Exometabolomic study of extracellular metabolites in tobacco plant induced by ethyl acetate extracts of *Streptomyces diastatochromogenes* KX852460

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**ABSTRACT**

*Streptomyces diastatochromogenes* KX852460, produced induce systemic resistance against *Rhizoctonia solani* AG-3. SA from the strain was given as treatment to the tobacco plants. Activity of the defense enzymes was analyzed by spectrophotometer, while extracellular metabolites were analyzed by Liquid Chromatography Tandem Mass spectrometer (LCMS-MS). Defense-related enzymes glutathione peroxidase (GR), glutathione peroxide (GPX), and peroxidase (POD) had maximum activity after treated with extract. Same as was observed in inoculated with *R. solani*, and extract induced the high activity of glutathione reductase (GSR), catalase (CAT), phenylalanine ammonia-lyase (PAL), and polyphenol oxidase (PPO). While PPO had moderate activity even though with untreated samples (control). Metabolic profile of the treated sample was compared with control (untreated). Exometabolomic study reveal that in the treated sample amino acid (L-cysteine, N,5-di(trifluorocetetyl)-, trimethylsilyl ester), mammalian steroid 5α-Androstone-3α,17β-diol, bis(pentafluoropropionate), flavonoids (Rutin), amines and amides were identified. Current study determined that ethyl acetate extract could induce the resistance by activating the enzymes and by regulating the metabolism.

1. **Introduction**

Antagonistic microorganisms contribute a significant part in disease management of plants, by inducing the resistance in the plants, and unfolding the biological control mechanism (Ab Rahman, Singh, Pieterse, & Schenk, 2018). Tobacco plants have the ability to regulate the physiological and metabolic processes, by adjusting a sequence of mechanisms (Liu, Wang, Wang, Li, & Xin, 2014; Zhang, Zhang, Du, Chen, & Tang, 2011). Action of numerous enzymes (including superoxide dismutase, SOD; peroxidase, POD; catalase, CAT; glutathione-S- transferase, GST; glutathione reductase, GR; glutathione peroxidase, GPX; and non-enzymatic GSH; vitamin C, vitamin E, Ve; carotenoids; and antioxidants) alleviated the disturbed homeostatic mechanism of cells and stress damages (Anjum et al., 2015; Chen et al., 2016). Polyphenol oxidase (PPO) and proline oxidase (POX) have the role to build the defensive mechanisms by changing the composition of cell, and have the ability to catalyze the generation of oxidative phenols and lignin, to stimulate in contradiction to phytopathogens (Thilagavathi, Saravanakumar, Ragupathi, & Samiyappan, 2007). In the schematic biosynthesis of phenylpropanoid PAL enzyme have main role, and have key influence in the formation of flavonoid and in the biosynthesis of lignin (Podile & Laxmi, 1998). In the stress conditions, response mechanism of metabolites in tobacco leaf, allied with the perception of cure and period (Zhang et al., 2011). Phyto metabolomics in combination with the genomics, transcriptomics, and proteomics, analysis inclusive statics, for the broad spectrum determination of plant growing, enlargement, resistance and production (Misra, Assmann, & Chen, 2014). Several bacterial metabolites besides phyto hormones have been investigated (Amin et al., 2015; Johnson, Kidou Soule, & Kujawinski, 2016; Paerl et al., 2017). Recently, many researchers investigated the plants and microbes exometabolomic study (Jacoby, Martyn, & Kopriva, 2018; Wienshausen, Noriega-Ortega, Niggemann, Dittmar, & Simon, 2017). Metabolic responses of tobacco to the environment have been vastly investigated, however, comparatively few works have been done to analyze the metabolic changes induced by microbial extract in tobacco plants. In our previous study, we isolated, identified, and characterized *Streptomyces diastatochromogenes* KX852460 strain for the biological control of tobacco target spot. (Ahsan, Chen, Wu, Irfan, & Shafi, 2017; Ahsan, Chen, Zhao, Irfan, & Wu, 2017; Ahsan, Chen, Wu, & Irfan, 2017). The aim of the present study is to induce the systemic resistance in tobacco against *Rhizoctonia solani* AG-3.

2. **Materials and methods**

2.1. **Microorganism and antimicrobial extract**

*R. solani* AG-3 and ethyl acetate extract used in this study was obtained as described in our earlier reports.
(Ahsan et al., 2017). Given the name to the ethyl acetate extract of S. diastatochromogenes KX852460 as SA.

2.2. Enzyme extraction

Treatments were given in replica 24-h pre-inoculation of R. solani AG-3 and 24-h post inoculation of R. solani. Plants were inoculated and treated at the same time. Treatment with distilled water was considered as control. Plants were kept in hygienic conditions at 28°C. Selected the leave randomly from different plants to make the homogenate for the analysis. For total enzyme extracts, 0.2 g of callus were ground to fine powder with liquid nitrogen and homogenized with a mortar and pestle in 2 ml of ice cold 50 mM Tris-HCl buffer (pH 6.8) containing 0.1 mM ethylene-diaminetetraacetate acid (EDTA), 5 mM cysteine and 1% PVP. The homogenate was centrifuged at 8000 g for 15 min, and the supernatant fraction was filtered through a column containing 1 ml of Sephadex G-50 equilibrated with the same buffer was used for homogenization. All operations were performed at 4°C. The filtered fraction was used for enzyme activity (Zhao et al., 2012).

2.3. Determination of enzyme activities

GST, GR, and GPX activity was assayed spectrophotometrically as described by the method of (Drotar, Phelps, & Fall, 1985). POD activity was determined following the method described by (Yao & Tian, 2005), CAT, SOD, phenylalanine ammonia-lyase (PAL) and PPO by the method of (Li & Steffens, 2002).

2.4. Analysis of metabolites changes in tobacco plant induced by the SA

Plant treated with SA and plant without treatment was considered as control (CK). After 36 h, leaf sample was taken and ground in different fractions of methanol followed by centrifugation at 10,000 rpm for 15 min. Mass spectrometer operating in electrospray ionization (ESI) negative mode and hyphenated with an Agilent 1290 ultra-high performance liquid chromatography system was used. The high purity nitrogen gas for the mass spectrometer was set at 30 psi for source gas, 30 psi for the heating gas and high for collision gas with a source temperature of 500°C. The setting for ESI voltage was set to 4500 kV. The collision energy to attain fragmentation was set at 35 eV with a spread of ±15 eV. Mass range for MS/MS scan was set from 50 to 1000 m/z while mass range for full scan was set from 50–1000 m/z while scan speed was set at 1000 m/z per second. A DIKMA Leapil C18 (150 mm × 4.6 mm i.d. 2.7 µm particle size was used to obtain separation. The mobile phase was made up of aqueous ammonium formate (5 mmol/L) with 0.1% formic acid (solvent A) and acetonitrile with ammonium formate (5 mmol/L) with 0.1% formic acid (solvent B). The compounds were separated with the following linear-programmed solvent gradient: 0 min (10% B), 10 min (95% B), 2 min (95% B) then equilibrating back to 10% B for 3 min. The flow rate for the column was set at 0.4 ml/min while the column temperature was set at 30°C and injection volume at 20 µL. Data were matched with NIST chemistry Web Book.

2.5. Statistical analysis

All the data were statistically analyzed using Minitab software version 17. The data presented were the mean of triplicates.

3. Results

3.1. Activity of GST in tobacco leaves of different treatments

When the SA sprayed over the leaves of tobacco plants, activity of GST were increased up to 4 days and then decreased in untreated (Ck) samples. When tobacco leaves were treated with culture filtrate of Streptomyces sp. (T), the enzyme activity was at its peak on 6th day and then declined up to 14th day. When tobacco leaves were inoculated with R. solani AG-3 (Y) the enzyme showed similar pattern like untreated samples. Tobacco leaves treated with both culture filtrate and R. solani AG-3 (TY), the GST activity become highest at 2nd day and then reduced with increased time as shown in Figure 1.

3.2. Activity of GR in tobacco leaves of different treatments

Activity of GR was increased up to 4 days and then become decreased in untreated (Ck) samples. When tobacco leaves were treated with SA (T) the enzyme activity was at its peak on 6th and 12th days and then declined up to 14th day. When tobacco leaves were inoculated with R. solani AG-3 (Y) the enzyme activity increase on 12th day and then declined on 14th day. Tobacco leaves treated with both SA and inoculated by R. solani AG-3 (TY), the GR activity become highest at 10th and 12th days and then reduced with increased time as shown in Figure 2.

3.3. Activities of GPX in tobacco leaves of different treatments

Activity of GPX was increased on 2nd, 4th, 6th, 8th, and 12th days, while the activity of GPX decreased on 10th and 14th day in untreated (Ck) samples. When tobacco leaves were treated with SA (T) the enzyme activity was at its peak on 2nd day and then declined up to
14th days except on the 8th day there was slightly increased in activity. When tobacco leaves were inoculated with *R. solani* AG-3 (Y), the enzyme activity increased on 2nd, 4th, and 8th day, while on 6th, 10th, 12th, and 14th day decreased. Tobacco leaves treated with both SA and *R. solani* AG-3 (TY), the GPX activity was highest at 0, and 6th day, while on 14th days the activity was maxima. Whereas decline in GPX activity was observed on 2nd, 8th, 10th, and 12th day as shown in Figure 3.

Figure 1. Changes of activities of glutathione-s-transferase (GST) in tobacco leaves of different treatments. Untreated control (CK). Treated with SA (T). Treated with SA and Inoculated with *R. solani* (TY). Inoculated with *R. solani* (Y).

Figure 2. Changes of activities of glutathione reductase (GR) in tobacco leaves of different treatments. Untreated control (CK). Treated with SA (T). Treated with SA and Inoculated with *R. solani* (TY). Inoculated with *R. solani* (Y).

Figure 3. Changes of activities of glutathione peroxide (GPX) in tobacco leaves of different treatments. Untreated control (CK). Treated with SA (T). Treated with SA and Inoculated with *R. solani* (TY). Inoculated with *R. solani* (Y).
3.4. Activity of POD in tobacco leaves of different treatments

POD activity was increased on 2\textsuperscript{nd}, 4\textsuperscript{th}, and 6\textsuperscript{th} day and then decreased up to 12\textsuperscript{th} day, while on the 14\textsuperscript{th} day again activity increased in untreated (Ck) samples. When tobacco leaves were treated with SA (T), the enzyme activity was at its peak on 4\textsuperscript{th}, 6\textsuperscript{th}, 10\textsuperscript{th}, and 12\textsuperscript{th} day, whereas declined the activity on 0, 2\textsuperscript{nd}, 8\textsuperscript{th}, and 14\textsuperscript{th} day. When tobacco leaves were inoculated with \textit{R. solani} AG-3 (Y), the enzyme showed similar pattern like untreated samples. Tobacco leaves treated with SA and \textit{R. solani} AG-3 (TY), the GST activity was high from 2\textsuperscript{nd} to 10\textsuperscript{th} day and then reduced with increased time as shown in Figure 4.

3.5. Activity of CAT in tobacco leaves of different treatments

Activity of CAT was increased on 2\textsuperscript{nd} and 4\textsuperscript{th} day and then decreased in untreated (Ck) samples. When tobacco leaves were treated with SA (T), the enzyme activity was at its peak on 6\textsuperscript{th} day and then declined up to 14\textsuperscript{th} day. When tobacco leaves were inoculated with \textit{R. solani} AG-3 (Y), the enzyme showed good activity on 2\textsuperscript{nd} and 4\textsuperscript{th} day, while afterwards decreased the activity up to 14\textsuperscript{th} day. Tobacco leaves treated with both SA and \textit{R. solani} AG-3 (TY), the GST activity was high at 2\textsuperscript{nd} day and then reduced with increase in time as shown in Figure 5.

3.6. Activity of SOD in tobacco leaves of different treatments

Activity of SOD was increased on 12\textsuperscript{th} day and then decreased in untreated (Ck) samples. When tobacco leaves were treated with SA (T), the enzyme activity was at its peak on 6\textsuperscript{th} day and then declined up to 14\textsuperscript{th} day. When tobacco leaves were inoculated with \textit{R. solani} AG-3 (Y), activity of SOD on 8\textsuperscript{th} and 12\textsuperscript{th} day increased. Tobacco leaves treated with both SA and \textit{R. solani} AG-3 (TY), the SOD activity was observed high at 10\textsuperscript{th} and 14\textsuperscript{th} day increased as shown in Figure 6.

3.7. Activity of PAL in tobacco leaves of different treatments

Activity of PAL was increased up to 6 days and then decreased in untreated (Ck) samples. When tobacco leaves were treated with SA (T), the enzyme activity was at its peak on 4\textsuperscript{th} to 6\textsuperscript{th} day, and as well again increased

![Figure 4](image1.png) Changes in the activities of peroxidase (POD) in tobacco leaves by different treatments. Untreated control (CK). Treated with SA (T). Treated with SA and Inoculated with \textit{R. solani} (TY). Inoculated with \textit{R. solani} (Y).

![Figure 5](image2.png) Changes in the activities of catalase (CAT) in tobacco leaves by different treatments. Untreated control (CK). Treated with SA (T). Treated with SA and Inoculated with \textit{R. solani} (TY). Inoculated with \textit{R. solani} (Y).
on 10<sup>th</sup> and 12<sup>th</sup> day, whereas declined up to 14<sup>th</sup> day. When tobacco leaves were inoculated with <i>R. solani</i> AG-3 (Y), the enzyme showed overall good activity, but high activity was observed on 8<sup>th</sup> day. Tobacco leaves treated with both SA and <i>R. solani</i> AG-3 (TY), the PAL activity become high at 2<sup>nd</sup> to 10<sup>th</sup> day and then reduced with increased time as shown in Figure 7.

### 3.8. Activity of PPO in tobacco leaves of different treatments

Activity of PPO was increased on 4<sup>th</sup> and 12<sup>th</sup> day, while throughout all other days decreased in untreated (Ck) samples. When tobacco leaves were treated with SA (T) the enzyme activity was at its peak up to 2 days and then declined up to 14<sup>th</sup> day. When tobacco leaves were inoculated with <i>R. solani</i> AG-3 (Y), the activity of PPO was increased on 0 day. Tobacco leaves treated with both SA and <i>R. solani</i> AG-3 (TY), the PPO activity was high at 0 to 2<sup>nd</sup> day and again raised on 14<sup>th</sup> day, whereas activity decreased with increased time as shown in Figure 8.

### 3.9. Analysis of metabolites changes in tobacco plant induced by the SA

Exometabolomic study was carried out for the identification and quantification of metabolic changes in tobacco leave by treating with SA. Liquid Chromatography-Mass Spectrometry (LC-MS/MS) was used to analyze the profile of metabolites in treated leave and non-treated leave (control). Mass spectrum of the identified extracellular metabolites is given in Figure 9. In the treated sample amino acids, organic acids and alkanes, derivatives, amides and their derivatives, derivatives of amines, mammalian steroid, and flavonoids were determined. Long chain fatty acid such as Oleic acid, 2,3-bis-(OTMS) propyl ester was found. The most important was the presence of 5α-Androstane-3α, 17β-diol, bis (pentafluoropropionate) steroid in the treated sample. As by comparison to the non-treated sample (Ck), the amino acids were identified in higher quantity. L-Cystathionine,N,N-bis(tert-butyldimethylsilyl)-,bis(tert butyldimethylsilyl) ester, Sebacic acid, di(2,2,3,3,4,4,5,5-octafluoropentyl) ester, and L-Methionine, N-heptfluorobutryyl-, heptadecyl ester, organic acids and alkanes were found in non-treated sample. None of the steroids, flavonoids, amines, and amides was determined in the control (Table 1).

Exometabolomic study of tobacco leaf by the LC-MS/MS indicated that metabolic flux of treated sample with ethyl extract was different from control (Ck). In the treated sample, there were two amino acids L-Cysteine, N,N-di(trifluoroacetyl)-, trimethylsilyl ester and D-Alanine, N-(4-ethyl benzoyl)-, tridecyl ester were determined in the MS/MS analysis. Presence of cysteine derivative in the treated sample indicated that metabolism of L-Methionine, N-heptfluorobutryyl-, butyl ester, L-

Figure 6. Changes in the activities of superoxide dismutase (SOD) in tobacco leaves by different treatments. Untreated control (CK). Treated with SA (T). Treated with SA and Inoculated with <i>R. solani</i> (TY). Inoculated with <i>R. solani</i> (Y).

Figure 7. Changes in the activities of phenylalanine ammonia-lyase (PAL) in tobacco leaves by different treatments. Untreated control (CK). Treated with SA (T). Treated with SA and Inoculated with <i>R. solani</i> (TY). Inoculated with <i>R. solani</i> (Y).
Methionine, N-heptafluorobutyryl-, heptadecyl ester and L-Cystathionine, N, N-bis(tert-butyldimethylsilyl)-bis (tert-butyldimethylsilyl)ester has been occurred. As these amino acids were found in control sample, which was extracted from the same growing conditions plants of tobacco and these amino acids, are the precursor of cysteine and derivatives of cysteine.

4. Discussion

To considering this point of view to induced the systemic resistance in tobacco against tobacco target spot. A systemic analysis of enzymes and metabolism was conducted. In the current study, eight kinds of defense enzymes were analyzed: GST, GR, GPX, POD, CAT, SOD, PAL and PPO. Defense-related enzymes GR, GPX, and POD had more activity after treated with secondary metabolites. Whereas GST, CAT, PAL, and PPO had more efficiency treated with secondary metabolites spray and inoculation at the same time. While PPO had moderate activity even though with untreated samples (control), indicated that PPO activity level was near the same to the each other. Activation of these defense enzymes demonstrated, that defensive enzymes burst the ROS and protect the plant cells from the oxidative damage.

It was observed that the tobacco plants showed restricted adaptation against the \textit{R. solani} AG-3 inoculation. Induction of GST by the ROS due to the oxidative stress, metabolomic regulation of toxic substances, and former molecules was done by GST enzyme (Liu et al., 2013). GR also has a key role to poise the redox phenomenon in the cell (Mohan et al., 2015). Reactive oxygen species (ROS) caused oxidative rupture, so the plants adopt intricate defensive pathway to foraging the ROS. This mechanism comprises of enzymatic scavenging systems, likewise POD, CAT, and SOD (Howe & Schilmiller, 2002). Catabolism of
Table 1. Identified extracellular metabolic profile through mass spectrometry.

| Metabolites identified in Treated (T) | Metabolites identified in Control (Ck) |
|--------------------------------------|----------------------------------------|
| **Amino Acids and Derivatives of Amino acids** | **Amino Acids and Derivatives of Amino acids** |
| L-Cysteine, N,S-di(trifluoroacetyl)-, trimethylsilyl ester | L-Methionine, N-heptafluorobutyryl-, butyl ester |
| 2-C$_{4}$H$_{2}$F$_{8}$NO$_{3}$S | C$_{4}$H$_{4}$F$_{9}$NO$_{3}$S |
| D-Alanine, N-(4-ethyl benzoyl)-, tridecyl ester | L-Methionine, N-heptafluorobutyryl-, heptadecyl ester |
| C$_{8}$H$_{2}$NO$_{3}$S | C$_{8}$H$_{2}$F$_{14}$NO$_{3}$S |
| **Organic acids and Alkanes** | **Organic acids and Alkanes** |
| Dibutyl acetal | Benzamide, 2,4,5-trifluoro-3-methoxy-N-octadecyl- |
| C$_{10}$H$_{2}$O$_{2}$ | C$_{10}$H$_{12}$N$_{2}$ |
| Ethanone, 2-hydroxy-1,2-bis(4-methoxyphenyl)- | Nicotyrine |
| C$_{2}$H$_{2}$O$_{2}$ | C$_{2}$H$_{2}$N$_{2}$ |
| Docosanoic acid | Triphenyleno-dio[1,12-bcd8,9-bcd] thiphene |
| C$_{3}$H$_{2}$O$_{2}$ | C$_{12}$H$_{11}$Si$_{2}$ |
| Hexacontane | 1,2-dibromido-benzio-dioxin |
| C$_{3}$H$_{2}$O$_{2}$ | C$_{14}$H$_{13}$Si$_{2}$ |
| Methoxethoxyether/ethoxy/ethoxy/ethoxy/ethoxy/ethoxy| Ethylmalonic acid, phenethyl undecyl ester |
| C$_{3}$H$_{2}$O$_{2}$ | C$_{16}$H$_{15}$Si$_{2}$ |
| Benzamide, N,N-didecyl-3-trifluoromethyl- | Diethylmalonic acid, 1-bromo-3,3,3-trifluoropropyl tridecyl ester |
| (Z)-Aconitic acid, DMTBS | Sebacic acid, di(2,3,3,4,4,5-octfluoropentyl) ester |
| C$_{2}$H$_{2}$O$_{2}$ | C$_{20}$H$_{20}$OSi$_{7}$ |
| **Fatty Acids** | **Glucoheptonic acid** |
| Oleic acid, 2,3-bis-(OTMS) propyl ester | Glucoheptonic acid 7TMS |
| C$_{2}$H$_{2}$O$_{2}$ | Not identified |
| Steroid Isomers | Not identified |
| 5α-Androstane-3α,17β-diol, bis(pentafluoropropionate) | Not identified |
| C$_{2}$H$_{2}$O$_{2}$ | Not identified |
| **Flavonoids** | **Amides and Amines** |
| Rutin | Phosphoric triamide, n,n'-tri-p-dodecyl phenyl- |
| C$_{2}$H$_{2}$O$_{2}$ | C$_{24}$H$_{20}$N$_{2}$OP |
| **Organic acids and Alkanes** | **Amides and Amines** |
| Dibutyl acetal | Perfluorotributylamine |
| C$_{10}$H$_{2}$O$_{2}$ | C$_{10}$F$_{17}$N |
| Ethanone, 2-hydroxy-1,2-bis(4-methoxyphenyl)- | C$_{10}$F$_{17}$Cl$_{2}$N$_{2}$ |
| Docosanoic acid | 0$_{3}$ |
| C$_{2}$H$_{2}$O$_{2}$ | 704.3 |
| Hexacontane | (2-Chloro-4-{3-chloro-4-[(2,2,3,3,3-pentafluoromethyl)-N-fluorobutyryl-} C$_{38}$H$_{19}$F$_{17}$O$_{2}$N$_{2}$ |
| Methoxethoxyether/ethoxy/ethoxy/ethoxy/ethoxy | 0$_{3}$ |
| C$_{3}$H$_{2}$O$_{2}$ | 704.3 |
| Benzamide, N,N-didecyl-3-trifluoromethyl- | (Z)-Aconitic acid, DMTBS |
| (Z)-Aconitic acid, DMTBS | C$_{2}$H$_{2}$O$_{2}$ |
| C$_{2}$H$_{2}$O$_{2}$ | C$_{24}$H$_{20}$N$_{2}$OP |
| **Phosphoric triamide, n,n'-tri-p-dodecyl phenyl-** | **Phosphoric triamide, n,n'-tri-p-dodecyl phenyl-** |
| C$_{2}$H$_{2}$O$_{2}$ | C$_{24}$H$_{20}$N$_{2}$OP |

Homeostasis of cysteine has been acting as a vital protagonist in the plant immunity (Álvarez, Ángeles, Romero, Gotor, & García, 2012). Organic acids and alkane were identified in both samples. As the organic acids have essential role in various plant metabolic pathways (Ludwig, 2016). Higher alkane has the inhibitory effects against the microbial pathogens. Presence of long chain fatty acid derivatives (Oleic acid, 2,3-bis-(OTMS) propyl ester) in the treated sample indicated that ethyl acetate extract induced the immunity in the tobacco plant. Biosynthesis pathway of long chain fatty acids belongs to the resistance of the plant against the pathogens (Raffaele, Leger, & Roby, 2009). Presence of 5α-Androstane-3α,17β-diol, bis(pentafluoropropionate) steroids in the treated sample is the key point.

According to recent reports, derivatives of androstane are endogenous antagonist of constitutive receptor in human, and CAR is a sensor of exobiotic and endobiotic (Wada, Gao, & Xie, 2009). Whereas some researchers reveal that mammalian steroids have effects on the plant growth, cell divisions, root and shoot growth, embryo growth, flowering, and pollen tube growth and callus proliferation (Lindemann, 2015). Rutin, a flavonoid, was detected in the treated sample, and the flavonoids are the groups of secondary biosynthetic suberin and lignin can be by POD (Ingham, Parker, & Waldron, 1998). Homeostatic mechanism of PPO has a variety of phenomena, and stimulates the reaction to respond the mechanical burst, invasion of fungus and bacteria, physiological stresses, and signs to the jasmonic acid, SA, Ethylene and CAMP (Thipyapong & Steffens, 1997). PAL has significant role in the metabolic process of phenyl propanoid and causes the production of phenols (Massala, Legrand, & Fritig, 1980). Ethyl acetate extracts of *S. diastatochromogenes* KXS52460 showed on tobacco leaf. Exometabolomic study of tobacco leaf by the LC-MS/MS indicated that metabolic flux of treated sample with ethyl extract was different than control (Ck). In the treated sample, there were two amino acids L-Cysteine, N,S-di(trifluoroacetyl)-, trimethylsilyl ester and D-Alanine, N-(4-ethyl benzoyl)-, tridecyl ester were determined in the MS/MS analysis. As these amino acids were found in control sample, which was extracted from the same growing conditions plants of tobacco and these amino acids, are the precursor of cysteine and derivatives of cysteine. Important biomolecules such as vitamins, cofactors, antioxidants, and many defense compounds originated from the metabolism of cysteine (Droux, 2004; Wirtz & Droux, 2005).
metabolites, which are mostly exist in the leaves of the plants. These flavonoids not only building blocks of the plants, but also has the immunity against the pathogens (Galeotti, Barile, Curir, Dolci, & Lanzotti, 2008). Derivatives of amines and amides were also identified in the treated sample. Amines and amides are the secondary metabolites and plant secondary metabolites are antimicrobial. Results of MS/MS spectrum of the control sample showed only amino acids, organic acids, and alkanes. None of the fatty acid, flavonoid, amines, and amides was identified in the non-treated sample. By comparison to the treated sample, the profile of non-treated sample was basic compounds amino acids and alkanes, which could induce the systemic resistance in the tobacco (Sharma, Vineet, Dubey & Maheshwar, 2018). Exometabolomic study revealed that the treatment of ethyl acetate extract of *S. diastatochromogenes* KX852460 induced the inhibitory metabolites in the tobacco plants. Several studies indicated that in plant diversity, bacterial microbes can induce the defense substances for the soil borne disease management (Nagór ska, Bikovski, & Obuchowski, 2007). Current study demonstrated that ethyl acetate extract of *S. diastatochromogenes* KX852460 can induce the defense response in tobacco plant.

5. Conclusion

Ethyl acetate extracts of *S. diastatochromogenes* KX852460 induced the resistance by activate the defense enzymes. Defense-related enzymes GR, GPX, and POD had more activity after treated with microbial extract. Whereas GST, CAT, PAL, and PPO had more efficiency treated with secondary metabolites spray and inoculation at the same time. While PPO had moderate activity even though with untreated samples (control), indicated that PPO activity level was near the same to each other. Activation of these defense enzymes demonstrated that defensive enzymes showed restricted adaptation against the *R. solani* AG-3 inoculation. Exometabolomic study of plant leaf indicated after treated the extracts of ethyl acetate that metabolism changes have been occurred in tobacco leaves and flux of the metabolites were changed. Amino acid (L-Cysteine, N,N-dinitrifluoracetyl)-, trimethylsilyl ester), mammalian steroid 5α-Androstan-3α,17β-diol, bis(1-pentafluoropropionate), flavonoids (Rutin), amines, and amides were identified. The current study determined that ethyl acetate extract of *Streptomyces diastatochromogenes* KX852460 activated the defense enzymes. Modulation in metabolic profile of tobacco could produce immune response against the pathogen.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Ab Rahman, S. F. S., Singh, E., Pieterse, C. M., & Schenk, P. M. (2018). Emerging microbial biocontrol strategies for plant pathogens. *Plant Science*, 267, 102–111.

Ahsan, T., Chen, J., Wu, Y., & Irfan, M. (2017). Application of response surface methodology for optimization of medium components for the production of secondary metabolites by *Streptomyces diastatochromogenes* KX852460. *AMB Express*, 7, 96.

Ahsan, T., Chen, J., Wu, Y., Irfan, M., & Shafi, J. (2017). Screening, identification, optimization of fermentation conditions, and extraction of secondary metabolites for the biocontrol of *Rhizoctonia Solani* AG-3. *Biotechnology & Biotechnological Equipment*, 31, 91–98.

Ahsan, T., Chen, J., Zhao, X., Irfan, M., & Wu, Y. (2017). Extraction and identification of bioactive compounds (ecosane and dibutyl phthalate) produced by Streptomycies strain KX852460 for the biological control of *Rhizoctonia solani* AG-3 strain KX852461 to control target spot disease in tobacco leaf. *AMB Express*, 7, 54.

Álvarez, C., Ángeles, B. M., Romero, L. C., Gotor, C., & García, I. (2012). Cysteine homeostasis plays an essential role in plant immunity. *New Phytologist*, 193, 165–177.

Amin, S. A., Hmeló, L. R., Van Tol, H. M., Durham, B. P., Carlson, L. T., Heal, K. R., … Armbrust, E. V. (2015). Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature*, 522, 98.

Anjum, S. A., Tanveer, M., Hussain, S., Bao, M., Wang, L., Khan, I., … Shahzad, B. (2015). Cadmium toxicity in Maize (Zea mays L.): Consequences on antioxidative systems, reactive oxygen species and cadmium accumulation. *Environmental Science and Pollution Research*, 22, 17022–17030.

Chen, W., Guo, C., Hussain, S., Zhu, B., Deng, F., Xue, Y., … Wu, L. (2016). Role of xylo-oligosaccharides in protection against salinity-induced adversities in Chinese cabbage. *Environmental Science and Pollution Research*, 23, 1254–1264.

Drotar, A., Phelps, P., & Fall, R. (1985). Evidence for glutathione peroxidase activities in cultured plant cells. *Plant Science*, 42, 35–40.

Droux, M. (2004). Sulfur assimilation and the role of sulfur in plant metabolism: A survey. *Photosynthesis Research*, 79, 331–348.

Galeotti, F., Barile, E., Curir, P., Dolci, M., & Lanzotti, V. (2008). Flavonoids from carnation (*Dianthus caryophyllus*) and their antifungal activity. *Phytochemistry Letters*, 1, 44–48.

Howe, G. A., & Schilmiller, A. L. (2002). Oxylipin metabolism in response to stress. *Current Opinion in Plant Biology*, 5, 230–236.

Ingham, L. M., Parker, M. L., & Waldron, K. W. (1998). Peroxidase: Changes in soluble and bound forms during maturation and ripening of apples. *Physiologia Plantarum*, 102, 93–100.

Jacoby, R. P., Martyn, A., & Kopriva, S. (2018). Exometabolomic profiling of bacterial strains as cultivated using arabinodiose root extract as the sole carbon source. *Molecular Plant-Microbe Interactions*, 31, 803–813. PMPI-10.

Johnson, W. M., Kido Soule, M. C., & Kujawinski, E. B. (2016). Evidence for quorum sensing and differential metabolite production by a marine bacterium in response to DMSP. *The ISME Journal*, 10, 2304–2316.

Li, L., & Steffens, J. C. (2002). Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. *Plant*, 215, 239–247.
Lindemann, P. (2015). Steroidogenesis in plants–Biosynthesis and conversions of progesterone and other pregnane derivatives. Steroids, 103, 145–152.

Liu, D., Liu, Y., Rao, J., Wang, G., Li, H., Ge, F., & Chen, C. (2013). Overexpression of the glutathione S-transferase gene from Pyrus pyrifolia fruit improves tolerance to abiotic stress in transgenic tobacco plants. Molecular Biology, 47, 515–523.

Liu, H. H., Wang, Y. G., Wang, S. P., Li, H. J., & Xin, Q. G. (2014). Improved zinc tolerance of tobacco by transgenic expression of an allene oxide synthase gene from hexaploid wheat. Acta Physiologica Plantarum, 36, 2433–2440.

Ludwig, M. (2016). The roles of organic acids in C4 photosynthesis. Frontiers in Plant Science, 7. doi:10.3389/fpls.2016.00647

Massala, R. A., Legrand, M., & Fritig, B. (1980). Effect of -aminooxyacetate, a competitive inhibitor of phenylalanine ammonia-lyase, on the hypersensitive resistance of tobacco to tobacco mosaic virus. Physiological Plant Pathology, 16, 213–226.

Misra, B. B., Assmann, S. M., & Chen, S. (2014). Plant single-cell and single-cell-type metabolomics. Trends in Plant Science, 19, 637–646.

Mohan, V., Achary, M., Reddy, C. S., Pandey, P., Islam, T., Kaul, T., & Reddy, M. K. (2015). Glutathione reductase a unique enzyme: Molecular cloning, expression and biochemical characterization from the stress adapted C^4^ sub 4^h^ plant, Pennisetum glaucum (L.) R. Br. Molecular Biology Reports, 42, 947.

Nagórska, K., Bikowski, M., & Obuchowski, M. (2007). Multicellular behaviour and production of a wide variety of toxic substances support usage of Bacillus subtilis as a powerful biocontrol agent. Acta Biochimica Polonica-English Edition, 54, 495.

Paerl, R. W., Bouget, F. Y., Lozano, J. C., Vergé, V., Schatt, P., Allen, E. E., ... Azam, F. (2017). Use of plankton-derived vitamin B1 precursors, especially thiazolerelated precursor, by key marine picoeukaryotic phytoplankton. The ISME Journal, 11, 753–765.

Podile, A., & Laxmi, V. (1998). Seed bacterization with Bacillus subtilis AF 1 increases phenylalanine ammonia-lyase and reduces the incidence of fusarial wilt in pigeonpea. Journal of Phytopathology, 146, 255–259.

Raffaele, S., Leger, A., & Roby, D. (2009). Very long chain fatty acid and lipid signaling in the response of plants to pathogens. Plant Signaling & Behavior, 4, 94–99.

Sharma, C. K., Vishnoi, V. K., Dubey, R. C., & Maheshwari, D. K. (2018). A twin rhizospheric bacterial consortium induces systemic resistance to a phytopathogen Macrophomina phaseolina in mung bean. Rhizosphere, 5, 71–75.

Thilagavathi, R., Saravanakumar, D., Ragupathi, N., & Samiyappan, R. (2007). A combination of biocontrol agents improves the management of dry root rot (Macrophomina phaseolina) in greengram. Phytopathologia Mediterranea, 46, 157–167.

Thipyapong, P., & Steffens, J. C. (1997). Tomato polyphenol oxidase (differential response of the polyphenol oxidase F promoter to injuries and wound signals). Plant Physiology, 115, 409–418.

Wada, T., Gao, J., & Xie, W. (2009). PXR and CAR in energy metabolism. Trends in Endocrinology & Metabolism, 20, 273–279.

Wienhausen, G., Noriega-Ortega, B. E., Niggemann, J., Dittmar, T., & Simon, M. (2017). The exometabolome of two model strains of the roseobacter group: A market-place of microbial metabolites. Frontiers in Microbiology, 8. doi:10.3389/fmicb.2017.01985

Wirtz, M., & Droux, M. (2005). Synthesis of the sulfur amino acids: Cysteine and methionine. Photosynthesis Research, 86, 345–362.

Yao, H., & Tian, S. (2015). Effects of pre-and post-harvest application of salicylic acid or methyl jasmonate on inducing disease resistance of sweet cherry fruit in storage. Postharvest Biology and Technology, 35, 253–262.

Zhang, J., Zhang, Y., Du, Y., Chen, S., & Tang, H. (2011). Dynamic metabolonomic responses of tobacco (Nicotiana tabacum) plants to salt stress. Journal of Proteome Research, 10, 1904–1914.

Zhao, J., Wang, Y., Zhang, J., Han, Y., Yang, Z., & Feng, W. (2012). Induction of defensive enzymes (isoenzymes) during defense against two different fungal pathogens in pear calli. African Journal of Biotechnology, 11, 13670–13677.