application(P0), eleven proteins differentially expressed in the leaf of cherry tomato on the application of PA and PW forms of illite, were characterized by 2-DE and MALDI-TOF-MS, respectively.

Key Words
Natural clay mineral, Illite, Soil conditioner, Cherry tomato, Proteome, 2-Dimensional gel electrophoresis(2-DE), Matrix-assisted laser desorption ionization-time of flight/time of flight mass spectrometry (MALDI-TOF-MS)

Introduction
The proteome is a collection of proteins expressed by the genome constituting all genes of living cells, the purpose of which is to evaluate the proteins in terms of the kinds and amount of protein and the environment in which the proteins are expressed. It is a technique used for studying the kind, distribution, location, amount, properties, network, and function of the proteins systematically and collectively expressed by the genome at the specific physiological conditions. Proteomics is often considered more of a technology than a science. The techniques of proteomics serve as a powerful tool by which the multi-proteins in a cell can be screened, to characterize not the behavior of the single protein molecule, but the entire network inherent to a biological system. Proteomics elucidates the cellular functions not at the gene levels but at the protein levels. Proteomics is a powerful tool that is widely used to evaluate the function of proteins related to specific metabolism in the biological sciences, such as plant, animal, and microbiology. Therefore, the purpose of this study is to elucidate and characterize the expressed the proteins extracted from the leaf samples of cherry tomato treated and untreated with the particulate (PA) and powder (PW) forms of illite through the use of 2-DE and MALDI-TOF-MS, respectively.

Materials and Methods
Materials
Two forms of illite, particulate (PA) and powder (PW), which are produced in the area of Yeongdong of Chungbuk province, Korea, were used as soil conditioners in this study.

Cultivation and treatment
The seedlings of cherry tomato were pre-cultivated in the box with dimension of 60cm X 30cm for two

weeks packed with soil used for horticultural purposes. Then, the healthy seedlings were selected and transplanted to pots with dimensions of 7cm in diameter and 7 cm in height. Before they are transplanted, the PA and PW forms of illite were mixed well in the pots as standard application [P1 (PA1, PW1), 1:30(w/w)], two times[P2(PA2, PW2), 1:15(w/w)], and four times[P4(PA4, PW4), 1:7.5(w/w)] of standard application .

Cherry tomato used

Academic name - Lycopersicon esculentum

Classification - Solanaceae

Native source –Latin America

Methods

1. Pretreatment

Proteins were extracted from the leaves of cherry tomato using a modified protocol according to the previous report (Choi *et al.* 2008). Two hundred milligrams of cherry tomato leaves were ground into powder with liquid nitrogen in a mortar and homogenized. One hundred and one hundred fifty mg of the homogenaized sample were added to the Micro centrifuge tubes in 1.6 ml of TCAAEB buffer containing 10%TCA, 0.07% β-mercaptoethanol, 89.93% acetone and centrifuged at the 15,000 rpm and 4^{o}C and maintained for 1 hour at -20^{o}C for the removal of salt and impurities and the proteins were precipitated. The supernatant was discarded and the precipitate was washed three times with 1.6 ml of wash buffer containing0.07% β-mercaptoethanol, 99.93% acetone and centrifuged at 15,000rpm at 4^{o}C for removal of colour and impurities. The pellet was washed three times with the lysis buffer and lyophilized prior to 2-DE analysis.

2. MALDI-TOF-MS:

Matrix absorbing UV is added to the protein sample which is crystallized and ionized by the laser beam and molecular mass estimated based on the flight time of m/z. Unlike the GPC/SEC, it is useful for measuringe the absolute mass of high molecular weight molecules in natural compounds such as proteins and synthetic compounds. MALDI-TOF is an ionization method developed by Hillenkamp in Germany in 1988 and is a rapid method for analyzing proteins more than 200,000 Da in molecular weight.

3. Identificaition of proteins by Mascot search

The collected m/z data are then evaluated using a search engine, i.e., MASCOT (http://www.matrixscience.com) in order to construct reports regarding the statistical summary of peptide mass finger printing(PMF) in the order of best-fitting scores.

Results

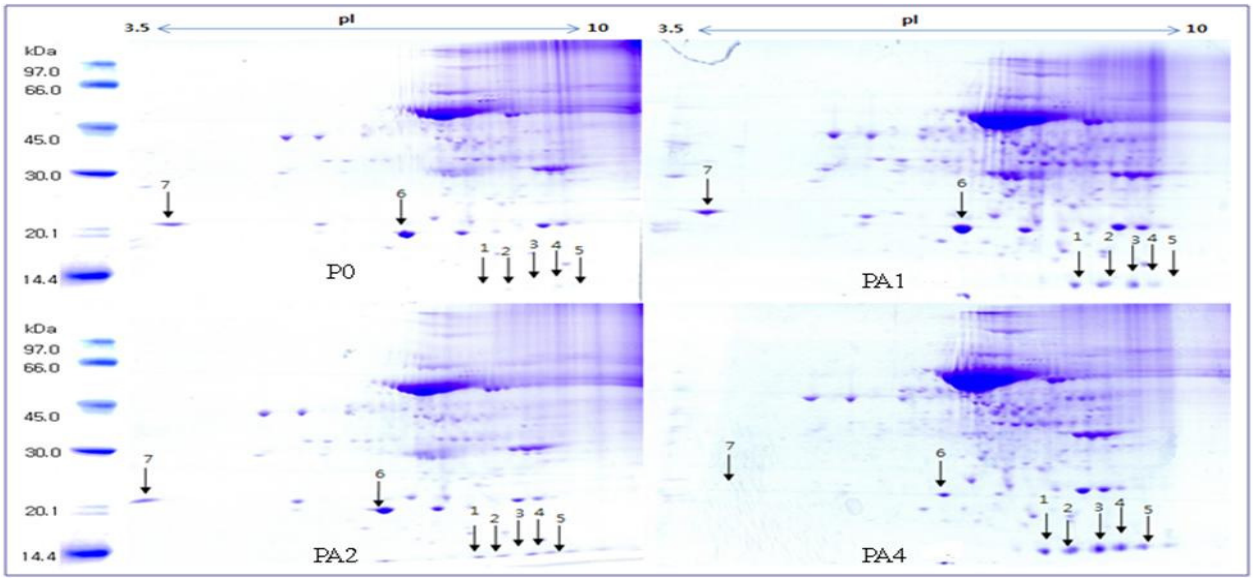

Figure 1. Two-dimensional electrophoresis pattern for proteins extracted from the cherry tomato samples treated with illite.

Table 1. List of the protein spots extracted from cherry tomato samples and characterized by two-dimensional electrophoresis analysis and MALDI-TOF.

Spot No	Accession No	Protein Name	Taxonomy	Molecular Weight (Da)	Score	Sequence Coverage (%)	Peptide count	Peptide Match
1	Q5GA69_LYCES	Putative polyprotein	Lycopersicon esculentum	78757	21	3	20	5
2	B59346	seed storage protein Lec2SA1 small chain	Lycopersicon esculentum	3943	16	18	6	1
3	Q157M6_LYCES	33kDa protein of oxygen-evolving complex	Lycopersicon esculentum	19038	22	8	20	3
4	HS22M_LYCES	Heat shock 22 kDa protein, mitochondrial	Lycopersicon esculentum	6445	24	19	8	2
5	gi|61374852	putative alcohol dehydrogenase class III	Lycopersicon peruvianum	10819	30	17	19	3
6	Q9SDY0_LYCES	Phosphoenolpyruvate carboxylase kinase	Lycopersicon esculentum	30967	19	3	21	3
7	T06398	reverse transcriptase	Lycopersicon peruvianum	10725	23	18	21	3
	Q2MI76_LYCES	Clp protease proteolytic subunit	Lycopersicon esculentum	23174	22	5	21	3
	Q53J22_LYCES	Hypothetical protein	Lycopersicon esculentum	38218	24	5	20	4
	Q1W7H8_LYCCI	Proteasome regulatory particle subunit	Lycopersicon chilense	23820	23	10	22	4
	Q1W377_LYCES	Phosphomannomutase	Lycopersicon esculentum	28538	21	9	22	4

Conclusion

The particulate(PA) and powder(Pw) illite-treated leaf samples of cherry tomato were carefully collected, frozen for three days and powderized under the liquid nitrogen. After electrophoresis, 0.1% colloidial coomassie brilliant blue was added as a dye for visualization of the sample. After the dye was added to gel, the protein patterns from PA treated samples were similar to those from PW treated samples. Therefore, the protein samples treated with only the PA illite were further used for the characterization of protein. After the dying were applied to the gel sample extracted from PA sample, the difference in the intensity of proteins from the gel was clearly demonstrated, according to the illite application rate (Figure 1). The intensity of proteins in the first five spots in the gel in order was increased as the application rate was increased, whereas, that of the sixth and seventh spots decreased as the application rate increased. Seven protein spots showing differences in the amount of proteins expressed were separated from the gel according to Fukuda *et al*. (2003), and the proteins were purified and characterized by MALDI-TOF-MS. Based on the data base of cherry tomato, twelve proteins from these spots were characterized and their names and functions are classified as the proteins as in Table 1: 1) Ribosomal protein L3: Structural constituent of ribosome (Mitterberbauer *et al.* 2004), 2) Putative polyprotein. DNA-binding(Guyo *et al.* 2005), 3) Seed storage protein Lec2SA1 small chain, 4) 33kDa protein of oxygen evolving complex : Calcium ion binding(Cai *et al.* 2006), 5) Heat shock 22 kDa protein: the antioxidant function(Banzet *et al.* 1998), 6) Mitochondrial, putative alcohol dehydrogenase class III: Oxidoreductase activity, zinc ion binding (Baudry *et al.* 2001), 7) Phosphoenolpyruvate carboxylase kinase : ATP binding, Protein serine/threonine kinase activite(Hartwell *et al.* 1999), 8) Reverse transcriptase: Nucleotidyltransferase (Kuioers *et al.* 1998), 9) Clp protease proteolytic subunit: cut to the various protein in ATP hydrolysis fixations (Kahlau *et al.* 2006), 10) Hypothetical protein: Proteosome regulatory particle subunit(Kawagoe *et al.* 1991), 11) Chlorophyll a-b binding protein CP24 10B, chloroplastic: chlorophyll binding (Egbert *et al.* 1990), 12) Phosphomannomutase: phosphomannomutaes activity(Qian *et al.* 2007). Consequently, it appears that the application of illite stimulates twelve specific proteins involved in the enhanced growth of cherry tomato.

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