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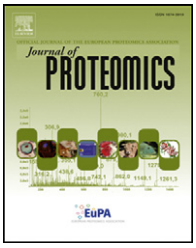

Differential lysine acetylation profiles of *Erwinia amylovora* strains revealed by proteomics

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ABSTRACT

Protein lysine acetylation (LysAc) has recently been demonstrated to be widespread in *E. coli* and *Salmonella*, and to broadly regulate bacterial physiology and metabolism. However, LysAc in plant pathogenic bacteria is largely unknown. Here we first report the lysine acetylome of *Erwinia amylovora*, an enterobacterium causing serious fire blight disease of apples and pears. Immunoblots using generic anti-lysine acetylation antibodies demonstrated that growth conditions strongly affected the LysAc profiles in *E. amylovora*. Differential LysAc profiles were also observed for two *E. amylovora* strains, known to have differential virulence in plants, indicating translational modification of proteins may be important in determining virulence of bacterial strains. Proteomic analysis of LysAc in two *E. amylovora* strains identified 141 LysAc sites in 96 proteins that function in a wide range of biological pathways. Consistent with previous reports, 44% of the proteins are involved in metabolic processes, including central metabolism, lipopolysaccharide, nucleotide and amino acid metabolism. Interestingly, for the first time, several proteins involved in *E. amylovora* virulence, including exopolysaccharide amylovoran biosynthesis- and type III secretion-associated proteins, were found to be lysine acetylated, suggesting that LysAc may play a major role in bacterial virulence. Comparative analysis of LysAc sites in *E. amylovora* and *E. coli* further revealed the sequence and structural commonality for LysAc in the two organisms. Collectively, these results reinforce the notion that LysAc of proteins is widespread in bacterial metabolism and virulence.

Abbreviations: 2DE, two-dimensional gel electrophoresis; AcK, acetyl-lysine; IEF, isoelectric focusing electrophoresis; LC-MS/MS, liquid chromatography and tandem mass spectrometry; LysAc, lysine acetylation; MALDI-TOF, matrix-assisted laser desorption/ionization- time-of-flight mass spectrometer; MW, molecular weight; pI, Isoelectric point; PTM, post-translational modification; SDS -PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis

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1. Introduction

Post-translational modifications (PTM) frequently occur to proteins and mediate their biological functions. Lysine acetylation (LysAc), a dynamic and reversible PTM, has emerged as a major PTM in both eukaryotes and prokaryotes [1,2]. Lysine acetylation normally refers to N^{ε}-acetylation, the transfer of an acetyl group from the acetyl donor acetyl-CoA to the ε-amino group of a specific lysine residue in a specific protein, resulting in acetyllysine (AcK) [3,4]. LysAc was first discovered in histone proteins about half a century ago [5,6], and has since been known to be crucial in regulating the functions of histones and transcription factors [7,8]. Comprehensive lysine acetylomics studies have significantly expanded the scope of LysAc beyond histone proteins and a more complete lysine acetylome has been reported from human, mouse, drosophila, plant, protozoan, and bacteria [1,2,9–14]. These proteomics studies revealed that LysAc is an evolutionarily conserved and widespread PTM [15,16] and demonstrated new roles of LysAc [1,2,7,17,18]. It is also well established that acetylation of proteins not only results in stimulation of DNA binding and thus gene expression, but also in regulating protein–protein interactions, protein stability and mRNA stability [3,4,15,19,20]. Furthermore, new findings point to an unexpected importance of LysAc in metabolic control and coordination of different metabolic pathways [2,21]. It is also expected that studies of LysAc will shed light on disease therapy or prevention by targeting lysine acetyltransferase and deacetylase, the enzymes that mediate the reversible protein LysAc [15].

Since the discovery of N^{ε}-acetylation of *Salmonella enterica* acetyl-CoA synthase (Acs) in 2002, several proteomics studies of α and γ proteobacteria, including *Escherichia coli* and *Salmonella enterica*, have identified several hundred acetylated proteins [2,13,22,23], indicating that a wide range of prokaryote proteins can be acetylated. Interestingly, about 50% of those identified proteins in bacteria are enzymes participating in multiple metabolic pathways and are important for the control of central metabolism, particularly energy, fatty acids and nucleotide metabolism. In addition, biochemical analysis of lysine acetylation on bacterial enzymes Acs and chemotaxis protein CheY demonstrated that site specific lysine acetylation directly modulated activities of both enzymes [24–26], indicating LysAc in bacteria can be functionally important. Moreover, given the fact that mitochondria, which are evolutionarily derived from bacteria, contain many acetylated proteins in mammals, LysAc in bacteria may be ubiquitous across genera and families [1,3,20,21]. However, no studies of protein LysAc on plant pathogenic bacteria have been reported so far.

Erwinia amylovora, an enterobacterium belonging to γ proteobacteria, causes fire blight disease, an economic important plant disease of the Rosaceae crops, including apples, pears and raspberries. The disease costs millions of dollars of crop losses annually around the world and its control has become a major concern for apple and pear industry [27]. Genetics studies in *E. amylovora* indicate that hypersensitive response and pathogenicity (*hrp*) -type III secretion system (T3SS) and the exopolysaccharide (EPS) amylovoran production are the two major virulence factors [28,29]. The genomic sequences of at least four *E. amylovora* isolates have been reported, which share more than 99.99% sequence identity, and more than 98% of proteins are identical [27,30,31]. However, differential virulence has been observed for *Erwinia* isolates, while the underlying mechanism for the differences in virulence was largely unclear [32]. On the other hand, amylovoran is a carbon compound derived from primary carbon metabolites [33], and LysAc was recently shown to dynamically regulate enzymes in carbon metabolism [2,21].We thus hypothesized that LysAc on metabolic enzymes in *E. amylovora* may play a regulatory role in its amylovoran production, and therefore may affect its virulence.

The objective of this study was to investigate the abundance of protein LysAc in *E. amylovora*, and compare the LysAc profiles of two natural isolates of *E. amylovora* strains, Ea273 from USA and Ea1189 from Germany, which have differential virulence in different host plants [32]. Using proteomic approaches, differential LysAc profiles for two *E. amylovora* isolates were documented and for the first time, several proteins involved in *E. amylovora* virulence were found to be lysine acetylated, including EPS amylovoran biosynthesis- and T3SS-associated proteins. The proteomics data of this study was acquired at a high resolution LTQ-FTICR mass spectrometry, which fully distinguishes LysAc from lysine trimethylation.

2. Materials and methods

2.1. Bacterial strains and culture media

The *E. amylovora* strains Ea1189 and Ea273 were either grown in LB medium or in MBMA medium (3 g KH_2PO_4, 7 g K2HPO_4, 1 g $[NH_4]_2SO_4$, 2 ml glycerol, 0.5 g citric acid, 0.03 g $MgSO_4$) plus 1% sorbitol [34] as described previously [32]. Bacterial growth was monitored by measuring OD_{600} and harvested at log or stationary phase as indicated. Samples for *E. amylovora* strains were processed simultaneously to allow side-by-side comparison.

2.2. Protein extraction

The *E. amylovora* cells were harvested by centrifugation and cell pellets were directly lysed by boiling in 2× SDS sample buffer and protein concentration was measured by Bradford assay (Bio-Rad, Hercules, CA). Thirty micrograms of total soluble proteins were loaded in each lane for SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) analysis. Alternatively, cells were lysed by sonication in a buffer containing competitive protease and deacetylase inhibitors (100 mM Tris pH 8.0, 5 mM caproic acid, 1 mM para-aminobenzamidine, 2 mM leupeptin, 5 μM PTACH and 2 μg/ml apicidin) (Sigma-Aldrich). Protein extracts were further fractionated by differential centrifugation at 20,000*g* and 100,000*g* (Beckman Coulter) for mass spectrometry analysis.

2.3. Anti-lysine acetylation immunoblots and acetyl-lysine peptide preparations

The generic anti-acetyl lysine antibodies (ImmuneChem Pharmaceuticals, Burnaby, CA) were used at a 1:1250