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Acetylome analysis of lysine acetylation in the plant pathogenic bacterium *Brenneria nigrifluens*

Yong Li | Han Xue | Dan-ran Bian | Guantang Xu | Chungen Piao

The Key Laboratory of National Forestry and Grassland Administration on Forest Protection, Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing, China

Correspondence
Yong Li, The Key Laboratory of State Forestry Administration on Forest Protection, Research Institute of Forest Ecology Environment and Protection, Chinese Academy of Forestry, Beijing 100091, China.
Email: lylx78@hotmail.com

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Abstract

Protein lysine acetylation, a dynamic and reversible posttranslational modification, plays a crucial role in several cellular processes, including cell cycle regulation, metabolism, enzymatic activities, and protein interactions. *Brenneria nigrifluens* is a pathogen of walnut trees with shallow bark canker and can cause serious disease in walnut trees. Until now, a little has been known about the roles of lysine acetylation in plant pathogenic bacteria. In the present study, the lysine acetylome of *B. nigrifluens* was determined by high-resolution LC-MS/MS analysis. In total, we identified 1,866 lysine acetylation sites distributed in 737 acetylated proteins. Bioinformatics results indicated that acetylated proteins participate in many different biological functions in *B. nigrifluens*. Four conserved motifs, namely, LK^{ac}, K^{ac}*F, I*K^{ac}, and L*K^{ac}, were identified in this bacterium. Protein interaction network analysis indicated that all kinds of interactions are modulated by protein lysine acetylation. Overall, 12 acetylated proteins were related to the virulence of *B. nigrifluens*.

KEYWORDS
Brenneria nigrifluens, interaction network, lysine acetylome, plant pathogen, virulence

1 | INTRODUCTION

Brenneria nigrifluens (synonym: *Erwinia nigrifluens*) (Hauben et al., 1998) is the pathogen of walnut trees with shallow bark canker. The disease and its causal agent were first described in California (USA) (Wilson, Stake, & Berger, 1957). Then, the shallow bark cankers of walnut trees caused by *B. nigrifluens* were identified in Spain in 1994 (Lopez, De Simone, & Gallelli, 1994). Subsequently, the microbe was also found in young and adult trees (Morone, Janse, & Scortichini, 1998; Saccardi, Bonetti, Melegatti, & Cristanini, 1998) in Italy. In recent years, reports about this canker disease have become more frequent and widespread; for example, the disease was found in France (2004), Iran (2009), Serbia (2013), and Hungary (2014) (Ménard, Delort, Baudry, & Le Saux, 2004; Popović, Ivanović, Žlvković, Trkulja, & Ignjatov, 2013; Roshangar & Harighi, 2009; Végh, Tóth, Zámbó, Borsos, & Palkovics, 2014). This pathogen has led to economic losses because of the weakening of trees and the consequent reduction in timber production and in the number of nuts as well as the importance of walnut tree losses from the landscape (Biosca & López, 2012).

Lysine acetylation is one of the most common posttranslational modifications (PTMs) of proteins in prokaryotes and eukaryotes. Protein acetylation has been found to be involved in the cytoplasm, mitochondria, and other compartments of a cell and plays important roles in cell morphology, cell cycle regulation, metabolic pathways, enzymatic activities, and protein interactions (Donadio, Monciardini, & Sosio, 2007; Kotan, Dikbas, & Bostan, 2009; Wolfe, Conley, & Berg, 1988; Yang, Guo, & Zhang, 2008). In the late 1990s, CheY was the first identified acetylated protein in bacteria (Barak, Welch, Yanovsky, Oosawa, & Eisenbach, 1992; Wolfe et al., 1988). Yu et al. performed the first bacterial lysine acetylome analysis and identified 125 lysine acetylation sites distributed on 85 proteins (2.1%) among

4,146 proteins derived from *Escherichia coli*. (Yu, Kim, Moon, Ryu, & Pan, 2015). Recent advances in highly sensitive MS-based proteomics and antibody-based affinity enrichment have been very conducive to the comprehensive analysis of lysine acetylomes in bacteria (Christensen et al., 2019).

Until now, only one lysine acetylation report, the lysine acetylome of *Erwinia amylovora*, was associated with plant pathogenic bacteria. This report identified only 141 acetylated (K^{ac}) sites on 96 proteins among proteins derived from two *E. amylovora* strains. Interestingly, their results indicated that several acetylated proteins involved in *E. amylovora* virulence, including exopolysaccharide amylovoran biosynthesis- and type III secretion-associated proteins, may play a major role in bacterial virulence (Wu et al., 2013). Lysine acetylation in the plant pathogenic bacterium *B. nigrifluens* remains unknown. In the present study, we performed the first proteome-wide analysis of lysine acetylation in *B. nigrifluens* using high-resolution liquid chromatography tandem mass spectrometry (LC-MS/MS) and immune-affinity enrichment. To our knowledge, this is the second lysine acetylome associated with plant pathogenic bacteria.

2 | MATERIALS AND METHODS

2.1 | Cell culture and protein extraction

The *B. nigrifluens* strain LMG 2,694 was cultured in tryptic soy broth (TSB, BD, USA) medium by shaking at 200 rpm for 24 hr at 30°C. The harvested cells were washed twice using cold PBS (phosphate-buffered saline) by centrifuging at 1,000 × g for 3 min at 4°C. Then, the centrifuged cell pellet was resuspended in 8 M urea supplemented with 0.1% Protease Inhibitor Cocktail III, 65 mM DTT, 3 μM trichostatin A (TSA), 2 mM EDTA, and 50 mM nicotinamide. After the remaining debris was removed by centrifuging at 20,000 × g for 10 min at 4°C, the proteins were precipitated by 15% cold trichloroacetic acid for 2 hr at −20°C. The precipitate was washed and dried. The redissolved proteins in buffer (8 M urea, 100 mM NH4CO3, pH 8.0) were defined with a 2-D Quant Kit (GE Healthcare) based on the manufacturer's instructions. The digestion of the protein was performed according to the method described by Reverdy, Chen, Hunter, Gozzi, & Chai, 2018. A total of 12 mg of protein were used for digestion and divided into 6 components, each of which was enriched with 2 mg of peptide. The tests were performed once. The disulfide bonds were reduced with 10 mM dithiothreitol (DTT, Sigma) for 1 hr at 37°C and alkylated with 20 mM iodoacetamide (IAA, Sigma) for 45 min at room temperature in darkness. Then, the protein sample was diluted by adding 100 mM NH_4CO_3 to achieve a final concentration of urea less than 2 M. Finally, trypsin was added at a 1:50 trypsin-to-protein mass ratio for the first digestion (overnight) and a 1:100 trypsin-to-protein mass ratio for a second, 4 hr digestion.

2.2 | Enrichment of lysine-acetylated peptide

The enrichment of lysine-acetylated peptides was performed as described by Liu, Wang, Song, Lv, & Liang, 2016. In brief, tryptic peptides were dissolved in NETN buffer (100 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl, 0.5% NP-40, pH 8.0) and incubated with prewashed anti-acetyl lysine antibody-conjugated agarose beads (PMT104, PTM Bio, Hangzhou, China) at 4°C overnight with gentle shaking. The beads were washed four times with NETN buffer and twice with ddH_2O. The bound peptides were eluted by using 0.1% trifluoroacetic acid. The eluted peptides were combined and vacuum-dried. The peptides were desalted and cleaned with C18 ZipTips (Millipore) and were analyzed by LC-MS/MS analysis.

2.3 | LC-MS/MS analysis

LC-MS/MS analysis was performed as described by Liu et al., 2016. In brief, 4 μL of enriched peptide was used for analysis. The peptides were dissolved in 0.1% formic acid and directly loaded onto a reversed-phase precolumn (Acclaim PepMap 100, Thermo Scientific). Peptide separation was performed using a reversed-phase analytical column (Acclaim PepMap RSLC, Thermo Scientific). The gradient was an increase from 7% to 20% solvent B (0.1% formic acid in 98% acetonitrile) for 20 min, an increase from 20% to 35% for 8 min and a climb to 80% in 2 min, where the percentage was held at 80% for the last 5 min; all steps were performed at a constant flow rate of 300 nl/min on an EASY-nLC 1000 UPLC system. The resulting peptides were analyzed by Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific).

Intact peptides were detected in the Orbitrap at a resolution of 70,000. Peptides were selected for MS/MS using a normalized collision energy of 30; daughter ions were detected in the Orbitrap at a resolution of 17,500. A data-dependent procedure that alternated between one MS scan and 20 MS/MS scans was applied for the top 20 precursor ions above a threshold ion count of 1E4 in the MS survey scan with 10.0 s dynamic exclusion. The electrospray voltage applied was 2.0 kV. Automatic gain control (AGC) was used to prevent overfilling of the ion trap; 5E4 ions were accumulated for generation of MS/MS spectra. For MS scans, the m/z scan range was 350 to 1,800. MS/MS fixed first mass set as 100.

2.4 | Database search

MaxQuant with an integrated Andromeda search engine (v.1.4.1.2) was used to analyze MS/MS data. Tandem mass spectra were searched against the UniProt database for *Brenneria* (EniD312). The detailed method was the same as the description by Liu et al., 2016. Trypsin/P was specified as a cleavage enzyme, and up to 4 missing cleavages, 5 modifications per peptide and 5 charges were allowed. Mass error was set to 20 ppm for first-search precursor ions, 5 ppm for second-search precursor ions and 0.02 Da for fragment ions. Carbamidomethylation on Cys was specified as a fixed modification, and oxidation on Met, acetylation on Lys and acetylation on the N-terminal protein were specified as variable modifications. False discovery rate (FDR) thresholds for protein, peptide, and modification site were specified at 1%. The minimum peptide length was set at

7. All the other parameters in MaxQuant were set to default values. The site localization probability was set as >0.75.

2.5 | Protein annotation and subcellular localization predication

The identified acetylated proteins were classified on the basis of the gene ontology (GO) (http://www.ebi.ac.uk/GOA) (Hulsegge, Kommadath, & Smits, 2009). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were investigated according to the method described by Kanehisa, Goto, Kawashima, Okuno, & Hattori, 2004. Identified functional protein domains were classified using InterProScan on the basis of the protein sequence alignment method and the InterPro domain database (http://www.ebi.ac.uk/interpro/) (Hunter et al., 2009). Subcellular localization of the proteins was predicted by Wolfpsort (Horton et al., 2007).

2.6 | Motif analysis, functional enrichment analysis, and motif logo-based clustering analysis

The 10 amino acids upstream and downstream of acetylation sites were analyzed for potential motifs using Motif-X software. The background database parameters were all set to default.

Enrichment analysis was performed by the functional annotation tool DAVID Bioinformatics Resources 6.7 (Huang et al., 2007). The detailed settings were the same as those in the description by Yu et al., 2008. In brief, a two-tailed Fisher's exact test was employed to test the enrichment of the identified modified protein against all proteins of the species from the UniProt database. Correction for multiple hypothesis testing was carried out using standard false discovery rate control methods. A corrected p value <.05 was considered significant for all the enrichment analyses.

Motif logo-based clustering analysis was performed according to the description by Li, Wang, Li, Yang, & Wang, 2018. All the acetylation substrate categories obtained after enrichment were collated along with their P values and then filtered for those categories that were enriched in at least one of the clusters with a p value <.05. This filtered p value matrix was transformed by the function x = −log10 (p value). Finally, these x values were z-transformed for each category. These z scores were then clustered by one-way hierarchical clustering (Euclidean distance, average linkage clustering) in Genesis. Cluster membership was visualized by a heat map using the "heatmap.2" function from the "gplots" R-package.

2.7 | Method for protein interaction network and secondary structure analysis

Protein–protein interactions were determined by searching in the STRING database (v10.0) using all differentially modified protein name identifiers. We selected the protein interactions belonging to the searched data set and excluded external candidates. Only predicted interactions with STRING high confidence scores (score ≥ 0.7) were selected as interactions. The results of the interaction network were visualized using Cytoscape (v3.3.0). The densely connected regions (clusters) were analyzed by Molecular Complex Detection (MCODE, v1.3). Secondary structure was analyzed with NetSurfP. Only predictions with a minimum probability of 0.5 for one of the different secondary structures (coil, α-helix, β-sheet) were considered for analysis.

3 | RESULTS

3.1 | Generation and analysis of *B. nigrifluens* lysine acetylome

The protein acetylome of *B. nigrifluens* was determined based on high-resolution LC-MS/MS and sensitive immune-affinity purification. We identified 1,866 lysine acetylation sites in 737 acetylated proteins (detailed in Table S1). The acetylated proteins account for 17.3% (737/4251) of the total proteins in *B. nigrifluens*. To ensure the mass accuracy of the MS data, the distribution of mass error was <0.02 Da. The length of most peptides ranged from 7 to 26, which agrees with the properties of tryptic peptides. Analysis of acetylated lysine sites revealed that the number of lysine acetylation sites in

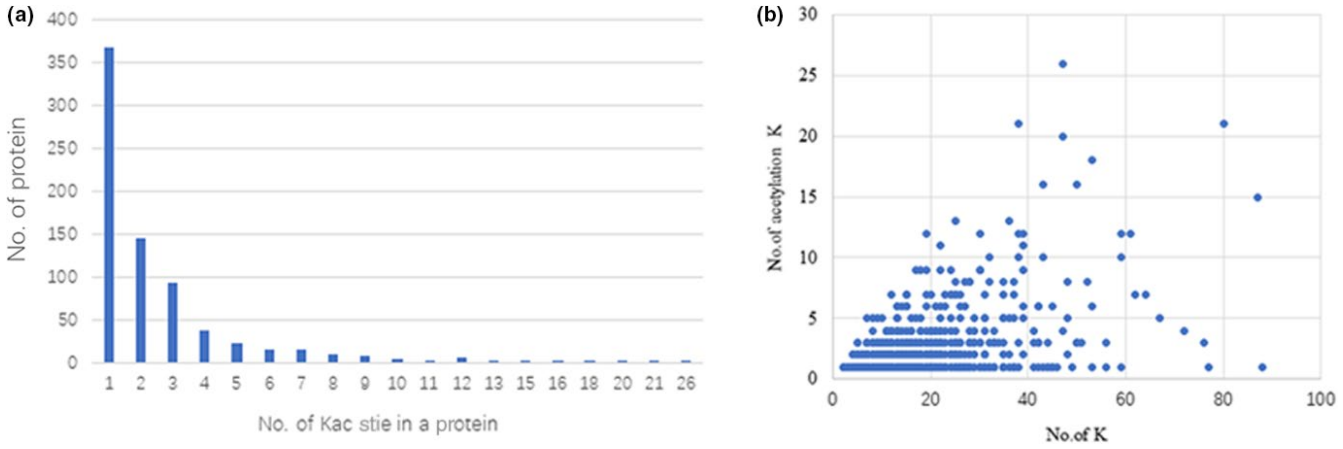

FIGURE 1 Identification of lysine acetylation sites in acetylome of *Brenneria nigrifluens*. (a) The number of lysine acetylation sites in each protein. (b) The number of lysines and acetylated lysines in proteins

each protein ranged from 1 to 26 (Figure 1a, 1). There were 367 proteins (more than 49.8% of the proteins) containing only one lysine acetylation site, which agrees with studies of other bacteria (Liu et al., 2016; Sun et al., 2018). The proportions of proteins harboring 2, 3, 4, 5 and more lysine acetylation sites were 19.7%, 12.8%, 5.2%, 3%, and 9.6% (Figure 1c), respectively. There were more than 15 lysine acetylation sites in the protein chaperones GroEL, ClpB, and DnaK; pyruvate dehydrogenase E1; DNA-directed RNA polymerase subunit beta; enolase; and formate acetyltransferase. The chaperonin GroEL is often identified with various acetylated sites in many bacteria, including *Thermus thermophilus* (12 sites), *Mycobacterium tuberculosis* (13 sites), and *Pseudomonas aeruginosa* (13 sites) (Ouidir, Kentache, & Hardouin, 2016).

3.2 | Functional annotation and cellular localization of acetylated proteins in *B. nigrifluens*

All the identified K^{ac} proteins in the lysine acetylome of *B. nigrifluens* were classified using the predicted features of GO terms, motif logos, and subcellular localizations (Figure 2a-2c, Table S2). GO analysis showed that the K^{ac} proteins were associated with a variety of molecular functions and biological processes. The results revealed that the largest group of acetylated proteins was involved in cellular and metabolic processes, which accounted for 38% and 36% of all the acetylated proteins, respectively (Figures 2a and 3b).

A molecular function analysis indicated that catalytic enzymes and binding proteins were the largest groups of acetylated proteins, accounting for 44% and 38% of all of the identified proteins, respectively (Figure 2a and 2b). The cellular component category comprised cell (57%), macromolecular complex (16%), membrane (15%), and organelle (12%) functions.

We also predicted the subcellular localization of the acetylated proteins, and the results revealed that the K^{ac} proteins were mainly distributed in the cytoplasm (83%), followed by the outer membrane (8%), periplasm (7%), inner membrane (2%), and extracellular space (only 1 K^{ac} site) (Figure 2d).

3.3 | Kac motifs and structural properties

The Motif-X program was used to analyze the sequence motifs in all the identified acetylated peptides. Fifteen conserved lysine-acetylated motifs were identified (Figure 3a, Table S3). Four of the conserved identified motifs, namely, LK^{ac}, K^{ac}*F, I*K^{ac}, and L*K^{ac} (* represents a random amino acid residue, and K^{ac} indicates the acetylated lysine) accounted for 21.7%, 9.7%, 10.1%, and 12.9% of the total acetylated peptides, respectively (Figure 3b).

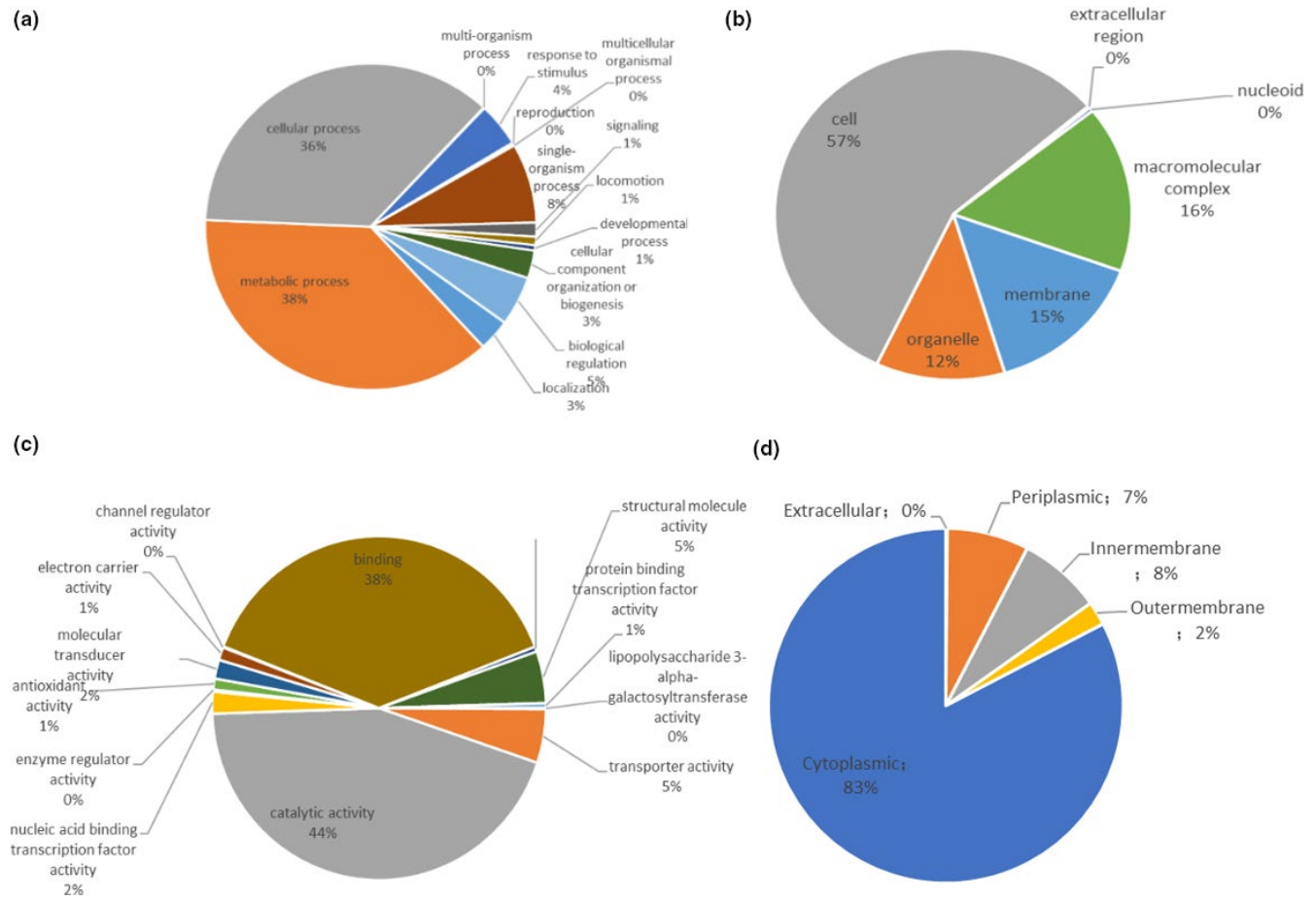

FIGURE 2 The distribution of all the identified acetylated proteins categorized by (a) biological process, (b) molecular function, (c) cellular component, (d) subcellular localization

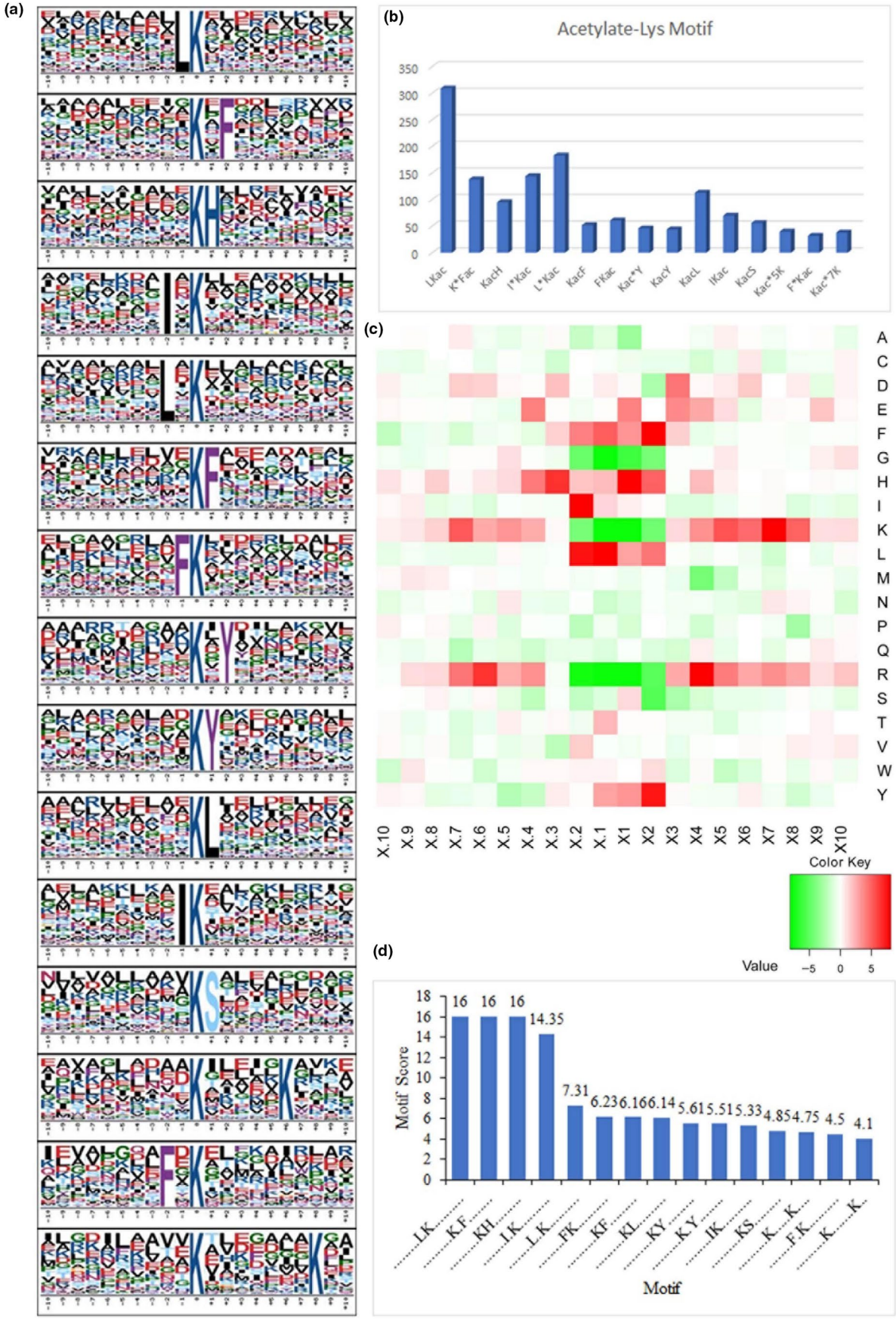

FIGURE 3 Properties of acetylated peptides. (a) Acetylation motifs and conservation of acetylation sites. (b) Number of identified peptides. (c) Heat map of the amino acid compositions around the lysine acetylation sites. (d) The motif scores

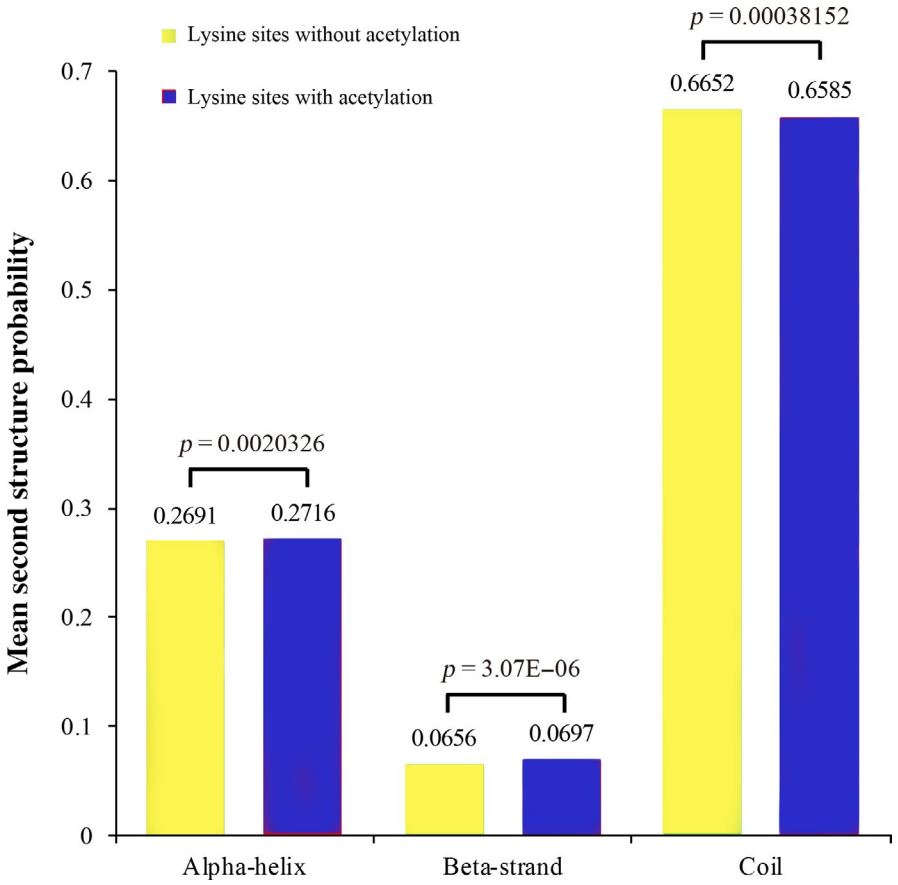

FIGURE 4 Distribution of secondary structures containing lysine acetylation sites. Probabilities of lysine acetylation in different protein secondary structures (α-helix, beta-strand, and coli). Secondary structure analysis was performed using NetSurfP. Only predictions with a minimum probability of 0.5 for one of the different secondary structures (coil, α-helix, β-strand) were considered for analysis

As illustrated in Figure 3b, the highest enrichment of phenylalanine (F) and leucine (L) was observed in the −2 to +2 positions. The enrichment significance between different motifs is shown in Figure 3c, and we provide an illustration sorting the motif scores (the motif score is the value of *p* after performing -log10) from high to low in Figure 3d.

Motifs K^{ac}*Y and K^{ac}H have been reported in other species of bacteria and eukaryotes (Kim et al., 2006; Zhang et al., 2009; Shao et al., 2012; Pan et al., 2014; Huang, Li, You, Zhou, & Ye, 2015; Xie et al., 2008; Hong et al., 2016), indicating that the two motifs are conserved and widespread. The motifs K^{ac}F, K^{ac}*F, K^{ac}*5K, F*K^{ac}, I*K^{ac}, and L*K^{ac} have been found in human cells (Kim et al., 2006; Shao et al., 2012). The motif K^{ac}F has also been found in *Schistosoma japonicum* (Hong et al., 2016). Moreover, Motif K^{ac}*F has been reported in *Bacillus nematocidal* and *Vibrio parahaemolyticus* (Pan et al., 2014; Sun et al., 2018). The other motifs, K^{ac}*Y, K^{ac}L, K^{ac}S, K^{ac}*7K, F^{ac}*K, F^{ac}K, IK^{ac}, I *K^{ac}, LK^{ac}, and L*K^{ac}, are rarely identified in other microbe species.

3.4 | Kac protein interactions of *B. nigrifluens*

To show the relationship between acetylation and protein structure in *B. nigrifluens*, the secondary structures of acetylated proteins were investigated in this study. The results showed that 34.2% of the acetylated lysine sites were located in ordered regions of protein secondary structure, including approximately 27.2% of acetylated lysine sites being located in α-helixes and 7.0% of sites being located in beta-strands. The remaining 65.9% of the sites were located in disordered regions of the protein (Figure 4). The acetylation sites in this study were found to be located primarily in disordered protein regions rather than in ordered regions, suggesting that secondary structure preferences of lysine-acetylated sites vary among different species. Some acetylation sites located in an important functional protein domain, for example, the acetylation of YciA (acyl-CoA thioesterase) at position 66, have catalytic efficiency toward many intermediates, including acetyl-CoA, acetoacetyl-CoA, both configurations of 3HB, crotonate, and butyrate (Clomburg, Vick, Blankschien, Rodriguez-Moya, & Gonzalez, 2012).

A global network of K^{ac} protein interactions of *B. nigrifluens* is shown in Table S4 and Figure 5a. Ten highly interconnected clusters of K^{ac} proteins were retrieved using Cytoscape software. There are 206 K^{ac} proteins that vary in molecular function, cell component, KEGG pathway, and biological process. There were 12 acetylated proteins discovered to be involved in multiple pathways, including plant–pathogen interactions, two-component systems, bacterial secretion systems, and several metabolic pathways. Interestingly, in the plant–pathogen interaction pathway, 12 lysine acetylation sites were distributed in the virulence factor HtpG, a stress response protein that helps bacteria overcome stressful environmental conditions (Arnold, Jackson, Waterfield, & Mansfield, 2007; Bocsanczy, Achenbach, Mangravita-Novo, Chow, & Norman, 2014).

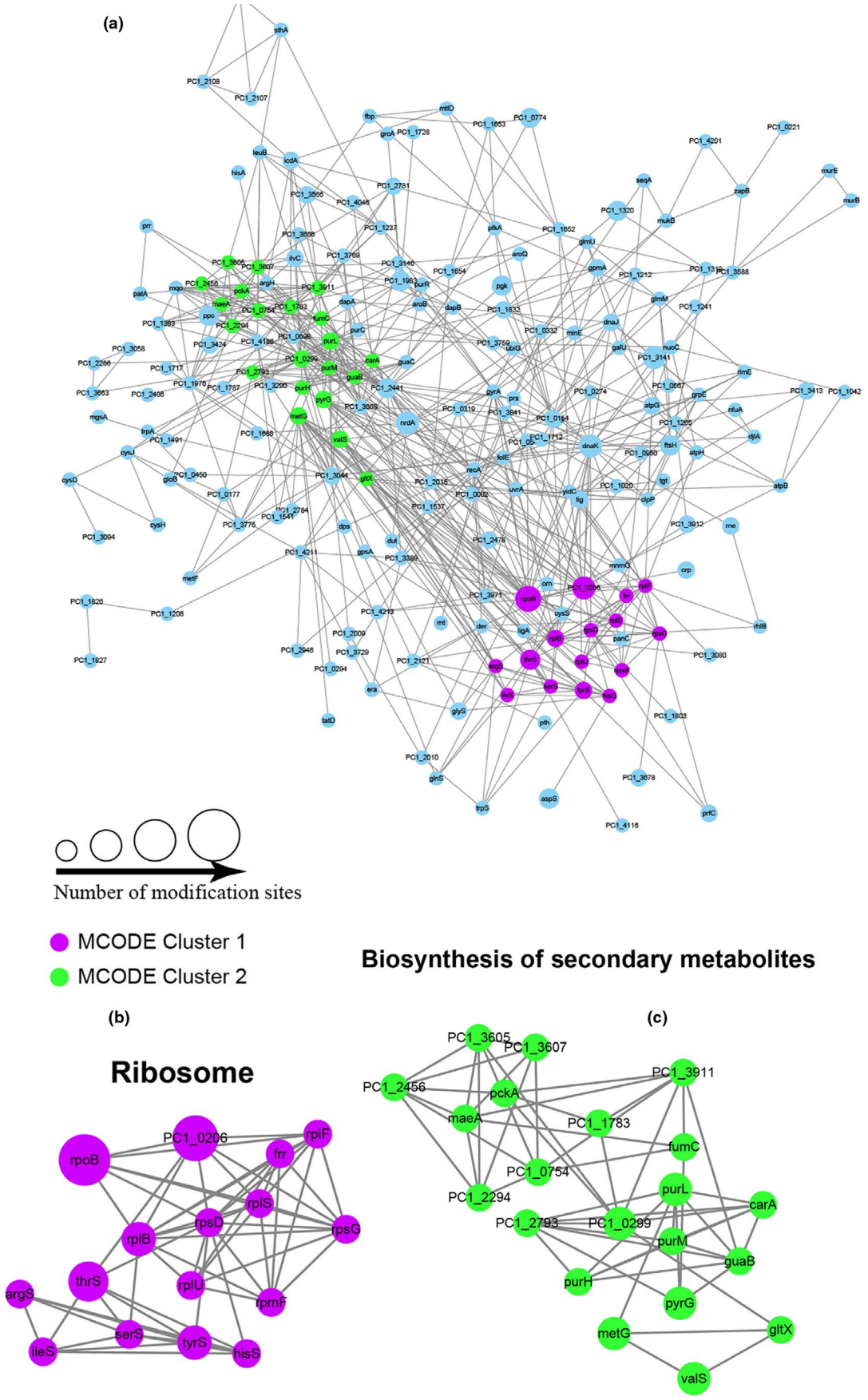

FIGURE 5 Interaction networks of acetylated proteins in *Brenneria nigrifluens*. (a) Interaction networks of all acetylated proteins. (b) Interaction network of acetylated proteins associated with ribosome. (c) Biosynthesis of secondary metabolites

TABLE 1 Acetylated proteins involved in the virulence of *Brenneria nigrifluens*

Gene	Position	Pathway	Reference
TolC: outer membrane protein	342	Bacterial secretion system	Al-Karablieh et al., 2009
ThrC: threonine synthase	2,7,203	Metabolic pathways	Guo et al., 2012
FliNY: flagellar motor switch protein FliN/FliY	82	Flagellar assembly	Khayi et al., 2015
TrpA: tryptophan synthase alpha chain	9	Metabolic pathways	Guo et al., 2012
PurF: amidophosphoribosyltransferase	53,114	Metabolic pathways	Guo et al., 2012
PurH: Bifunctional purine biosynthesis protein	359,380	Metabolic pathways	Chatterjee, S, 2005
GPI: glucose−6-phosphate isomerase	28, 35, 80, 65, 140, 228, 234, 238	Metabolic pathways	Tsuge et al., 2004
HtpG: molecular chaperone HtpG	18	Plant–pathogen interaction	Bocsanczy et al., 2014
Virulence-related outer membrane protein	45	Metabolic pathways	Holtappels et al., 2016
GroEL: chaperonin GroEL	7, 28, 42, 51, 65, 75, 117, 122, 132, 160, 272, 321, 362, 364, 432, 443	RNA degradation	Chaudhary et al., 2014
LeuA: 2-isopropylmalate synthase	249	Metabolic pathways	Lee et al., 2013
SerB: phosphoserine phosphatase	248	Metabolic pathways	Lee et al., 2013

4 | DISCUSSION

Lysine acetylation is a dynamic, reversible and regulatory posttranslational modification in both prokaryotes and eukaryotes with multiple functions (Huang et al., 2007; Liu et al., 2016). Analysis of the acetylome in bacteria revealed that protein lysine acetylation occurs on proteins associated with diverse functions, including cell size regulation, DNA binding, motility, transcription and central metabolism (Carabetta & Cristea, 2017; Ouidir, Cosette, Jouenne, & Hardouin, 2015; Post et al., 2017). In many countries, shallow bark canker, which is a serious disease of walnut trees, is caused by *B. nigrifluens*. However, the function of lysine acetylation in the virulence of this plant pathogen is elusive. In this study, we performed the first comparative, high-throughput analysis of the acetylome of *B. nigrifluens*. A total of 1,866 lysine acetylation sites distributed in 737 acetylated proteins were identified. The identified proteins account for 17.3% (737/4251) of the total proteins in *B. nigrifluens*. These modified proteins are involved in many biological processes and located in different cellular compartments. These results suggest a regulatory role of lysine acetylation in *B. nigrifluens*.

Lysine acetylation motifs have been reported to play important roles in acetylation and recognition. Motif PX4GK has been proven to be a recognition motif for protein acetyltransferase. Crosby and Escalante-Semerena (2014) proposed PK/RTXS/ T/V/NGKX2K/R as substrate recognition motifs for acyl-CoA synthetase B. In the present study, fifteen conserved lysine-acetylated motifs were identified. The motifs I*K^{ac} and FK^{ac} were localized in an important family of proteins, those containing the GNAT domain, which includes more than 100,000 members among eukaryotes and prokaryotes (Favrot, Blanchard, & Vergnolle, 2016). Another motif, K^{ac}H, was localized in the glucosamine-1-phosphate acetyltransferase domain, which is a target involved in cell wall biosynthesis in gram-negative and gram-positive bacteria. A little is known about the roles of these motifs, and they are worth studying.

In 2013, the first lysine acetylome of the bacterial plant pathogen *E. amylovora*, a pathogen of fire blight disease in pears and apples, was reported (Wu et al., 2013). The report identified 141 acetylated sites in 96 proteins derived from two *E. amylovora* strains. With the development of accurate mass spectrometry methods, increasing numbers of acetylated sites and proteins have been identified. In the present study, we identified 1,866 lysine acetylation sites distributed in 737 proteins (17.3%) among 4,251 proteins derived from *B. nigrifluens*. Almost eight times more acetylated proteins and sites were identified in *B. nigrifluens* than in *E. amylovora*, which were involved in cell cycle regulation and metabolic pathways, protein interactions, and enzymatic activity. In particular, 38% of the identified proteins were involved in metabolic processes such as central carbon, lipid, nucleotide, and amino acid metabolism, which agreed with research in *E. amylovora* (Wu et al., 2013). Twenty-five sites from 24 LysAc proteins were commonly identified in both *E. amylovora* and *B. nigrifluens*, and another 10 proteins were commonly identified as LysAc proteins but with different LysAc sites.

Lysine acetylation has been reported to play an important role in the virulence and pathogenicity of *V. parahaemolyticus* and *Streptococcus pneumoniae* (Liu et al., 2018; Trosky et al., 2007). Wu X et al. reported that seven acetylated proteins, including two proteins associated with the type III secretion system (T3SS), an effector protein (YopH) and a *Hrp*-associated systemic virulence B (HsvB) protein, are directly involved in pathogenesis in *E. amylovora* (Wu et al., 2013). In the present study, we found 58 lysine acetylation sites distributed in 12 proteins to be involved in the virulence of *B. nigrifluens* (Table 1). For bacterial secretion system pathways,

proteins belonging to the type I and II secretion systems were found to be acetylated, including the type I secretion outer membrane protein TolC, which is an important virulence factor in plant pathogens (Al-Karablieh, Weingart, & Ullrich, 2009). Other acetylated virulence factor proteins are involved in metabolic pathways, including PGI (Tsuge et al., 2004), TrpA, ThrC, PurF, PurH (Guo et al., 2012), LeuA, and SerB (Lee et al., 2013). Proteins PurH, LeuA, and SerB, associated with nutrient utilization, were shown to be important for bacterial pathogenicity (Lee et al., 2013). The acetylated protein FliN, a motility-related protein, is an important virulence factor in plant pathogens (Khayi et al., 2015). Protein GroEL, a widely conserved bacterial protein, has 15 lysine acetylation sites and can trigger host pattern-triggered immunity (Chaudhary et al., 2014). Thus, we hypothesize that protein acetylation may play important roles in the virulence regulation of plant pathogens.

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ETHICS STATEMENT

None required.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

YL and CGP conceived and designed the experiments. HX, DRB, and GTX have done the experiment. YL wrote the paper.

DATA AVAILABILITY STATEMENT

Supporting data are available in the supplementary file. All raw mass spectrometry data files are available via ProteomeXchange with identifier PXD014046.

ORCID

Yong Li https://orcid.org/0000-0002-4406-1329

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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