**Original Article**
J Vet Sci 2018, 19(2), 188-199 · https://doi.org/10.4142/jvs.2018.19.2.188

# *In silico* analysis of putative drug and vaccine targets of the metabolic pathways of *Actinobacillus pleuropneumoniae* using a subtractive/comparative genomics approach

Biruk T. Birhanu[1], Seung-Jin Lee[1], Na-Hye Park[1], Ju-Beom Song[2], Seung-Chun Park[1,*]

[1]*Laboratory of Veterinary Pharmacokinetics and Pharmacodynamics, College of Veterinary Medicine, and* [2]*Department of Chemistry Education, Teachers College, Kyungpook National University, Daegu 41566, Korea*

*Actinobacillus pleuropneumoniae* is a Gram-negative bacterium that resides in the respiratory tract of pigs and causes porcine respiratory disease complex, which leads to significant losses in the pig industry worldwide. The incidence of drug resistance in this bacterium is increasing; thus, identifying new protein/gene targets for drug and vaccine development is critical. In this study, we used an *in silico* approach, utilizing several databases including the Kyoto Encyclopedia of Genes and Genomes (KEGG), the Database of Essential Genes (DEG), DrugBank, and Swiss-Prot to identify non-homologous essential genes and prioritize these proteins for their druggability. The results showed 20 metabolic pathways that were unique and contained 273 non-homologous proteins, of which 122 were essential. Of the 122 essential proteins, there were 95 cytoplasmic proteins and 11 transmembrane proteins, which are potentially suitable for drug and vaccine targets, respectively. Among these, 25 had at least one hit in DrugBank, and three had similarity to metabolic proteins from *Mycoplasma hyopneumoniae*, another pathogen causing porcine respiratory disease complex; thus, they could serve as common therapeutic targets. In conclusion, we identified glyoxylate and dicarboxylate pathways as potential targets for antimicrobial therapy and tetra-acyldisaccharide 4′-kinase and 3-deoxy-D-manno-octulosonic-acid transferase as vaccine candidates against *A. pleuropneumoniae*.

**Keywords:** *Actinobacillus pleuropneumoniae*, drug target, *in silico*, metabolic networks and pathways, vaccine target

## Introduction

The respiratory disease known as porcine respiratory disease complex (PRDC) is a widespread problem on intensive pig farms worldwide. PRDC is a polymicrobial disease that is caused by various viral and bacterial agents, including *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*, which are considered to be the primary pathogen in pigs [27]. Coinfection with these two pathogens is known to cause more severe disease than infection with either pathogen alone or with other agents [5].

*A. pleuropneumoniae* is a Gram-negative, facultative anaerobic bacterium that belongs to the family *Pasteurellaceae*. The organism is known to cause porcine pleuropneumonia, a severe contagious respiratory disease that often leads to very rapidly evolving pleuropneumonia, which is characterized by hemorrhagic necrotizing pneumonia and fibrinous pleuritis, and most commonly affects pigs aged 18 to 20 weeks [14]. The polysaccharide capsule and exotoxins of *A. pleuropneumoniae* are the major virulence determinants and are responsible for the pathogenesis of pleuropneumonia [2,15].

Over the last two decades, increasing numbers of *A. pleuropneumoniae* strains have been isolated that are resistant to a number of commonly utilized drugs for treating pleuropneumonia in pigs [19,37,40]. This alarming increase in the incidence of antimicrobial resistance has initiated a search for new therapeutics against these 'superbugs'. Current developments in the fields of genomics and proteomics have opened new avenues into the development of new antimicrobial agents and vaccine targets for combating drug resistance [31].

Different researchers have discussed the significance of *in silico* approaches for the identification of vaccine and drug targets. Morya *et al*. [24] identified drug targets in *Staphylococcus aureus* by analyzing its metabolic pathways. Likewise, vaccine and therapeutic drug targets in methicillin-resistant *S. aureus* [35], *Mycobacterium abscessus* [32], *Mycobacterium ulcerans*

Received 24 May 2017, Revised 4 Aug. 2017, Accepted 7 Oct. 2017
*Corresponding author: Tel: +82-53-950-5964; Fax: +82-53-950-5955; E-mail: parksch@knu.ac.kr
pISSN 1229-845X
eISSN 1976-555X

[3], *Vibrio cholerae* [4], and *Mycoplasma hyopneumoniae* [6] were identified by using the same approach.

However, there are no reported therapeutic target data available for the metabolic pathways in *A. pleuropneumoniae*. Herein, using the available genome sequences and genetic/proteomic database resources, we identified putative targets for antibiotics and vaccine therapy in *A. pleuropneumoniae* by undertaking an *in silico* comparative and subtractive genomic/proteomic metabolic pathway analysis approach.

## Materials and Methods

### Comparative metabolic pathway analysis of the pathogen and its hosts

The genomic nucleotide and protein sequences of three strains of *A. pleuropneumoniae* (serovar 5, [Refseq: NC_009053.1], serovar 7 [NC_010939.1], and serovar 3) and the genome sequences of pig (host; taxid: 9821) and human (*Homo sapiens*) were downloaded from the National Center for Biotechnology Information (NCBI) database. The metabolic pathways of host and pathogen were compared to distinguish the unique and common metabolic pathways by using BLASTP (NCBI, USA). To identify potential drug targets in the pathogen, search engines like NCBI-BLAST and saved databases were used (Fig. 1).

### KEGG comparison of the metabolic pathways in the pathogen and its host

The sequences of proteins in the metabolic pathways of the pathogen and its host were compared. The Kyoto Encyclopedia of Genes and Genomes (KEGG) databases (KEGG, Japan) were used to retrieve and compare the metabolic pathways in the whole genome sequences of three strains of *A. pleuropneumoniae* [17]. A manual comparison of the metabolic pathways of the natural host (pig), human, and *A. pleuropneumoniae* was conducted. Pathways that were not present in pig and human, but were present in the pathogen, were considered unique pathways, while the others were considered common pathways. The amino acid sequences of the proteins in the common and unique pathways of the pathogen were identified and downloaded from the NCBI database.

### Non-homologous essential pathogen protein selection

A two-step comparison method was used to identify the non-homologous essential proteins in the bacterium. Proteins of *A. pleuropneumoniae* were first compared to the host proteome to select non-homologous proteins, then, the identified

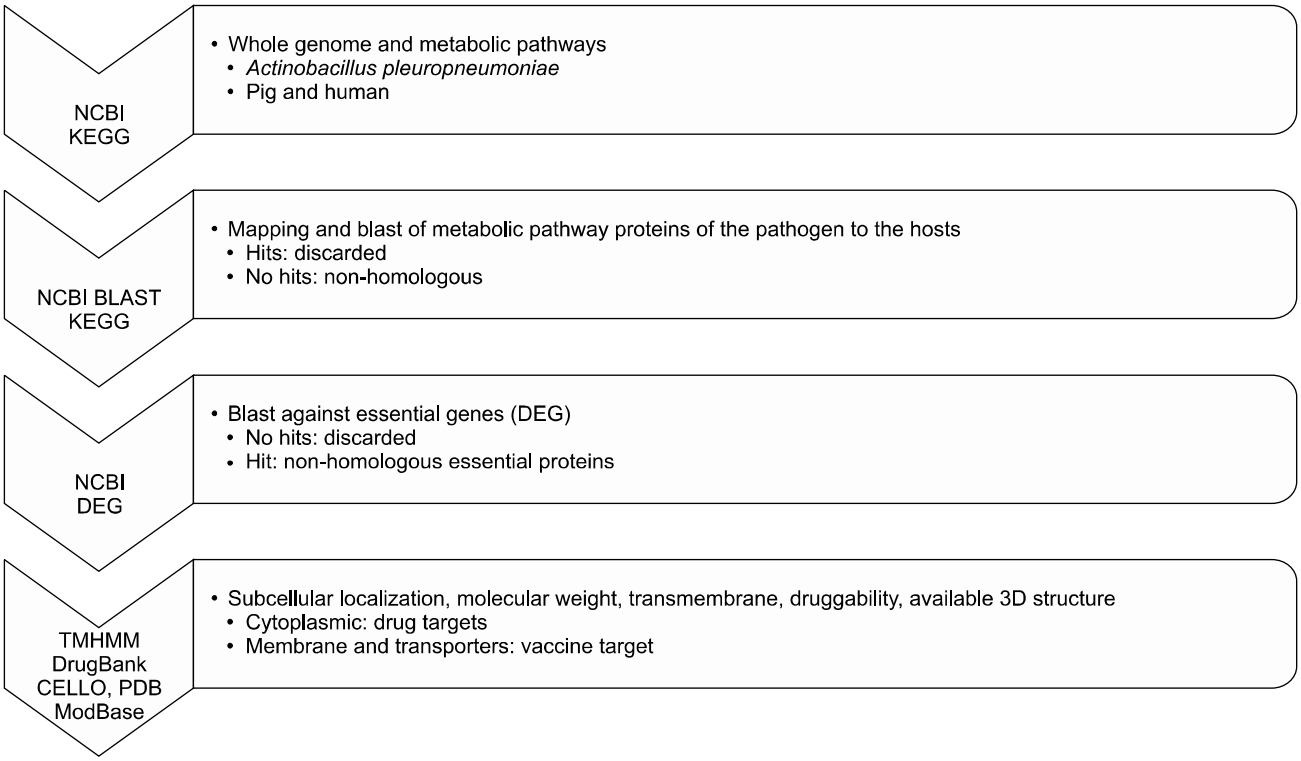


**Fig. 1.** Schematic of the *in silico* method used. Each protein was checked for homology in the respective databases. NCBI, National Center for Biotechnology Information; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEG, Database of Essential Genes; PDB, Protein Data Bank; 3D, three-dimensional.

non-homologous proteins were compared with the essential proteins in the Database of Essential Genes (DEG). The non-homologous sequences of *A. pleuropneumoniae* serotype 5B were aligned with the experimentally verified essential genes of *Haemophilus influenzae* Rd KW20 (NC000907) and with the protein sequences from 36 others Gram-positive and -negative bacteria in the DEG [22].

Comparative searches of host proteins were limited by using the available options under BLASTP criteria. Screening of hits was based on a threshold expectation value (e-value) of 0.005, matching similarity of $\leq$ 35%, and minimum bit score of 100. The identified proteins were further filtered using DEG microbial BLASTP based on their essentiality, with a cutoff e-value of $10^{-10}$ and a least possible bit score of 100 [22].

### Prioritizing the essential non-homologous proteins as drug targets

The identified essential non-homologous proteins of the bacterium were prioritized as potential therapeutic targets, based on their molecular and structural organization. Protein molecular weight was determined with the computational tools and drug target-associated data available in the Swiss-Prot database (UniProt) [36]. The biological significance and subcellular localization of the proteins was predicted by CELLO v.2.5 (multi-class support vector machine classification system) [41], and the transmembrane regions were predicted by using TMHMM v2.0 [20]. In addition, the experimentally and computationally solved three-dimensional structures were determined by using the Protein Data Bank (PDB, USA) [1] and ModBase [28], respectively. To predict the protective antigens and subunit vaccines, VaxiJen v2.0 was used [8].

### Druggability of non-homologous essential proteins of *A. pleuropneumoniae*

DrugBank (ver. 4.3) [38], which contains unique bioinformatic and cheminformatic data on drugs and drug targets, was used to determine the druggability of the identified essential proteins. The proteins were aligned by using the default parameters with the available drug entries, which included U.S. Food and Drug Administration (FDA)-approved small molecule drugs, biotech (protein/peptide) drugs, nutraceuticals, and experimental drugs.

## Results

In this study, *A. pleuropneumoniae* L20 (serotype 5b), with 104 identified pathways, was selected. The sequences of three different *A. pleuropneumoniae* strains are deposited in the NCBI-KEGG database. *A. pleuropneumoniae* JL03 (serotype 3) and *A. pleuropneumoniae* AP76 (serotype 7) have 105 and 106 pathways, respectively. We selected the strain with all of the common pathways for further analysis.

Comparison of the selected pathways in the pathogen to the 295 and 299 pathways in pig and human, respectively, showed that 29 pathways were unique to the pathogen. Twenty of these pathways were metabolic pathways (Table 1). More than 900 proteins were involved in the common and unique metabolic pathways; however, only 273 of them were non-homologous to both pig and human, based on the established KEGG cutoff value.

We aligned these non-homologous proteins with the essential protein sequences of *Haemophilus influenzae* Rd KW20 (NC000907), which shares 85% genome similarity with *A. pleuropneumoniae*, in the DEG and identified 122 essential non-homologous proteins in *A. pleuropneumoniae*. These essential proteins were involved in 40 different metabolic pathways, and, of these 122 proteins, 13 were from 6 different unique metabolic pathways, which were only present in the pathogen.

These non-homologous proteins were compared with all of the essential proteins of the 37 bacteria in the DEG. The greatest numbers of homologous proteins were found for the essential

**Table 1.** Unique metabolic pathways of *Actinobacillus pleuropneumoniae*

| No. | Name | KEGG entry |
|---|---|---|
| 1 | Monobactam biosynthesis | apl00261 |
| 2 | Geraniol degradation | apl00281 |
| 3 | Carbapenem biosynthesis | apl00332 |
| 4 | Benzoate degradation | apl00362 |
| 5 | Novobiocin biosynthesis | apl00401 |
| 6 | Phosphonate and phosphinate metabolism | apl00440 |
| 7 | D-Alanine metabolism | apl00473 |
| 8 | Streptomycin biosynthesis | apl00521 |
| 9 | Lipopolysaccharide biosynthesis | apl00540 |
| 10 | Peptidoglycan biosynthesis | apl00550 |
| 11 | Chloroalkane and chloroalkene degradation | apl00625 |
| 12 | Naphthalene degradation | apl00626 |
| 13 | Aminobenzoate degradation | apl00627 |
| 14 | Glyoxylate and dicarboxylate metabolism | apl00630 |
| 15 | Nitrotoluene degradation | apl00633 |
| 16 | Ethylbenzene degradation | apl00642 |
| 17 | C5-Branched dibasic acid metabolism | apl00660 |
| 18 | Methane metabolism | apl00680 |
| 19 | Limonene and pinene degradation | apl00903 |
| 20 | Caprolactam degradation | apl00930 |
| 21 | Biosynthesis of secondary metabolites | apl01110 |
| 22 | Microbial metabolism in diverse environments | apl01120 |

The unique pathways were identified from the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database of the *A. pleuropneumoniae* L20 (serotype 5b) strain, which has 104 metabolic pathways. The pathways in this strain were compared with the 295 and 299 metabolic pathways of pig and human, respectively.

proteins of *Synechococcus elongate* PCC 7942, *Mycobacterium tuberculosis* H37Rv II, and *Acinetobacter baylyi* ADP1, with 130, 127, and 126 hits, respectively. The smallest number of hits was for *Salmonella enterica* subsp. enterica serovar Typhimurium str. 14028S, with only nine homologous proteins (Fig. 2).

The cellular localization of each non-homologous protein was determined by using the CELLO database, and the results showed 95 cytoplasmic, 11 transmembrane, 4 periplasmic, and 2 outer membrane proteins as well as 10 proteins with undetermined localization. In the unique pathways, there were 7 cytoplasmic, 2 transmembrane, 1 periplasmic, and 1 outer membrane proteins and 2 with undetermined cellular localization. However, the TMHMM server predicted 14 transmembrane proteins, and 9 (64.3%) of them matched with the CELLO prediction (Table 2). Furthermore, 11 of them showed an antigenicity prediction > 0.4 in VaxiJen v2.0 (Table 3).

DrugBank was used to categorize the druggability of the identified essential proteins. Of the non-homologous essential proteins, 24 had a hit in DrugBank of an approved, nutraceutical, investigational, or experimental drug (Table 4). Six genes, appser1_11470, *acc*C, *gua*A, *rpo*A, *asd*, and *mur*C, had hits in DrugBank, with an e-value limit of $10^{-25}$. Each of these genes had at least a one hit of an approved, nutraceutical, or experimental drug.

The molecular weight of each essential protein was determined by using UniProt. Among the 122 essential non-homologous proteins, 120 had a low molecular weight (< 110 kDa; Table 2).

The essential proteins of *A. pleuropneumoniae* were then cross-checked for similarity to the proteins of *M. hyopneumoniae*, which is a major component of PRDC. Three proteins with similarity to essential proteins of *M. hyopneumoniae* were identified: DNA-directed RNA polymerase subunit alpha, rpoA; methionine-tRNA ligase, metG; and glutamate-tRNA ligase, gltX.

## Discussion

Previously, the development of new antimicrobial agents and vaccine therapies was limited by our understanding of the biology of the target microbial agents. However, in this post-genomic era, advances in the fields of genomics and proteomics have allowed for *in silico* investigations of new drugs and vaccine targets, by using genomic and protein sequence resources [6]. *A. pleuropneumoniae*, a bacterium known to cause PRDC in pigs [27], is often resistant to most of the drugs commonly used to treat the disease [37]. Thus, alternative therapeutic agents are greatly needed. In this study, by using an *in silico* approach, we identified unique proteins in the metabolic pathways of *A. pleuropneumoniae* as potential targets for antimicrobial and vaccine therapy.

Multiple unique metabolic pathways were identified in *A. pleuropneumoniae* that are not present in their natural host. The presence of these unique pathways offers an opportunity to identify antimicrobials that specifically target the pathogen, and

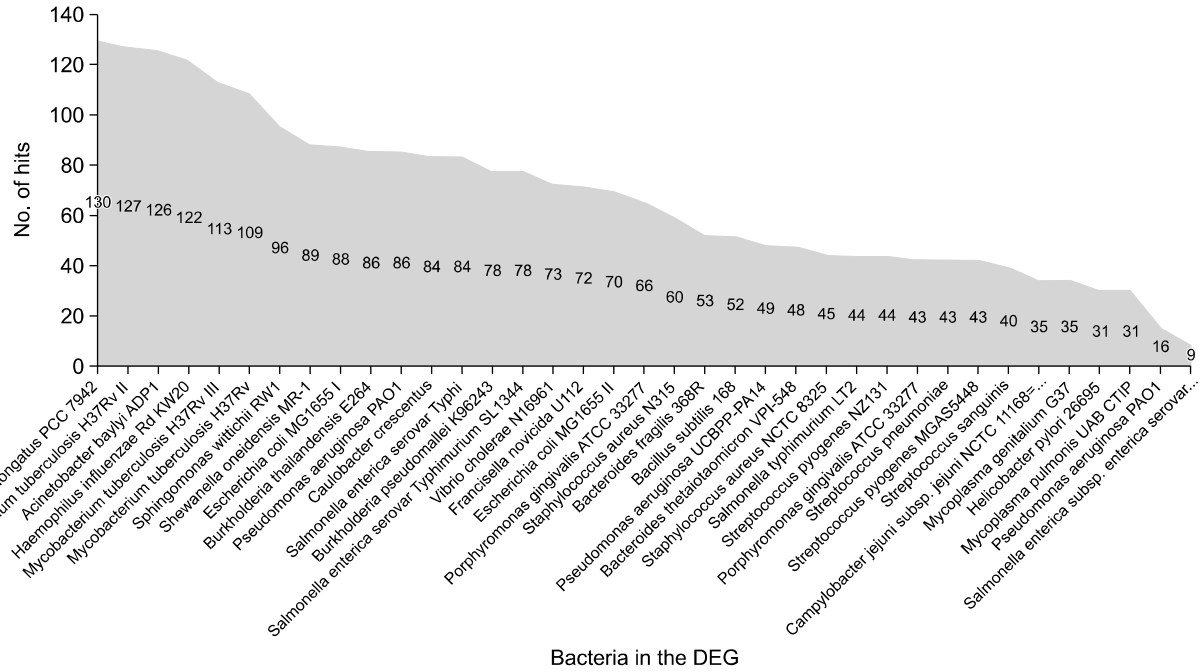


**Fig. 2.** Number of *Actinobacillus pleuropneumoniae* genes with essential gene hits in the Database of Essential Genes (DEG). The essential genes of *A. pleuropneumoniae* were identified by comparison to those of all 36 bacteria in the DEG.

**Table 2.** Cellular localization of non-homologous essential proteins of *Actinobacillus pleuropneumoniae* and their molecular weight (MW)

| No. | Gene | Protein ID/ UniProt | UniProt protein name | Subcellular localization | TMHMM | MW (Da) | Length (bp) |
|---|---|---|---|---|---|---|---|
| 1 | *glp*X | A3MZZ0 | Fructose-16-bisphosphatase | Cytoplasmic | No | 35,851 | 332 |
| 2 | *pyk*A | A3MYQ9 | Pyruvate kinase | Cytoplasmic | No | 51,512 | 479 |
| 3 | *ace*F | A3N0D4 | Acetyltransferase component of pyruvate dehydrogenase complex | Cytoplasmic | No | 66,145 | 632 |
| 4 | appser12_8180 | E0FG80 | Dihydrolipoyl dehydrogenase | Cytoplasmic | No | 50,552 | 474 |
| 5 | appser12_20010 | E0FJK9 | Alcohol dehydrogenase zinc-binding domain protein | Cytoplasmic | No | 36,767 | 349 |
| 6 | *suc*B | A3MZH2 | Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex | Cytoplasmic | No | 45,119 | 409 |
| 7 | *frd*D | D9PCW1 | Fumarate reductase subunit D | Transmembrane | 3 | 12,563 | 114 |
| 8 | *tkt*A | A3N0Z1 | Transketolase | Cytoplasmic | No | 73,263 | 668 |
| 9 | *rpi*A | E0E9U4 | Ribose-5-phosphate isomerase A | Cytoplasmic | No | 23,255 | 219 |
| 10 | appser10_16360 | A3N2M1 | 23-diketo-L-gulonate reductase | Periplasmic/ cytoplasmic | No | 25,933 | 235 |
| 11 | *pgm* | A3MZV7 | Phosphoglucomutase/phosphomannomutase | Periplasmic/ cytoplasmic | No | 59,776 | 552 |
| 12 | *fuc*I | A3N2Y2 | L-fucose isomerase | Cytoplasmic | No | 65,176 | 588 |
| 13 | *fuc*K | A3N2Y3 | L-fuculokinase | External/outer membrane | No | 52,186 | 477 |
| 14 | *glg*B | A3MZ64 | 14-alpha-glucan branching enzyme GlgB | Cytoplasmic | No | 89,300 | 777 |
| 15 | appser1_18790 | E0EAV7 | N-acetylglucosamine-6-phosphate deacetylase | Cytoplasmic | No | 41,320 | 381 |
| 16 | appser1_11470 | E0E8N3 | Formate acetyltransferase | Cytoplasmic | No | 86,174 | 770 |
| 17 | appser9_7010 | E0EX94 | Phosphate acetyltransferase | Cytoplasmic | 1 | 76,723 | 712 |
| 18 | *acc*C | A3N3G1 | Biotin carboxylase | Cytoplasmic | No | 49,234 | 447 |
| 19 | *ppc* | A3MZ57 | Phosphoenolpyruvate carboxylase | Cytoplasmic | No | 99,144 | 879 |
| 20 | *leu*A | A3MZB1 | 2-isopropylmalate synthase | Cytoplasmic | No | 55,881 | 513 |
| 21 | *ldh*A | A3N3X7 | Glycerate dehydrogenase | Cytoplasmic | No | 34,312 | 316 |
| 22 | *leu*C | A3MYL1 | 3-isopropylmalate dehydratase large subunit | Cytoplasmic | No | 50,705 | 469 |
| 23 | *leu*B | E0FF97 | 3-isopropylmalate dehydrogenase | Cytoplasmic | No | 39,118 | 360 |
| 24 | *cyd*A | A3MZ15 | Cytochrome oxidase subunit 1 | Transmembrane | 9 | 57,173 | 516 |
| 25 | appser1_15650 | E0E9U3 | D-3-phosphoglycerate dehydrogenase | Cytoplasmic | No | 44,368 | 409 |
| 26 | *nap*A | D9P8F5 | Nitrate reductase | Periplasmic | No | 93,572 | 827 |
| 27 | *cys*T | A3N3E2 | Sulfate transport system permease protein cysT | Transmembrane | 7 | 29,770 | 270 |
| 28 | *cys*A | A3N3E4 | Sulfate/thiosulfate import ATP-binding protein CysA | Cytoplasmic | No | 40,250 | 356 |
| 29 | *glp*E | E0E5N2 | Thiosulfate sulfurtransferase GlpE | Cytoplasmic | No | 12,403 | 108 |
| 30 | appser9_16390 | E0EZX2 | Serine acetyltransferase | Cytoplasmic | No | 29,518 | 271 |
| 31 | *dms*C | A3N2X4 | Anaerobic dimethyl sulfoxide reductase chain C | Transmembrane | 8 | 30,080 | 277 |
| 32 | *fab*D | A3N3T1 | Malonyl CoA-acyl-carrier-protein transacylase | Cytoplasmic | No | 32,654 | 311 |
| 33 | appser12_20350 | E0FJP3 | 3-oxoacyl-[acyl-carrier-protein] reductase | Cytoplasmic | No | 25,133 | 241 |
| 34 | *fab*A | E0FJD8 | 3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase | Cytoplasmic | No | 19,371 | 176 |
| 35 | *fab*Z | E0E6N4 | 3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ | Cytoplasmic | No | 17,481 | 154 |
| 36 | appser1_4350 | E0E6M1 | Long-chain-fatty-acid--CoA ligase | Cytoplasmic | No | 63,288 | 563 |
| 37 | *glp*K | A3MZ93 | Glycerol kinase | Cytoplasmic | No | 55,663 | 503 |
| 38 | appser4_15300 | E0ELZ7 | 1-acyl-sn-glycerol-3-phosphate acyltransferase | Transmembrane | 2 | 27,292 | 244 |
| 39 | appser1_8310 | E0E7R7 | Diacylglycerol kinase | Transmembrane | 3 | 13,197 | 120 |
| 40 | *gps*A | E0EA03 | Glycerol-3-phosphate dehydrogenase [NAD(P)+] | Transmembrane | No | 36,052 | 336 |

**Table 2.** Continued

| No. | Gene | Protein ID/ UniProt | UniProt protein name | Subcellular localization | TMHMM | MW (Da) | Length (bp) |
|---|---|---|---|---|---|---|---|
| 41 | *glp*A | A3MZ97 | Glycerol-3-phosphate dehydrogenase | Cytoplasmic | No | 61,956 | 561 |
| 42 | appser1_4570 | E0E6P3 | Phosphatidylglycerophosphatase B | Transmembrane | 5 | 26,861 | 234 |
| 43 | *prs*A | A3N0D7 | Ribose-phosphate pyrophosphokinase | Cytoplasmic | No | 34,207 | 316 |
| 44 | *pur*T | E0E8V7 | Phosphoribosylglycinamide formyltransferase 2 | Cytoplasmic | No | 42,969 | 393 |
| 45 | *pur*K | A3N025 | N5-carboxyaminoimidazole ribonucleotide synthase | Cytoplasmic | No | 41,028 | 362 |
| 46 | *ush*A | A3N0D1 | UshA | Periplasmic | No | 60,896 | 547 |
| 47 | *maz*G | A3MZZ6 | Predicted pyrophosphatase | Cytoplasmic | No | 22,929 | 199 |
| 48 | *gpt* | E0E665 | Xanthine phosphoribosyltransferase | Cytoplasmic | No | 17,726 | 157 |
| 49 | *gua*A | A3MZV8 | GMP synthase [glutamine-hydrolyzing] | Cytoplasmic | No | 58,318 | 523 |
| 50 | appser1_11000 | E0E8I6 | Ribonucleoside-diphosphate reductase | Cytoplasmic | No | 85,074 | 756 |
| 51 | appser12_1810 | E0FEF1 | Ribonucleotide reductase alpha subunit | Outer membrane | No | 63,022 | 554 |
| 52 | *rpo*A | A3N382 | DNA-directed RNA polymerase subunit alpha | Cytoplasmic | No | 36,540 | 329 |
| 53 | *rpo*B | E0F6M5 | DNA-directed RNA polymerase subunit beta | Cytoplasmic | No | 149,710 | 1342 |
| 54 | *dna*E | A3N1B1 | DNA-directed DNA polymerase | Cytoplasmic | No | 129,802 | 1158 |
| 55 | appser1_170 | A3N1B1 | DNA polymerase III subunit beta | Cytoplasmic | No | 41,198 | 367 |
| 56 | *dna*X | A3MYY7 | DNA polymerase III subunit gamma/tau | Cytoplasmic | No | 77,048 | 688 |
| 57 | *cya*A | A3N160 | Adenylate cyclase | Outer membrane/ cytoplasmic | No | 97,029 | 842 |
| 58 | *pyr*H | A3MZT5 | Uridylate kinase | Cytoplasmic | No | 25,701 | 237 |
| 59 | *udk* | A3N0J4 | Uridine kinase | Cytoplasmic | No | 25,032 | 217 |
| 60 | appser12_5200 | E0FFD8 | Serine 3-dehydrogenase | Periplasmic | No | 27,004 | 249 |
| 61 | appser1_7720 | E0E7K8 | Aspartokinase | Cytoplasmic | No | 48,488 | 450 |
| 62 | appser1_2720 | E0E660 | Homoserine dehydrogenase | Cytoplasmic | No | 88,030 | 818 |
| 63 | *asd* | E0E5F8 | Aspartate-semialdehyde dehydrogenase | Cytoplasmic | No | 40,421 | 370 |
| 64 | *thr*C | A3N2E7 | Threonine synthase | Cytoplasmic | No | 46,446 | 426 |
| 65 | *trp*A | A3MZI8 | Tryptophan synthase alpha chain | Cytoplasmic | No | 28,934 | 269 |
| 66 | *trp*B | E0F308 | Tryptophan synthase beta chain | Cytoplasmic | No | 42,883 | 396 |
| 67 | *arg*D | A3N3R2 | Diaminobutyrate–2-oxoglutarate aminotransferase | Cytoplasmic | No | 46,677 | 431 |
| 68 | *met*C | A3MZ38 | Cystathionine beta-lyase | Cytoplasmic | No | 44,050 | 396 |
| 69 | appser1_7620 | E0E7I3 | O-acetylhomoserine/O-acetylserine sulfhydrylase | Cytoplasmic | No | 46,186 | 422 |
| 70 | APL_1197 | A3N1J9 | 3-hydroxyacid dehydrogenase | Cytoplasmic | No | 30,382 | 288 |
| 71 | *dap*A | A3N0Q9 | 4-hydroxy-tetrahydrodipicolinate synthase | Cytoplasmic | No | 31,419 | 295 |
| 72 | *arg*D | A3MYW6 | Acetylornithine aminotransferase | Cytoplasmic | No | 41,875 | 393 |
| 73 | *dap*F | E0EA26 | Diaminopimelate epimerase | Cytoplasmic | No | 30,412 | 274 |
| 74 | *mur*F | A3MY86 | UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase | Cytoplasmic | No | 50,346 | 464 |
| 75 | *his*G | E0ENG6 | ATP phosphoribosyltransferase | Cytoplasmic | No | 33,226 | 299 |
| 76 | *his*B | E0FDX6 | Histidine biosynthesis bifunctional protein HisB | Cytoplasmic | No | 41,284 | 363 |
| 77 | *his*C | A3N3V9 | Histidinol-phosphate aminotransferase | Cytoplasmic | No | 38,672 | 350 |
| 78 | *aro*B | E0E605 | 3-dehydroquinate synthase | Cytoplasmic | No | 40,043 | 366 |
| 79 | *aro*C | A3N0B0 | Chorismate synthase | Cytoplasmic | No | 38,893 | 360 |
| 80 | *trp*D | A3N1G7 | Anthranilate phosphoribosyltransferase | Cytoplasmic | No | 36,243 | 334 |
| 81 | *met*G | A3MZ70 | Methionine--tRNA ligase | Cytoplasmic | No | 77,118 | 679 |
| 82 | *mur*D | E0EHR5 | UDP-N-acetylmuramoylalanine--D-glutamate ligase | Cytoplasmic | No | 47,020 | 436 |
| 83 | *mur*C | E0E5H2 | UDP-N-acetylmuramate--L-alanine ligase | Cytoplasmic | No | 51,502 | 475 |
| 84 | *ddl* | E0E5H3 | D-alanine--D-alanine ligase | Cytoplasmic | No | 32,704 | 303 |
| 85 | *pep*A | A3N1A7 | Probable cytosol aminopeptidase | Cytoplasmic | No | 54,219 | 499 |
| 86 | *pep*N | A3N1Y8 | Aminopeptidase N | Cytoplasmic | No | 100,131 | 869 |

**Table 2.** Continued

| No. | Gene | Protein ID/ UniProt | UniProt protein name | Subcellular localization | TMHMM | MW (Da) | Length (bp) |
|---|---|---|---|---|---|---|---|
| 87 | appser1_2760 | E0E664 | Aminoacyl-histidine dipeptidase | Cytoplasmic | No | 56,563 | 515 |
| 88 | *gor* | A3N1P3 | Glutathione reductase | Cytoplasmic | No | 49,025 | 456 |
| 89 | *lpx*A | A3MZC5 | Acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase | Cytoplasmic | No | 28,735 | 264 |
| 90 | *lpx*D | A3MZC7 | UDP-3-O-acylglucosamine N-acyltransferase | Cytoplasmic | No | 35,947 | 341 |
| 91 | *lpx*K | E0FHM3 | Tetra-acyldisaccharide 4′-kinase | Transmembrane | 1 | 36,236 | 326 |
| 92 | *kdt*A | A3N1D6 | 3-deoxy-D-manno-octulosonic-acid transferase | Inner membrane/ cytoplasmic | 1 | 48,112 | 426 |
| 93 | APL_1131 | A3N1D5 | Uncharacterized protein | Transmembrane | 11 | 50,295 | 449 |
| 94 | appser4_16420 | E0EMA9 | Penicillin-binding protein 2 | Periplasmic/ outer membrane | 1 | 73,376 | 653 |
| 95 | *fts*I | A3MY84 | Peptidoglycan synthetase FtsI | Outer membrane | 1 | 76,609 | 686 |
| 96 | appser4_9750 | E0EKF0 | D-alanyl-D-alanine carboxypeptidase/D-alanyl-D-alanine-endopeptidase | Periplasmic | No | 52,514 | 480 |
| 97 | *thi*D | A3MZQ5 | Phosphomethylpyrimidine kinase | Cytoplasmic/ inner membrane/ periplasmic | No | 16,265 | 152 |
| 98 | *isc*S | E0FGP5 | Cysteine desulfurase IscS | Cytoplasmic | No | 45,699 | 408 |
| 99 | *rib*BA | P50855 | Riboflavin biosynthesis protein RibBA | Cytoplasmic | No | 44,740 | 401 |
| 100 | appser1_4210 | E0E6K7 | Riboflavin synthase alpha chain | Cytoplasmic | No | 23,390 | 215 |
| 101 | *pdx*Y | A3N2D3 | Pyridoxamine kinase | Cytoplasmic | No | 31,505 | 286 |
| 102 | appser1_610 | E0E5J9 | Transcriptional regulator nadR | Cytoplasmic | No | 51,844 | 439 |
| 103 | *coa*A | E0EA06 | Pantothenate kinase | Cytoplasmic | No | 36,457 | 316 |
| 104 | *coa*D | D9P743 | Phosphopantetheine adenylyltransferase | Cytoplasmic | No | 17,586 | 158 |
| 105 | appser12_9920 | E0FGQ4 | Dithiobiotin synthetase | Cytoplasmic | 1 | 25,342 | 219 |
| 106 | *bio*A | A3N0V2 | Adenosylmethionine-8-amino-7-oxononanoate aminotransferase | Cytoplasmic | No | 47,998 | 432 |
| 107 | *bio*D | E0FGQ2 | ATP-dependent dethiobiotin synthetase BioD | Cytoplasmic | No | 24,187 | 214 |
| 108 | *bio*D | E0E7I8 | ATP-dependent dethiobiotin synthetase BioD | Cytoplasmic | No | 26,692 | 239 |
| 109 | *bio*B | E0FEB9 | Biotin synthase | Cytoplasmic | No | 37,601 | 336 |
| 110 | appser1_9500 | E0E836 | Dihydrofolate reductase | Cytoplasmic | No | 18,632 | 162 |
| 111 | *que*F | A3N0K4 | NADPH-dependent 7-cyano-7-deazaguanine reductase | Outer membrane/ cytoplasmic | No | 32,106 | 279 |
| 112 | *glt*X | A3N1S5 | Glutamate--tRNA ligase | Cytoplasmic | No | 54,190 | 479 |
| 113 | *hem*L | A3N2K3 | Glutamate-1-semialdehyde 21-aminomutase | Peri/cytoplasmic | No | 45,267 | 426 |
| 114 | *hem*H | E0EB93 | Ferrochelatase | Outer membrane/ cytoplasmic | No | 36,279 | 319 |
| 115 | appser9_11630 | E0EYJ7 | Menaquinone-specific isochorismate synthase | Cytoplasmic | No | 48,461 | 426 |
| 116 | *men*D | A3N348 | 2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase | Transmembrane | No | 62,667 | 568 |
| 117 | *men*B | E0EAX5 | 14-dihydroxy-2-naphthoyl-CoA synthase | Cytoplasmic | No | 31,841 | 285 |
| 118 | *dxr* | A3MZC4 | 1-deoxy-D-xylulose 5-phosphate reductoisomerase | Cytoplasmic | No | 42,953 | 396 |
| 119 | *isp*E | A3N0D8 | 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase | Cytoplasmic | No | 31,309 | 285 |
| 120 | *isp*F | E0EXQ9 | 2-C-methyl-D-erythritol 24-cyclodiphosphate synthase | Cytoplasmic | No | 17,245 | 158 |
| 121 | *isp*H | A3N2G8 | 4-hydroxy-3-methylbut-2-enyl diphosphate reductase | Cytoplasmic | No | 34,035 | 314 |
| 122 | *isp*B | A3N3T3 | Octaprenyl-diphosphate synthase | Cytoplasmic | No | 36,102 | 331 |

**Table 3.** Antigenic prediction of the transmembrane proteins of *Actinobacillus pleuropneumoniae* using Vaxijen v.20 [8]

| Protein | Gene | TMHMM | Antigen probability* |
|---|---|---|---|
| APL_1526 | *frd*D | 3 | Antigen |
| APL_0644 | appser9_7010 | 1 | Antigen |
| APL_0297 | *cyd*A | 9 | Antigen |
| APL_1846 | *cys*T | 7 | Antigen |
| APL_1676 | *dms*C | 8 | Antigen |
| APL_1488 | appser4_15300 | 2 | Non-antigen |
| APL_0768 | appser1_8310 | 3 | Antigen |
| APL_0417 | appser1_4570 | 5 | Antigen |
| APL_1278 | *lpx*K | 1 | Antigen |
| APL_1132 | *kdt*A | 1 | Non-antigen |
| APL_1131 | APL_1131 | 11 | Antigen |
| APL_1599 | appser4_16420 | 1 | Antigen |
| APL_0012 | *fts*I | 1 | Antigen |
| APL_0940 | appser12_9920 | 1 | Non-antigen |

*Threshold value used was 0.4.

**Table 4.** Similarity of the non-homologous proteins of *Actinobacillus pleuropneumoniae* to the binding proteins of FDA-approved drugs from DrugBank [38]

| KEGG ID | Gene | DrugBank ID | Drug name | Drug group |
|---|---|---|---|---|
| APL_0771 | appser12_8180 | DB03147 | Flavin adenine dinucleotide | Approved |
| APL_0983 | *tkt*A | DB01987 | Thiamin Diphosphate | Experimental |
| APL_1573 | appser10_16360 | DB01694 | D-tartaric acid | Experimental |
| APL_1684 | *fuc*I | DB03815 | Fucitol | Experimental |
| APL_1036 | appser1_11470 | DB01992, DB03278, DB03940 | Coenzyme A, D-Treitol, Oxamic Acid | Nutraceutical, experimental, experimental |
| APL_1865 | *acc*C | DB08074, DB08075, DB08076, DB08144, DB08145, DB08146, DB08314, DB08315, DB08316, DB08317, DB08318 | 3-(3-methylbut-2-en-1-yl)-3H-purin-6-amine, 4-(2-amino-1,3-thiazol-4-yl)pyrimidin-2-amine, 4-[1-(2,6-dichlorobenzyl)-2-methyl-1H-imidazol-4-yl]pyrimidin-2-amine, 6-(2,6-dibromophenyl)pyrido[2,3-d]pyrimidine-2, 7-diamine, 6-(2,6-DIMETHOXYPHENYL)PYRIDO[2,3-D] PYRIMIDINE-2,7-DIAMINE, 7-(2,5-dihydropyrrol-1-yl)-6-phenyl-pyrido[6,5-d]pyrimidin-2-amine, (2-AMINO-1,3-OXAZOL-5-YL)-(3-BROMOPHENYL)METHANONE, 2-AMINO-N,N-BIS(PHENYLMETHYL)-1,3-OXAZOLE-5-CARBOXAMIDE, 4-amino-7,7-dimethyl-7,8-dihydroquinazolin-5(6H)-one, 5-methyl-6-phenylquinazoline-2,4-diamine, 6-(2-phenoxyethoxy)-1,3,5-triazine-2,4-diamine | Experimental |
| APL_0339 | *ppc* | DB04317 | 3,3-Dichloro-2-Phosphonomethyl-Acrylic Acid | Experimental |
| APL_1511 | appser9_16390 | DB01992 | Coenzyme A | Nutraceutical |
| APL_1992 | appser12_20350 | DB04450, DB08404, DB084505 | Heptyl 1-Thiohexopyranoside, S-(2-{[N-(2-HYDROXY-4-{[HYDROXY(OXIDO)PHOSPHINO]OXY}-3,3-DIMETHYLBUTANOYL)-BETA-ALANYL]AMINO}ETHYL) HEXANETHIOATE, S-(2-{[N-(2-HYDROXY-4-{[HYDROXY(OXIDO)PHOSPHINO]OXY}-3,3-DIMETHYLBUTANOYL)-BETA-ALANYL]AMINO}ETHYL) HEPTANETHIOATE | Experimental |

**Table 4.** Continued

| KEGG ID | Gene | DrugBank ID | Drug name | Drug group |
|---|---|---|---|---|
| APL_1889 | *fab*A | DB03813 | 2-Decenoyl N-Acetyl Cysteamine | Experimental |
| APL_0375 | *glp*K | DB02937, DB04551 | Gamma-Arsono-Beta, Gamma-Methyleneadenosine-5′-Diphosphate, Fructose-1,6-Diphosphate | Experimental |
| APL_0775# | *prs*A | DB02798, DB03148 | Alpha-Methylene Adenosine Monophosphate, Phosphomethylphosphonic Acid Adenosyl Ester | Experimental |
| APL_0255 | *gpt* | DB01972, DB02134, DB02377, DB03942 | Guanosine-5′-Monophosphate, Xanthine, Guanine, Carboxylic PRPP | Experimental |
| APL_0592 | *gua*A | DB04272 | Citric Acid | Nutraceutical |
| APL_1784 | *rpo*A | DB00615 | Rifabutin | Approved |
| APL_0250 | appser1_2720 | DB07118 | 7-hydroxy-4-methyl-2H-chromen-2-one | Experimental |
| APL_0005 | *asd* | Db03461, DB03502, DB04498 | 2′-Monophosphoadenosine 5′-Diphosphoribose, (4s)-4-{[(2s)-2-Amino-3-Oxopropyl]Sulfanyl}-L-Homoserinate, Aspartate-Semialdehyde | Experimental |
| APL_0352 | *met*G | DB02151, DB02229, DB03799, DB03816, DB04015 | Methionine Phosphonate, 5′-O-[(L-Methionyl)-Sulphamoyl]Adenosine, Trifluoromethionine, Difluoromethionine, Methionine Phosphinate | Experimental |
| APL_0016 | *mur*D | DB01673, DB02314, DB03801, DB08105, DB08106, DB08107, DB08108, DB08112 | Uridine-5′-Diphosphate-N-Acetylmuramoyl-L-Alanine, Uridine-5′-Diphosphate-N-Acetylmuramoyl-L-Alanine-D-Glutamate, Lysine Nz-Carboxylic Acid, N-[(6-BUTOXYNAPHTHALEN-2-YL) SULFONYL]-L-GLUTAMIC ACID, N-[(6-BUTOXYNAPHTHALEN-2-YL)SULFONYL]-D-GLUTAMIC ACID, N-{[6-(PENTYLOXY) NAPHTHALEN-2-YL]SULFONYL}-D-GLUTAMIC ACID, N-({6-[(4-CYANOBENZYL)OXY]NAPHTHALEN-2-YL}SULFONYL)-D-GLUTAMIC ACID, N-({6-[(4-CYANO-2-FLUOROBENZYL)OXY] NAPHTHALEN-2-YL}SULFONYL)-D-GLUTAMIC ACID | Experimental |
| APL_0019 | *mur*C | DB01673, DB03909, DB04395 | Uridine-5′-Diphosphate-N-Acetylmuramoyl-L-Alanine, Adenosine-5′-[Beta, Gamma-Methylene]Triphosphate, Phosphoaminophosphonic Acid-Adenylate Ester | Experimental |
| APL_1243 | *gor* | DB00336, DB03147 | Nitrofural, Flavin adenine dinucleotide | Approved, approved |
| APL_1599 | appser4_16420 | DB00303, DB00671 | Ertapenem, Cefixime | Approved and investigational, approved |
| APL_0012 | *fts*I | DB00303 | Ertapenem | Approved and investigational |
| APL_0776 | *isp*E | DB03687, DB04395 | 4-Diphosphocytidyl-2-C-Methyl-D-Erythritol, Phosphoaminophosphonic Acid-Adenylate Ester | Experimental |

FDA, U.S. Food and Drug Administration; KEGG, Kyoto Encyclopedia of Genes and Genomes.

thus, should be safe. Targeting essential genes/proteins in unique metabolic pathways, which are required for survival and replication, provides an extra advantage for designing potent therapeutic agents, since they should interfere with the survival and/or replication of the pathogen [23,42].

Determining the cellular localization of a protein is an essential step toward identifying it as a potential target for therapeutic intervention [33]. This leads to elucidation of the function of each protein, which helps to differentiate between targets for antimicrobial agents and those for vaccine therapy [12]. Most of the identified non-host essential cytoplasmic proteins could serve as targets for antimicrobial treatment,

while the transmembrane proteins, as suggested by the TMHMM, could be potential vaccine targets [16]. These transmembrane proteins may be selected toxins/surface-exposed proteins and may be used for the production of a preventive vaccine that initiates antibody-mediated immunity [7].

Most of the identified essential proteins of *A. pleuropneumoniae* (120/122) had a low molecular weight, thereby providing a broad opportunity for selecting and utilizing these proteins as targets for therapeutic intervention. Low molecular weight proteins are likely to be soluble and purified easily, which makes them suitable drug targets [9]. Moreover, the existence of FDA-approved, nutraceutical, or experimental drugs with the ability to bind proteins similar to the identified essential proteins of the pathogen demonstrates the potential druggability of these proteins as therapeutic targets and offers the opportunity of using different combinations of drugs to treat *Actinobacillus* infections in pigs. In fact, five FDA-approved drugs with hits in essential non-host *A. pleuropneumoniae* proteins were identified in this study.

Penicillin-binding protein 2 (PBP2) and peptidoglycan synthetase, which are from a unique pathway in *A. pleuropneumoniae*, have been previously characterized as drug targets in other pathogens. In *S. aureus*, PBP2 is the only bifunctional penicillin-binding protein [13,29], and the transpeptidase domain of the protein was reported to be critical for the survival and replication of the bacterium [28]. Peptidoglycan synthetase, FtsI, a cell division protein, is essential for the synthesis of peptidoglycan and catalyzes the synthesis of cross-linked peptidoglycan from lipid-linked precursors [25,34]. Hence, inhibition of these proteins/enzymes using one or more drugs might reduce the infection rate and incidence of drug resistance in *A. pleuropneumoniae*.

The remaining transmembrane proteins, either from unique or common pathways, which showed antigenic and MHC cleavability, have been suggested as targets for vaccine therapy. Specifically, tetra-acyldisaccharide 4′-kinase and 3-deoxy-D-manno-octulosonic-acid transferase, two transmembrane proteins in unique pathways, are involved in the synthesis of lipid A in the lipopolysaccharide layer [10]. Hence, these proteins could be targets for a host antibody response, since Gram-negative bacteria resist the host defense mechanism by upregulating their expression in the outer membrane. This upregulation results in an increased host response, which suggests the potential of these proteins as vaccine candidates [21,26,39].

Glycerate dehydrogenase, 3-isopropylmalate dehydratase large subunit, 3-isopropylmalate dehydrogenase, D-3-phosphoglycerate dehydrogenase, D-alanine--ligase, acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase, UDP-3-O-acylglucosamine N-acyltransferase, and D-alanyl-D-alanine carboxypeptidase/D-alanyl-D-alanine-endopeptidase are among the cytoplasmic proteins in unique metabolic pathways, and these can be targeted for the development of novel antimicrobial agents against *A. pleuropneumoniae*.

The cytoplasmic enzyme glycerate dehydrogenase (ldhA) is an essential protein from a unique pathway; moreover, it is involved in eight different metabolic pathways, but mainly in glyoxylate and dicarboxylate metabolism, which is crucial for the synthesis of carbohydrates in the anabolic pathway by converting acetyl CoA. Thus, blockage of this specific enzyme or the pathway might lead to bacterial cell death due to carbohydrate limitation. Therefore, the glyoxylate and dicarboxylate metabolic pathway could be a useful drug target for *A. pleuropneumoniae*.

Furthermore, three of the essential proteins of *A. pleuropneumoniae* are also essential for *M. hyopneumoniae*, which is another major component of PRDC [6]. The DNA-directed RNA polymerase subunit alpha rpoA, which is involved in purine and pyrimidine metabolism, had a hit with an FDA-approved drug. Methionine-tRNA ligase (metG), which is an essential protein in selenocompound metabolism, had a hit in DrugBank for an experimental drug. Glutamate-tRNA ligase (gltX), an essential protein in porphyrin and chlorophyll metabolism and aminoacyl-tRNA biosynthesis, catalyzes the binding of glutamate to tRNA (Glu) in a two-step reaction. Phosphorylation of this enzyme by HipA, a toxin and serine/threonine kinase, results in amino acid starvation; prevents replication, transcription, translation, and cell-wall synthesis, and it inhibits growth, leading to multidrug resistance and persistence [11,18,30]. Targeting these proteins in both pathogens could be beneficial for preventing antimicrobial resistance and pig loss due to PRDC.

In conclusion, *in silico* approaches are of paramount importance when identifying target proteins and metabolic pathways as potential drug and vaccine therapy targets. In this study, we identified the glyoxylate and dicarboxylate pathways and glycerate dehydrogenase as putative targets for antimicrobial therapy against *A. pleuropneumoniae*; moreover, we identified tetra-acyldisaccharide 4′-kinase and 3-deoxy-D-manno-octulosonic-acid transferase as prospective vaccine targets. In addition, we identified three non-host essential proteins that are common to both *A. pleuropneumoniae* and *M. hyopneumoniae*; proteins that could serve as targets for antimicrobial therapy against both pathogens. However, although an *in silico*-based approach involves a series of screens for proteins that can be used as potential drug targets and vaccine candidates, the method has a major limitation in that the identified target proteins require experimental confirmation of their potential. In addition, non-protein vaccine and drug targets cannot be identified by applying this method [31]. Thus, a study should be undertaken to identify any unlisted pathogenic genes of *A. pleuropneumoniae* and determine the practicability of using the proteins and pathways identified as targets for drug and vaccine therapies.

## Acknowledgments

This research was supported by the Kyungpook National University Bokhyeon Research Fund, 2016.

## Conflict of Interest

The authors declare no conflicts of interest.

## References

1. **Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE.** The protein data bank. Nucleic Acids Res 2000, **28**, 235-242.
2. **Bossé JT, Janson H, Sheehan BJ, Beddek AJ, Rycroft AN, Kroll JS, Langford PR.** *Actinobacillus pleuropneumoniae*: pathobiology and pathogenesis of infection. Microbes Infect 2002, **4**, 225-235.
3. **Butt AM, Nasrullah I, Tahir S, Tong Y.** Comparative genomics analysis of *Mycobacterium ulcerans* for the identification of putative essential genes and therapeutic candidates. PLoS One 2012, **7**, e43080.
4. **Chawley P, Samal HB, Prava J, Suar M, Mahapatra RK.** Comparative genomics study for identification of drug and vaccine targets in *Vibrio cholerae*: MurA ligase as a case study. Genomics 2014, **103**, 83-93.
5. **Chung WB, Bäckström LR, Collins MT.** Experimental model of swine pneumonic pasteurellosis using crude *Actinobacillus pleuropneumoniae* cytotoxin and *Pasteurella multocida* given endobronchially. Can J Vet Res 1994, **58**, 25-30.
6. **Damte D, Suh JW, Lee SJ, Yohannes SB, Hossain MA, Park SC.** Putative drug and vaccine target protein identification using comparative genomic analysis of KEGG annotated metabolic pathways of *Mycoplasma hyopneumoniae*. Genomics 2013, **102**, 47-56.
7. **Doro F, Liberatori S, Rodríguez-Ortega MJ, Rinaudo CD, Rosini R, Mora M, Scarselli M, Altindis E, D'Aurizio R, Stella M, Margarit I, Maione D, Telford JL, Norais N, Grandi G.** Surfome analysis as a fast track to vaccine discovery: identification of a novel protective antigen for Group B *Streptococcus* hypervirulent strain COH1. Mol Cell Proteomics 2009, **8**, 1728-1737.
8. **Doytchinova IA, Flower DR.** Bioinformatic approach for identifying parasite and fungal candidate subunit vaccines. Open Vaccine J 2008, **1**, 22-26.
9. **Duffield M, Cooper I, McAlister E, Bayliss M, Ford D, Oyston P.** Predicting conserved essential genes in bacteria: *in silico* identification of putative drug targets. Mol Biosyst 2010, **6**, 2482-2489.
10. **Garrett TA, Que NL, Raetz CR.** Accumulation of a lipid A precursor lacking the 4'-phosphate following inactivation of the *Escherichia coli* lpxK gene. J Biol Chem 1998, **273**, 12457-12465.
11. **Germain E, Castro-Roa D, Zenkin N, Gerdes K.** Molecular mechanism of bacterial persistence by HipA. Mol Cell 2013, **52**, 248-254.
12. **Glory E, Murphy RF.** Automated subcellular location determination and high-throughput microscopy. Dev Cell 2007, **12**, 7-16.
13. **Goffin C, Ghuysen JM.** Multimodular penicillin-binding proteins: an enigmatic family of orthologs and paralogs. Microbiol Mol Biol Rev 1998, **62**, 1079-1093.
14. **Gottschalk M.** Actinobacillosis. In: Zimmerman JJ, Karriker LA, Schwartz KJ, Stevenson GW (eds.). Diseases of Swine. 10th ed. pp. 653-669, Wiley-Blackwell, Chichester, 2012.
15. **Huang H, Potter AA, Campos M, Leighton FA, Willson PJ, Haines DM, Yates WD.** Pathogenesis of porcine *Actinobacillus pleuropneumonia*, part II: roles of proinflammatory cytokines. Can J Vet Res 1999, **63**, 69-78.
16. **Hung MC, Link W.** Protein localization in disease and therapy. J Cell Sci 2011, **124**, 3381-3392.
17. **Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M.** KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 2016, **44**, D457-462.
18. **Kaspy I, Rotem E, Weiss N, Ronin I, Balaban NQ, Glaser G.** HipA-mediated antibiotic persistence via phosphorylation of the glutamyl-tRNA-synthetase. Nat Commun 2013, **4**, 3001.
19. **Kim B, Min K, Choi C, Cho WS, Cheon DS, Kwon D, Kim J, Chae C.** Antimicrobial susceptibility of *Actinobacillus pleuropneumoniae* isolated from pigs in Korea using new standardized procedures. J Vet Med Sci 2001, **63**, 341-342.
20. **Krogh A, Larsson B, von Heijne G, Sonnhammer EL.** Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 2001, **305**, 567-580.
21. **Li C, Ye Z, Wen L, Chen R, Tian L, Zhao F, Pan J.** Identification of a novel vaccine candidate by immunogenic screening of *Vibrio parahaemolyticus* outer membrane proteins. Vaccine 2014, **32**, 6115-6121.
22. **Luo H, Lin Y, Gao F, Zhang CT, Zhang R.** DEG 10, an update of the database of essential genes that includes both protein-coding genes and noncoding genomic elements. Nucleic Acids Res 2014, **42**, D574-580.
23. **Mobegi FM, van Hijum SA, Burghout P, Bootsma HJ, de Vries SP, van der Gaast-de Jongh CE, Simonetti E, Langereis JD, Hermans PW, de Jonge MI, Zomer A.** From microbial gene essentiality to novel antimicrobial drug targets. BMC Genomics 2014, **15**, 958.
24. **Morya VK, Dewaker V, Mecarty SD, Singh R.** *In silico* analysis metabolic pathways for identification of putative drug targets for *Staphylococcus aureus*. J Comput Sci Syst Biol 2010, **3**, 62-69.
25. **Nguyen-Distèche M, Fraipont C, Buddelmeijer N, Nanninga N.** The structure and function of *Escherichia coli* penicillin-binding protein 3. Cell Mol Life Sci 1998, **54**, 309-316.
26. **Nishio M, Okada N, Miki T, Haneda T, Danbara H.** Identification of the outer-membrane protein PagC required for the serum resistance phenotype in *Salmonella enterica* serovar Choleraesuis. Microbiology 2005, **151**, 863-873.
27. **Opriessnig T, Giménez-Lirola LG, Halbur PG.** Polymicrobial respiratory disease in pigs. Anim Health Res Rev 2011, **12**, 133-148.
28. **Pieper U, Webb BM, Dong GQ, Schneidman-Duhovny D,**

**Fan H, Kim SJ, Khuri N, Spill YG, Weinkam P, Hammel M, Tainer JA, Nilges M, Sali A.** ModBase, a database of annotated comparative protein structure models and associated resources. Nucleic Acids Res 2014, **42**, D336-346.
29. **Pinho MG, Filipe SR, de Lencastre H, Tomasz A.** Complementation of the essential peptidoglycan transpeptidase function of penicillin-binding protein 2 (PBP2) by the drug resistance protein PBP2A in *Staphylococcus aureus*. J Bacteriol 2001, **183**, 6525-6531.
30. **Potrykus K, Cashel M.** (p)ppGpp: still magical? Annu Rev Microbiol 2008, **62**, 35-51.
31. **Seib KL, Dougan G, Rappuoli R.** The key role of genomics in modern vaccine and drug design for emerging infectious diseases. PLoS Genet 2009, **5**, e1000612.
32. **Shanmugham B, Pan A.** Identification and characterization of potential therapeutic candidates in emerging human pathogen *Mycobacterium abscessus*: a novel hierarchical *in silico* approach. PLoS One 2013, **8**, e59126.
33. **Simon I, Wright M, Flohr T, Hevezi P, Caras IW.** Determining subcellular localization of novel drug targets by transient transfection in COS cells. Cytotechnology 2001, **35**, 189-196.
34. **Spratt BG.** Distinct penicillin binding proteins involved in the division, elongation, and shape of *Escherichia coli* K12. Proc Natl Acad Sci U S A 1975, **72**, 2999-3003.
35. **Uddin R, Saeed K, Khan W, Azam SS, Wadood A.** Metabolic pathway analysis approach: identification of novel therapeutic target against methicillin resistant *Staphylococcus aureus*. Gene 2015, **556**, 213-226.
36. **UniProt Consortium.** UniProt: a hub for protein information. Nucleic Acids Res 2015, **43**, D204-212.
37. **Vanni M, Merenda M, Barigazzi G, Garbarino C, Luppi A, Tognetti R, Intorre L.** Antimicrobial resistance of *Actinobacillus pleuropneumoniae* isolated from swine. Vet Microbiol 2012, **156**, 172-177.
38. **Wishart DS, Knox C, Guo AC, Shrivastava S, Hassanali M, Stothard P, Chang Z, Woolsey J.** DrugBank: a comprehensive resource for *in silico* drug discovery and exploration. Nucleic Acids Res 2006, **34**, D668-672.
39. **Wu XB, Tian LH, Zou HJ, Wang CY, Yu ZQ, Tang CH, Zhao FK, Pan JY.** Outer membrane protein OmpW of *Escherichia coli* is required for resistance to phagocytosis. Res Microbiol 2013, **164**, 848-855.
40. **Yoo AN, Cha SB, Shin MK, Won HK, Kim EH, Choi HW, Yoo HS.** Serotypes and antimicrobial resistance patterns of the recent Korean *Actinobacillus pleuropneumoniae* isolates. Vet Rec 2014, **174**, 223.
41. **Yu CS, Chen YC, Lu CH, Hwang JK.** Prediction of protein subcellular localization. Proteins 2006, **64**, 643-651.
42. **Zhang R, Lin Y.** DEG 5.0, a database of essential genes in both prokaryotes and eukaryotes. Nucleic Acids Res 2009, **37**, D455-458.