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Original Article

Re-construction of Co-expression Network of Genes Involved in Bacterial Cell Wall Synthesis and Their Role in Penicillin Resistance

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Abstract

Background: Peptidoglycan (Murein), which consists of disaccharide and amino acid chain subunits, has a key role in bacterial survival and ranks first in the line defense system against drug therapy. In addition, the transpeptidase enzyme plays an important role in cross-linking in bacterial cell walls. In *Escherichia coli* bacteria, cross-linking happens by proteins that have a D-D transpeptidase role and bond two amino acids of D-alanine together. These proteins are characterized by their affinity for and binding of penicillin thus they are called penicillin-binding proteins (PBPs). It should be noted that this bonding formation is prevented by the beta-lactam family as they have a similar structure to the above-mentioned proteins. The product of the *idtD* gene by characteristics such as L-D transpeptidase can catalyze the peptidoglycan structure in the bacterial cell wall in the presence of beta-lactam antibiotics.

Methods: In this study, around 426 interactions were identified between genes and approximately 20 genes with a key role in the process of bacterial cell wall synthesis by the reconstruction of 44 genes involved in bacterial cell wall synthesis.

Results: The *idtD* gene locus at the reconstructed network clearly shows that its catalytic activity is the side activity, and there won't be a lag or disturbance in the procedure cell wall synthesis by removing it from the cycle. However, this side process causes the strengthening of the bacterial cell wall synthesis process against disorders arising by the presence of beta-lactam antibiotics.

Conclusions: These five genes in *E. coli* that furnish L-D transpeptidase properties include *IdtA, IdtC, IdtD, IdtE,* and *mrdA* out of which, *IdtD* is the most important gene in this process.

Keywords: Antimicrobial resistance, Cell wall, *E. coli*, Gene Network, Murein, Penicillin

Introduction

The cell wall plays a key role in bacterial survival and is considered as the first line of defense against drug therapies. In addition, gram-positive and Gram-negative bacteria have peptidoglycan structures in their cell walls which consist of disaccharide subunit repeat binding with cross-linked amino acids (1). Glycan strands are polymerized by the glycosyltransferase enzyme. Further, the transpeptidase enzyme has the main role in the creation and development of cross-linking in the bacterial cell wall. In the *Escherichia coli*, crossed bonds are controlled by proteins that have D-D transpeptidase properties and connect 2 D-alanine amino acids. Furthermore, these proteins have the ability to bind to penicillin, which is the reason they are called penicillin-binding proteins (PBPs). Moreover, these bonds are prevented by the beta-lactam family of antibiotics as they have similar structures to the above-mentioned bonds. The penicillin family of antibiotics has a single ring of beta-lactam in its structure thus it is also known as the beta-lactam antibiotic (2). Beta-lactam ring in the beta-lactam family of antibiotics and their similarity to the D-alanine/D-alanine structure can be observed in Figure 1. The similarity between the beta-lactam families of antibiotics and D-alanine/D-alanine in the cell wall prohibits the progress and function of the cell wall, and finally, causes cell death.

Unusual interconnections that connect the remaining of two diaminopropionic acids were identified in early 1969 in *E. coli*, but the primary enzyme which is responsible for their formation remained unknown until later decades (3). These crossed bonds of $DAP^3 \rightarrow DAP^3$ consists of 3 and 10% of available interconnections in the extracted peptidoglycan from bacterial in exponential and stationary growth phases, respectively (3). Recently, it has been elucidated that the L,D transpeptidase (*Idt*) enzyme is responsible for this unusual interconnection and, unlike

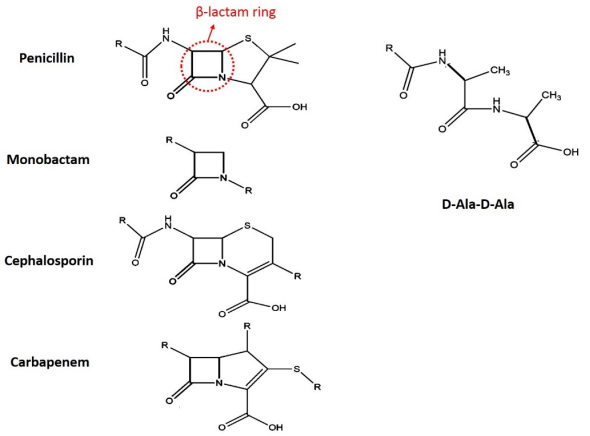

Figure 1. Beta-lactam Ring in the Beta-lactam Families of Antibiotics (penicillin) and Their Similarities to D-alanine/D-alanine Bindings.

the common belief, these proteins are not from the PBPs (4). Previous research on the *E. coli* chromosome showed that it has five genes that are responsible for the L,D transpeptidase. Among these genes, namely, *YcbB* and *YnhG* can catalyze DAP[3]→DAP[3] (5) and the other three genes (i.e., *YcfS, ErfK,* and *YbiS*) shall connect lipoproteins to peptidoglycans (6). Hugonnet et al demonstrated that *YcbB* encoded for a protein has the ability to function as a substitute for the D, D transpeptidase of the PBP family of proteins and thus causes the resistance to the beta-lactam family of antibiotics (7). Resistance to penicillin and other antibiotics from the beta-lactam family can be found in a wide range of gram-negative and gram-positive bacteria. Accordingly, the identification and study of genes involved in the resistance process can be a great help to overcome this problem and break the defense mechanism of bacteria against the beta-lactam family of antibiotics. To nail this aim using data deposited to databases and bioinformatics tools, the present study evaluated the co-expression network of genes involved in the synthesis of the bacterial cell wall and its role in antibacterial resistance.

Materials and Methods

In this study, published papers and findings and deposited data on UniProt were collected with regard to genes that were involved in the cell wall synthesis of bacteria (8), the details of which are presented in Table 1. Then, these genes were entered in the STRING database (9) and the network was expended three times. Finally, the network was obtained by 44 nodes with 426 edges. Additionally, other genes associated with this process were identified using parameters like co-expression and experiments. The obtained data from STRING analysis for the re-creation of the co-expression network purpose were uploaded into Cytoscape software (10). In addition, network topology was studied based on betweenness and closeness centrality parameters by Network Analyser software (11). An entity centrality of a complex network is named by betweenness centrality. Likewise, it is calculated based on all shortest edge pairs in a network. On the other hand, the closeness centrality is defined by the shortest distance mean from one node to all other nodes. In a simpler word, the higher

Table 1. The Initial Genes Involved in Peptidoglycan Anabolism Which Entered Into the STRING Database

Gene Name	Gene ID	Definition	Reference
mtgA	947728	Biosynthetic peptidoglycan transglycosylase	PubMed: 18165305
ftsW	946322	Probable peptidoglycan glycosyltransferase	PubMed: 11807049
mrcB	944843	Penicillin-binding protein 1B	PubMed: 19458048
ftsI	944799	Peptidoglycan D,D-transpeptidase	PubMed:9282742
mrcA	947907	Penicillin-binding protein 1A	PubMed: 7006606
murA	947703	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	PubMed: 20392080
murE	944791	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase	Pubmed: 11124264
dacB	947693	D-alanyl-D-alanine carboxypeptidase DacB	PubMed: 2046551
mrdB	945238	Peptidoglycan glycosyltransferase	PubMed: 27643381
pbpC	947152	Penicillin-binding protein 1C	PubMed: 10542235
dacB	947693	D-alanyl-D-alanine carboxypeptidase	PubMed: 2046551
dacC	945455	D-alanyl-D-alanine carboxypeptidase	PubMed: 2046551
ldtD	945541	L,D-transpeptidase	PubMed: 32486329
ycbB	945541	Probable L,D-transpeptidase	PubMed: 18456808
mrdA	945240	Peptidoglycan D,D-transpeptidase	PubMed: 20392080

Note. UDP-N: Uridine diphosphate N-acetylglucosamine.

amount of betweenness and closeness centrality for a node represents the importance of that node for the network (12,13). The ontology analysis of genes in the above network was performed by web-based software DAVID (14). Then, Benjamin *P* value was calculated for every single gene (15).

Results and Discussion

The mechanism of antimicrobial-resistance to beta-lactam antibiotics is evaluated in 3 different ways. First, the mechanism is related to gram-negative bacteria that block the entry of antibiotics into the cell by changes in the structure of purines (pores in the bacterial cell wall). The second mechanism linked to the antimicrobial-resistant is common between gram-positive and -negative bacteria. These antibiotic-resistant bacteria have the capacity to breakdown the C-N bond in the beta-lactam ring. Finally, the third mechanism in antimicrobial-resistant bacteria to the beta-lactam family, the bacteria change the transpeptidase enzyme structure in such a way that no antibiotic can enter the bacteria. The second and third mechanisms were already reported in different strains of *E. coli* (16).

In general, 44 genes with a role in peptidoglycan synthesis in *E. coli* were selected based on our data mining and evaluation and analysis on the UniProt database (Table 2). Further, the interactions between the genes were identified using the STRING database. Furthermore, the centrality of the nodes in the protein-protein interaction network was calculated by applying the Network Analyzer tool, and finally, the co-expression network of the above genes was re-created by Cytoscape software. As shown in Figure 2, a complex network of genes was involved in the synthesis of the peptidoglycan process. The topology analysis elucidated that the *murF* gene by 23 connections had the highest connections with other genes in the network and then *murA* by 22 connections and *murD*, *murE*, *murI*, and *ftsW* genes each with 20 connections. The *murA* gene, along with *mrcA*, *mrdB*, *mrdA*, *dacB*, and *ftsW* had 0.020, 0.018, 0.0137, 0.0133, 0.0099, and 0.0092 betweenness centrality, respectively. The closeness centrality of each gene was an amount between 0 and 1. Further, 13 genes had the highest amount of closeness centrality equal to 1, including *IdtD*, *murA*, *murC*, *murD*, *murE*, *murF*, and *murG*.

The product of the *murA* gene is an enzyme named 1-carboxy vinyl transferase which catalyzes the cornerstone of bacterial cell wall biosynthesis (17). As shown in Figure

Table 2. Some Hub nodes in Figure 2 Networks and the Metrical Information of Networks

Hub Nodes	Edge Count	Closeness Centrality	Betweenness Centrality
ldtD	3	1	0.00166113
pbpG	6	0.5	0.00221484
mepM	7	0.51851852	0.00415282
murJ	7	0.66666667	0.00198413
bacA	8	0.6	0.00913621
lpoB	8	0.58333333	0.00510863
alr	9	0.47692308	0.00909007
nagZ	10	0.5	0.00478564
dacB	11	0.60465116	0.00996678
mrcB	12	0.75	0.00361889
glmU	12	0.66666667	0.00293994
mrdA	15	0.78571429	0.01339187
mrcA	16	0.625	0.01889535
mrdB	17	1	0.01376101
murC	17	1	0.00277053
murG	19	1	0.00373359
ftsW	20	1	0.0092608
murD	20	1	0.00238161
murA	22	1	0.02042003
murF	23	1	0.00279689
Total Number of Nodes	Total Count of Edge	Closeness Centrality Average	Betweenness Centrality Average
44	426	0.645646082	0.003347428

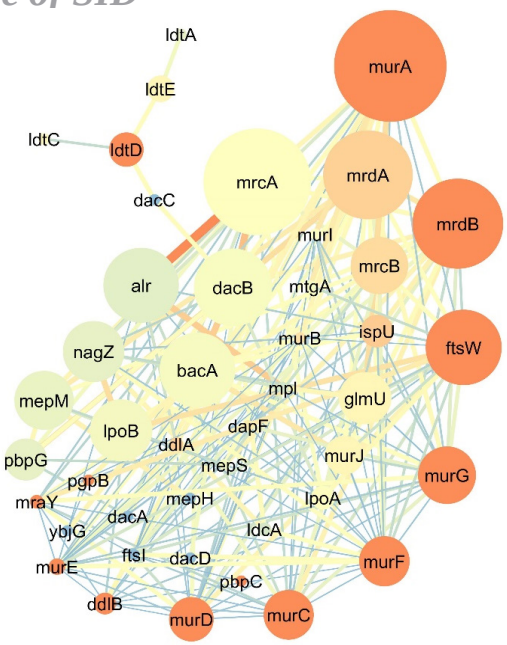

Figure 2. Genes Involved in Peptidoglycan Wall Synthesis in *E. coli*. *Note*. Node size depends on betweenness centrality (lower size for a lesser amount) and the color of the node varies from blue (the least closeness centrality) to red (highest closeness centrality). The color and thickness of each line (the connection between to genes) also vary from red and thick for a higher amount and blue and thin lines for fewer amounts.

3, this enzyme catalyzes the biosynthesis in the presence of phosphoenolpyruvate and the uridine diphosphate N-acetylglucosamine molecule yield (1-carboxy vinyl)-α-D-glucosamine. A free phosphate is also a byproduct of this reaction (18). The suppression of the *murA* gene by fosfomycin antibiotics prevents the cell wall synthesis and subsequently bacterial death (18). More precisely, the re-created network and the topology analysis results elucidate that the *murA* gene has a key role in the process. The other genes from the Mur gene family (e.g., *murB*, *murC*, and the like) all play a role in cell wall synthesis. Eventually, the gene *murG* catalyzes the last reaction of the process, and subsequently, causes the formation of disaccharide-pent peptide production which is a subunit of the peptidoglycan cell wall (19). Moreover, the products of *mrdA* and *mrdB* catalyze the crossed binding of peptidoglycan cell walls and have a role in the width synthesis of the peptidoglycan cell wall, and finally, preserve and protect the bacterial structure and firmness (20). Most of the genes in this network are sensitive to the beta-lactam family of antibiotics (21). As mentioned earlier, each gene has connections with a higher number of other genes in the network. For example, the *IdtD* gene via *dacC* and *dacB* genes is connected to the main network. Although the *dacB* gene does not have a direct role in transpeptidation, it exclusively catalyzes DD-carboxy-peptidase and DD-endopeptidase reactions (22). On the other hand, dacC discards the remaining of the C-terminal of D-alanine from the precursors of peptide-sugar cell walls.

In *E. coli* bacteria, the product of *IdtD* AKA *YcbB* has the ability to catalyze the reciprocal bindings of the amino acids in the peptidoglycan structure of the bacterial cell wall in the presence of the beta-lactam family of antibiotics since the catalyzed peptidyl binding is not D-D but is of L-D type (5). Despite the activity of *IdtD* in the presence of the beta-lactam family of antibiotics, it remains sensitive to the carbapenem, meropenem, and imipenem family of antibiotics. Additionally, it has been determined that copper can repress the activity of *IdtG* (7). The *IdtD* gene locus in the re-created network clearly demonstrates that the catalyzed reaction is a collateral process and there will be no disruption in the process of bacterial cell wall synthesis by removing this reaction. Conversely, this collateral process has a key role in bacterial resistance during the synthesis of the cell wall against the beta-lactam family of antibiotics. For a more detailed analysis of the role of the *IdtD* gene in *E. coli*, its exclusive co-expression network has been re-created, which is shown in Figure 4.

Based on the data in Figure 4, co-expression network topology analysis shows that the *IdtD* gene has the most connections (n=10) in the network and other genes like *ygaU*, *osmC*, *dps*, and *ydiZ* by 9, 8, 7, and 7 connections are placed 2 to 5 in the ranking, respectively. Additionally, *ycaC*, *osmC,* and *IdtD* by the value of 1 have the highest closeness centrality and, from the betweenness centrality viewpoint of genes like *IdtD* (0.0578), *IdtE* (0.0236), and

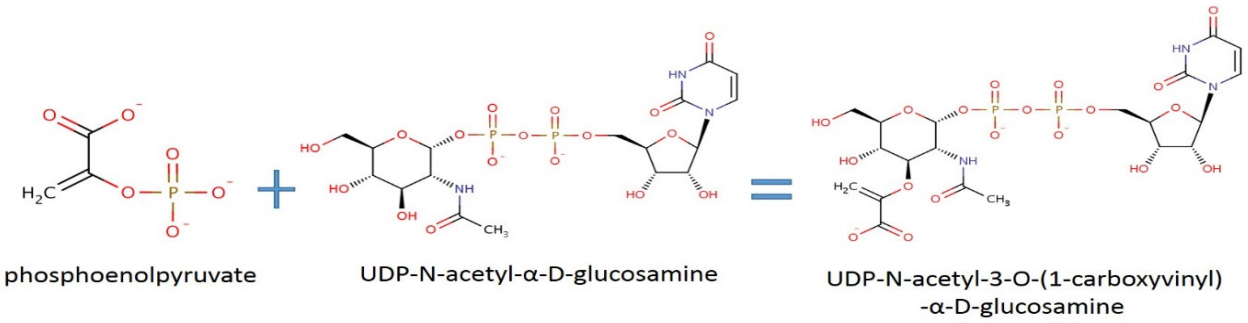

Figure 3. Catalyzed Reaction of *Carboxy Vinyl* Transferase. *Note*. This reaction is the first step in the synthesis of bacterial cell walls.

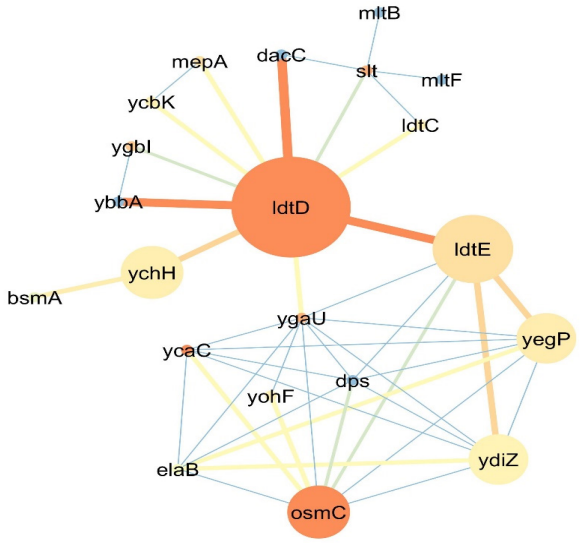

Figure 4. Co-expression Network of the *IdtD* Gene that Is Responsible for D-L Transpeptidase Reciprocal Binding in *E. coli* Bacteria. *Note*. Node size depends on betweenness centrality (lower size for a lesser amount) and the color of the node differs from blue (the least closeness centrality) to red (highest closeness centrality). The color and thickness of each line (the connection between to genes) also vary from red and thick for a higher amount and blue and thin lines for fewer amounts.

ychH (0.0078) have the highest value.

Based on the results in Figure 4, the *IdtD* gene had an important role in the L,D transpeptidase binding process. As previously mentioned, this gene catalyzes the DAP^3 → DAP^3. Another gene named *IdtE* AKA YnhG has the same function as the *IdtG* gene. According to a previous report, the upregulation of this gene in *E. coli* bacteria subsequently results in an increase in the DAP^3 → DAP^3 binding in the bacterial cell wall structure (5). In defense against oxidative stress from exposure to the organic hydro peroxidase, osmC gene plays an osmotic induction role and preferably metabolizes organic hydro peroxidases more than inorganic hydrogen peroxidase (23). The co-expression of this gene by the upregulation of the D-L transpeptidase process shall clarify that D-L transpeptidase is activated in unfavorable environmental conditions and substitutes with the D,D transpeptidase function. Nonetheless, it also has an activity of 3 to 10% in favorable environmental conditions.

The box plot (Figure 5) displays the ontology analysis of genes in the co-expression network of bacterial cell wall synthesis. Out of 44 identified genes by the DAVID database based on molecular function, penicillin-binding with a *P* value of 7.2E-14 and 9 connections had the highest molecular function among all genes involved in the bacterial cell wall synthesis process. This finding proved that bacterial cell wall synthesis is extremely under the influence of the beta-lactam family of antibiotics. Binding capability to the magnesium (Mg) ion by 6 genes and the *P* value of 8.2E-2 is one of the active molecular functions among bacterial cell wall synthesis. These genes include D-alanine ligase A (*ddlA*), D-alanine ligase B (*ddlB*), L-alanine ligase (*murC*), meso-diaminopimelate ligase (*murE*), glucosamine 1-phosphate acetyltransferase (*glmU*), and finally, undecaprenyl-pyrophosphate syntheses (ispU). By considering the obtained data, the Mg enzyme was the co-factor of these enzymes and had a key role in bacterial cell wall synthesis. In addition, five genes of *IdtA*, *IdtC*, *IdtD*, *IdtE*, and *mrdA* had the D-L transpeptidase function and were observed in the co-expression network (Figure 4). The *IdtD* gene is the most key gene and responsible for D-L transpeptidase functionality in *E. coli* bacteria. From the locus point of view, these genes were evaluated and the results showed that all the above-mentioned genes are located in a

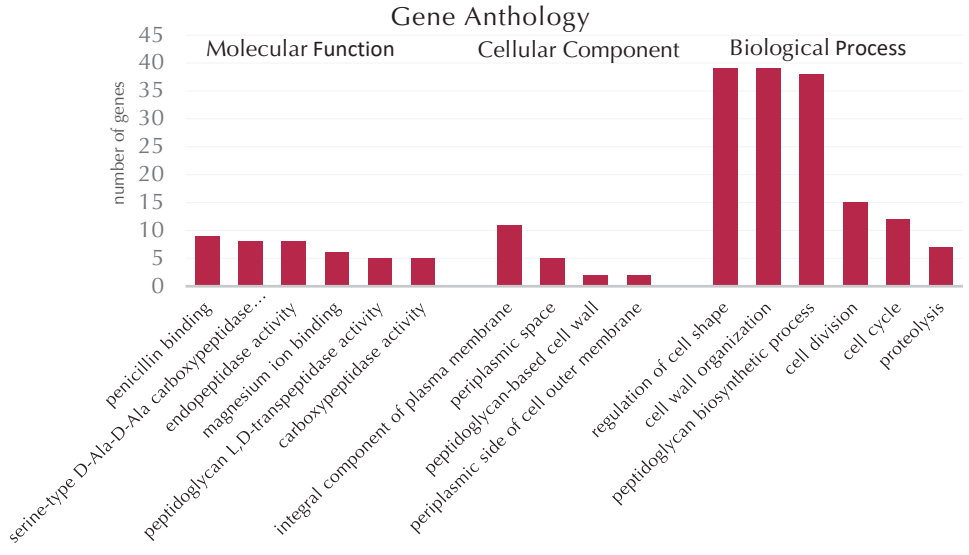

Figure 5. Ontology Analysis Chart of Genes Involved in Bacterial Cell Wall Synthesis Process

membrane or the cell wall itself. From the biopathway perspective, the cell shape regulatory and peptidoglycan biosynthesis pathways with 39 (*P* value of 4.6E-73) and 38 (*P* value of 1.2E-62) genes are the two most active biopathways, respectively. It should be noted that each gene can present in different pathways with numerous different functions.

Conclusions

In this study, about 426 interactions were identified between genes and approximately 20 genes with a key role in the process of bacterial cell wall synthesis by the reconstruction of 44 genes involved in bacterial cell wall synthesis. *E. coli* bacteria has the resistance machinery and mechanism to the penicillin (beta-lactam) family of anti-biotic. This process can proceed via different pathways. However, the cell wall structure changes, and the alteration and prevention of beta-lactam antibiotic efficacy in the bacterial cell wall synthesis process is one of the main mechanisms in bacterial resistance. In *E. coli* bacteria, the product of the *IdtD* gene has the ability to catalyze the reciprocal bindings of amino acids in the presence of the beta-lactam family of antibiotics. In addition, the *IdtD* gene locus in the re-created network clearly suggests that catalyzing activity is a collateral process, and there will be no disruption to the bacterial cell wall synthesis by removing it from the network. Nevertheless, this collateral process causes strengthening the synthesis pathway against disruption by the presence of the beta-lactam family of antibiotics. It seems that the D-L transpeptidase mechanism is activated in unfavorable environmental conditions, replacing the D, D transpeptidase mechanism. However, the amount of activity goes up to 10% in favorable environmental conditions. Further, the magnesium co-factor has a key role in bacterial cell wall synthesis. Overall, 5 genes in *E. coli* have D-L transpeptidase function, including *IdtA, IdtC, IdtD, IdtE*, and *mrdA.* Finally, the *IdtD* gene has an essential role in the D-L transpeptidase function in *E. coli*.

Conflict of Interests

The authors confirm that there is no conflict of interests.

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