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Bioinformatics analysis of *Klebsiella pneumoniae* and its Proteins

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Abstract | **Original Research Article**

Objective: To analyze the structure and properties of *Klebsiella pneumoniae* and its encoded proteins. ***Methods***: To analyze and predict the gene of *Klebsiella pneumoniae* and to analyze and predict the sequence and structure of its encoded proteins by using various information analysis tools in NCBI, ExPASy and other websites. The analysis of the gene sequence (homology analysis, multiple sequence comparison, conserved region analysis, gene structure prediction, gene annotation, enzyme cleavage site analysis, primer design, six-frame translation, etc.), protein sequence analysis and structure prediction (primary structure analysis, subcellular localization, signal peptide, transmembrane information, secondary structure prediction, three-dimensional structural homology modeling, etc.), molecular phylogenetic analysis (constructing phylogenetic tree), and molecular phylogenetic analysis (constructing phylogenetic tree). ***Conclusion***: We successfully analyzed and predicted the sequence and structure of *Klebsiella pneumoniae* and its encoded proteins, with a view to providing reference for the in-depth study of the biological properties of SapC, the ABC transporter permease of *Klebsiella pneumoniae*, the establishment of a rapid detection method for the bacterium, and the selection of targets for subunit and nucleic acid vaccines, and laying a foundation for the further understanding and utilization of this gene.

Keywords: *Klebsiella pneumonia*, ABC transporter protein, Permease SapC, Bioinformatic analysis.

1. INTRODUCTION

Klebsiella pneumoniae is one of the members of the genus Klebsiella in the family of Enterobacteriaceae, widely distributed in water and soil and other environments, and easy to colonize the respiratory tract and digestive tract of humans and animals, in the case of low immunity, can cause pneumonia, enteritis, inflammation of the urinary system and traumatic infections, and even cause peritonitis, meningitis, septicemia and so on. In recent years, due to the extensive use of antimicrobial drugs, the number of drug-resistant strains of *Klebsiella pneumoniae* has been increasing, resulting in the frequent occurrence of animal epidemics caused by this bacterium, which not only caused serious economic losses to the breeding industry, but also greatly increased the risk of animal-to-human transmission. Therefore, the study of *Klebsiella pneumoniae* will provide theoretical support for the development of rapid diagnostic methods and the establishment of effective prevention and control measures for the diseases caused by this bacterium.

Outer membrane proteins are the main components of Gram-negative bacteria, accounting for about 50% of the structure of the outer membrane, which is located on the surface of the cell membrane or inlaid therein, and play a role in maintaining the structure of the outer membrane, material transport, and bacterial infection and pathogenicity of the host process, such as bacterial physiological activities and pathogenicity, such as microporous proteins in the outer membrane to non-covalent bonds with peptidoglycan tightly coupled to the formation of a non-specific channel or permeable channels, with permeable The outer membrane proteins also play a role in bacterial resistance. The outer membrane proteins are also closely related to bacterial drug resistance and have excellent immunogenicity, which can induce a strong immune response in the body, and their crude extracts have shown good protective effects in immunized animals, thus becoming the main candidate antigens for subunit vaccine screening. The ABC transporter protein permease SapC is an important protein component of the extracellular membrane of *Klebsiella pneumoniae*, but few studies have been reported on this protein. studies have rarely been reported. In this study, we designed specific primers to amplify the CDS sequence of the ABC transporter permease SapC gene of Klebsiella pneumoniae and analyzed it bioinformatically, in order to provide reference for the in-depth study of the biological properties of ABC transporter permease SapC of *Klebsiella pneumoniae*, the establishment of a rapid

method for the detection of the bacterium, and the selection of targets for the subunit vaccine and nucleic acid vaccine.

Therefore, this paper analyzes and predicts the *Klebsiella pneumoniae* gene and analyzes and predicts the sequence and structure of its encoded proteins by using various information analysis tools from NCBI, ExPASy and other websites, which lays the foundation for further understanding and utilization of the gene.

2. EXPERIMENTAL MATERIALS AND METHODS

2.1 Experimental sequence

The gene sequence of *Klebsiella pneumoniae* was obtained from the NCBI database under the gene accession number BQSD01000004.1. The *Klebsiella pneumoniae* ABC transporter protein permease, SapC, was accessed under the accession number GKL76932 in NCBI.

3. Experimental methodology

3. 1 Gene sequence analysis

Using the basic local comparison search tool (Blast: http://www.ncbi.nlm.nih.gov/BLAST/) of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov), gene homology analysis was completed using Blastn, and protein homology analysis was completed using Blastp. The protein homology analysis was performed by Blastp. Three homologous genes were selected for multiple sequence comparison of their proteins and their conserved regions were found using the Clastal omega program from the EMBL website (http://www,ebi.ac.uk/embl/). The gene structure of this nucleic acid sequence was predicted using Genscan or Genemarks. Gene annotation was performed using KEGG or eggNOG. Gene sequences were analyzed for possible cleavage sites using NEBcutter (http://tools.neb.com/NEBcutter2/index.php). Primers were designed using primer 3.0 (http://bioinfo.ut.ee/primer3-0.4.0/). The ORF finder (http: //www.ncbi.nlm.nih.gov/gorf/ofig.cgi) was applied to find its open reading frame readable frames and deduce the codable protein sequences.

3.2 Protein sequence analysis and structure prediction

The relative molecular mass, theoretical pI value of the protein of *Klebsiella pneumoniae* ABC transporter protein permease SapC were analyzed by applying programs such as ProtParam, Proscale, etc. through the proteomics and analytical tools provided by the Expert System for Protein Analysis of the Swiss Bioinformatics Institute (ExPASy, https://www.expasy.org/), amino acid composition, atomic composition, extinction coefficient, half-life, instability coefficient, and basic physicochemical properties such as hydrophilicity. Protein signaling peptides, subcellular localization, subcellular localization, and transmembrane information were analyzed by SignalP4.1 server, TargetP1.1 server, TM HMM 2.0 server, and Protfun 2.2 server tools on the CBSPredictionServers (www.cbs.dtu.dk/services) website, hydrophobic regions, and transmembrane information. Prediction of the secondary structure of the *Klebsiella pneumoniae* ABC transporter protein permease SapC protein by the GOR program. Find out in the PDB database if the protein has been determined to have a three-dimensional structure, and if there is no known structure utilize SWISS-MODEL for homology modeling.

Search in protein family or classification databases to analyze proteins for structural domains, functional key sites, or protein families to which they belong (http://www.ebi.ac.uk/interpro).

3. 3 Molecular phylogenetic analysis

Phylogenetic trees were constructed using MEGA software.

4. RESULTS

4.1 Analysis of *Klebsiella pneumoniae* gene sequences

The sequence name of the *Klebsiella pneumoniae* gene was obtained from the information in NCBI as BQSD01000004, the sequence length is 223,883 bp, the molecular type is DNA, the date of the last revision is September 2, 2023, and the coding sequence starts at base 64784 and terminates at base 65674, and the product is the ABC transporter protein permease SapC.

Gene homology analysis was completed using Blastn, and the results showed that there are no similar genes in other species of microorganisms. However, the whole genome of *Klebsiella pneumoniae* has many other strains different from this sequence, such as Klebsiella phageST974oxa48phi18.2. Protein homology analysis was completed using Blastp, and the interval of the percentage of similarity was [36.62%, 92.57%]. The protein is SapC, the ABC transporter protein permease of *Klebsiella pneumoniae*, with an amino acid residue length of 296aa, and its similar homologous sequences are all of *Klebsiella pneumoniae*, such as Sample E16KP0287, Sample 20467, and Sample NKU_Kleb8A7.

Three homologous genes were selected for multiple sequence comparison of their proteins using the Clastal omega program from the EMBL website and their conserved regions were found as shown in Fig 1.

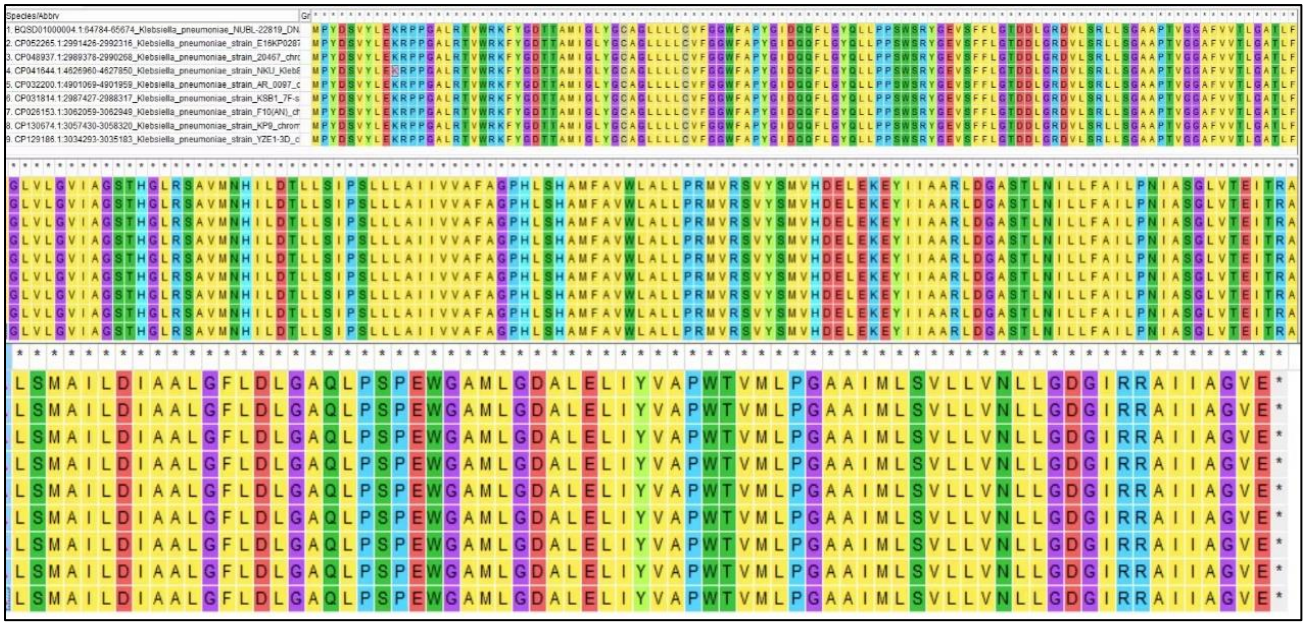

Fig 1: Conservation area

The gene structure of the nucleic acid sequence was predicted using Genscan, followed by gene annotation using KEGG. NEB cutter was used to analyze the possible enzyme cleavage sites of the gene sequence, the possible enzyme cleavage sites of the gene sequence were obtained, and three fragments could be obtained by enzyme cleavage using selected restriction endonucleases, and the gel graph of electrophoresis of enzyme cleavage fragments is shown in Fig 2.

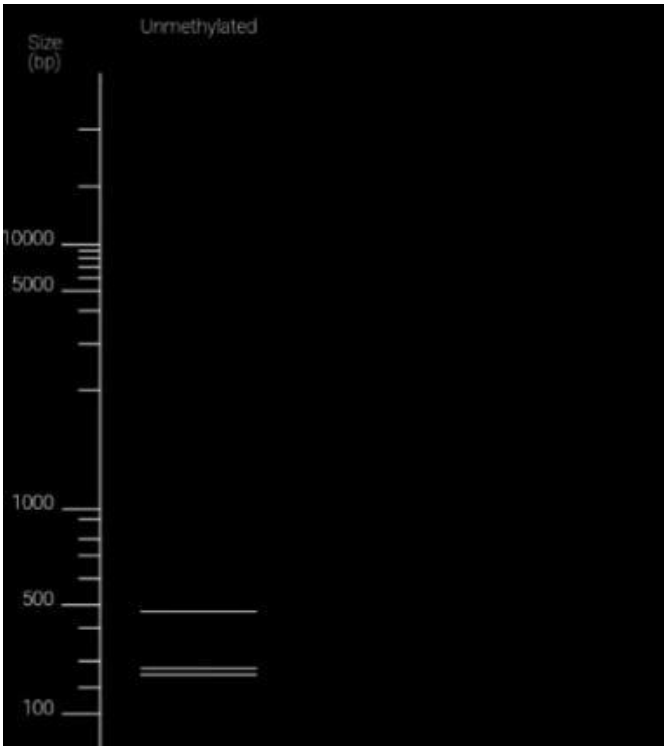

Fig 2: Gel diagram of electrophoresis of the enzyme-sectioned segments

Primers were designed using primer 3.0. According to the principle of primer design, the best primer sequence and its basic information were obtained, and ORF finder was applied to find its open reading frame readable frame, and six frames graphic and position start and length information were obtained, and the sequence of the encodable protein was deduced, which is a gene with the full length of 223,883bp, coding region of 64784~65674, coding for 296 amino acids, and the start codon AGT termination codon TAA.

4.2 Protein sequence analysis and structure prediction

4.2.1 Physicochemical properties of SapC, the ABC transporter protein permease from *Klebsiella pneumoniae*

The relative molecular mass, theoretical pI value, amino acid composition, atomic composition, extinction coefficient, half-life, instability coefficient, and hydrophobicity of the protein of *Klebsiella pneumoniae* ABC transporter permease SapC were analyzed by the proteomic and analytical tools provided by the Expert Protein Analyzer System (ExPASy) of the Swiss Institute of Bioinformatics (SEIB) using the programs such as ProtParam, Proscale, etc. The protein was also analyzed for its relative molecular mass and theoretical pI value. coefficient, half-life, instability, and basic physicochemical properties such as hydrophilicity.

The results showed that *Klebsiella pneumoniae* ABC transporter protein permease SapC has a molecular formula of$C_{1468}H_{2314}N_{362}O_{390}S_{12}$, with a relative molecular mass of 4546, a theoretical isoelectric point of 5.29, a total average hydrophilicity of 0.783, and is mainly composed of leucine (17.6%), alanine (11.1%) and glycine (10.5%). The number of positively charged amino acid residues in the sequence was 16 (arginine + lysine), and the number of negatively charged amino acid residues was 22 (aspartic acid + glutamic acid), and the total number of atoms was 296, with an extinction coefficient of 47,900 at A280 and an instability coefficient of 34.44, which was less than 40, indicating that the protein was a stable protein in vitro. The half-life in mammals is 30h in vitro, the half-life in yeast is >20 h, and the half-life in Escherichia coli is >20 h, which meets the expression conditions of the prokaryotic expression system, and can be used to induce the expression of SapC, the permease of ABC transporter protein of *Klebsiella pneumoniae*, in vitro.

The hydrophobicity prediction showed that the total average hydrophilicity of the protein sequence was 0.783, with the strongest hydrophilicity of -1.711 for tryptophan at position 76 and 3.600 for alanine at position 148. The protein was predicted to be hydrophobic, suggesting that the protein may exist in bacterial lysates as inclusion bodies, which can be verified by the E. coli prokaryotic expression system at a later stage. The presence of this protein can be verified by the prokaryotic expression system of Escherichia coli.

4.2.2 Transmembrane region, signal peptide and subcellular localization of SapC, an ABC transporter protein permease from *Klebsiella pneumoniae*

The signal peptide, subcellular localization, and transmembrane information of the protein were analyzed by SignalP4.1server, TargetP1.1 server, TM HMM 2.0 server, and Protfun 2.2 server tools from CBSPrediction Servers website.

The results showed that the protein has two parts, intracellular domain and extracellular domain, containing transmembrane region (Table 1, & Fig 3), and the probability of having signal peptide is 0.167%, which may be an intracellular protein. After analyzing the results of subcellular localization prediction, it was predicted that this protein existed in the cytoplasm. PeptideMass was used to analyze the endonucleated products of this protein after protease k treatment, and the results showed that there were 7 peptide fragments larger than 750Da.

Table 1: Starting and ending positions of transmembrane regions

Typology	Starting Position	Ending Position
inside	1	29
TMhelix	30	52
outside	53	98
TMhelix	99	121
inside	122	133
TMhelix	134	156
outside	157	197
TMhelix	198	217
inside	218	223
TMhelix	224	243
outside	244	257
TMhelix	258	280
inside	281	296

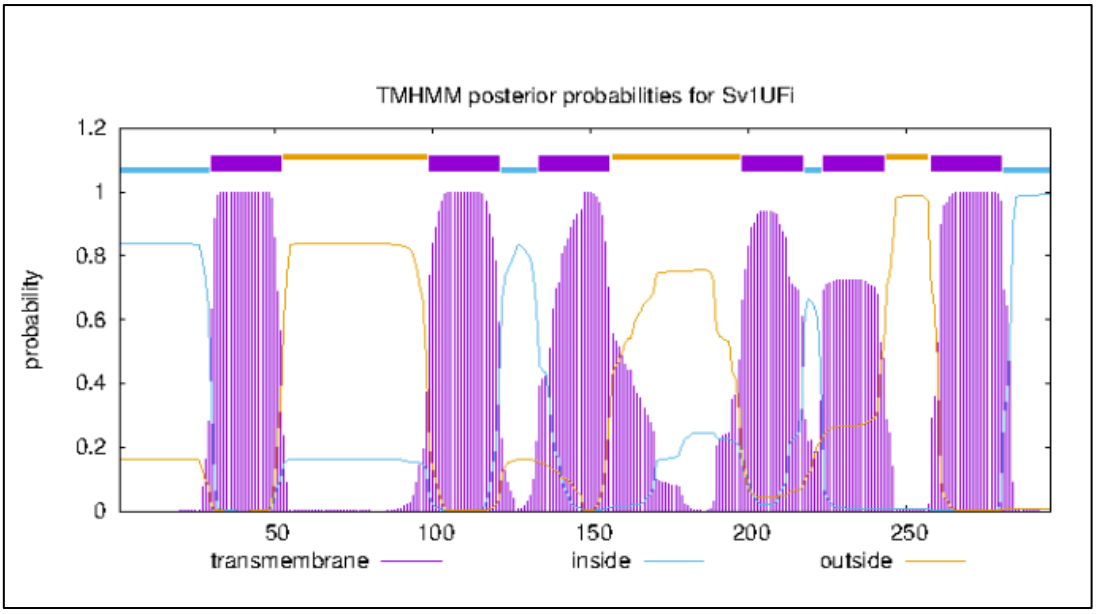

Fig 3: Predicted transmembrane region of the *Klebsiella pneumoniae* ABC transporter protein permease SapC

4.2.3 Structural analysis of SapC, an ABC transporter protein permease from *Klebsiella pneumoniae*

The secondary structure of *Klebsiella pneumoniae* ABC transporter protein permease SapC protein was predicted by the GOR program, and the results showed that the secondary structure of the protein consisted of 74 α-helices (64.91%) 9 β-turns (7.89%), 21 irregular convolutions (18.42%), and 10 elongated chains (8.77%) (Fig 4). The results of tertiary structure prediction showed that the protein contained a large number of α-helices and irregular coils (Fig. 5), which was consistent with the results of secondary structure prediction. Since α-helices, β-turns and irregular curls are easy to twist and fold, and are more likely to form structures that are chimeric with cell-surface receptors, the presence of more of the above structures in this protein suggests that more cellular antigenic epitopes may exist in this protein.

Fig 4: Predicted secondary structure of SapC, the ABC transporter protein permease of *Klebsiella pneumoniae*

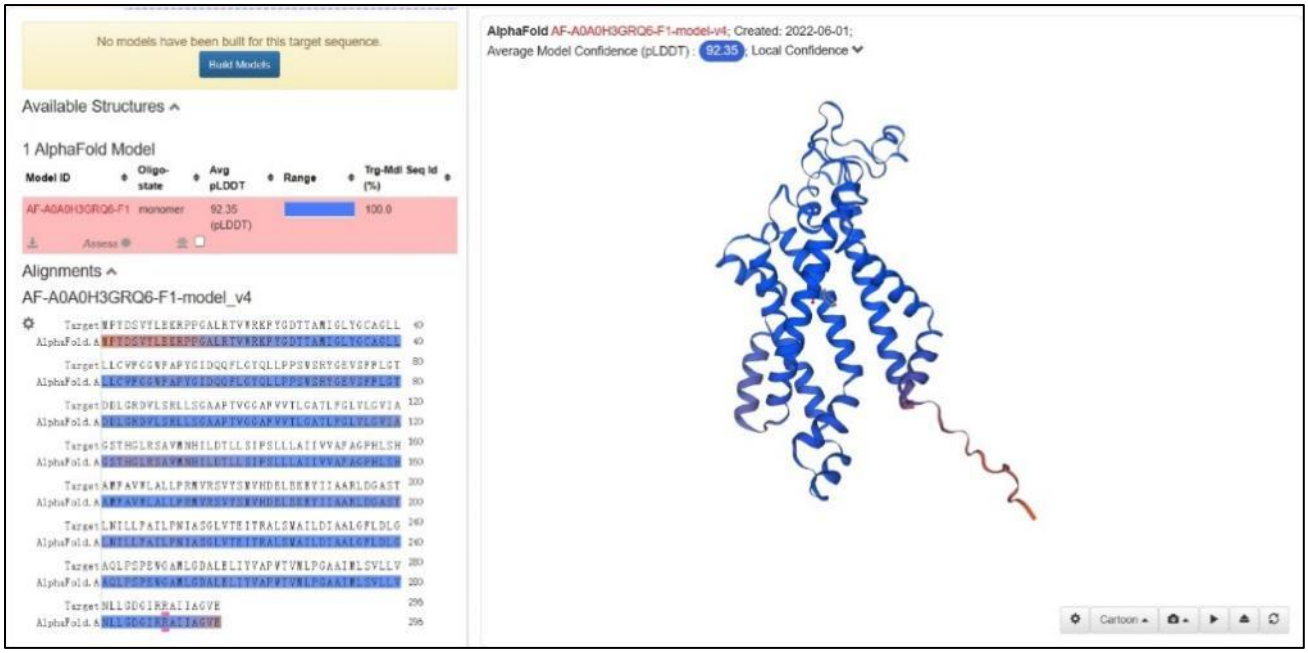

Fig 5: Predicted tertiary structure of SapC, the ABC transporter protein permease of *Klebsiella pneumoniae*

4.2.4 Functional site of the ABC transporter protein permease SapC from *Klebsiella pneumoniae*

A search in protein family or classification databases to analyze the structural domains, functional key sites, or protein families to which the proteins belonged showed that there were no functional key sites and no predicted protein families to which they belonged.

4.2.5 Prediction of proteins interacting with SapC, the ABC transporter protein permease of *Klebsiella pneumoniae*

As predicted by the STRING software, the proteins that interacted with the *Klebsiella pneumoniae* ABC transporter protein permease SapC were TLR4, SLC39A14, ATP7A, and so on (confidence level >0.4, Fig 6).

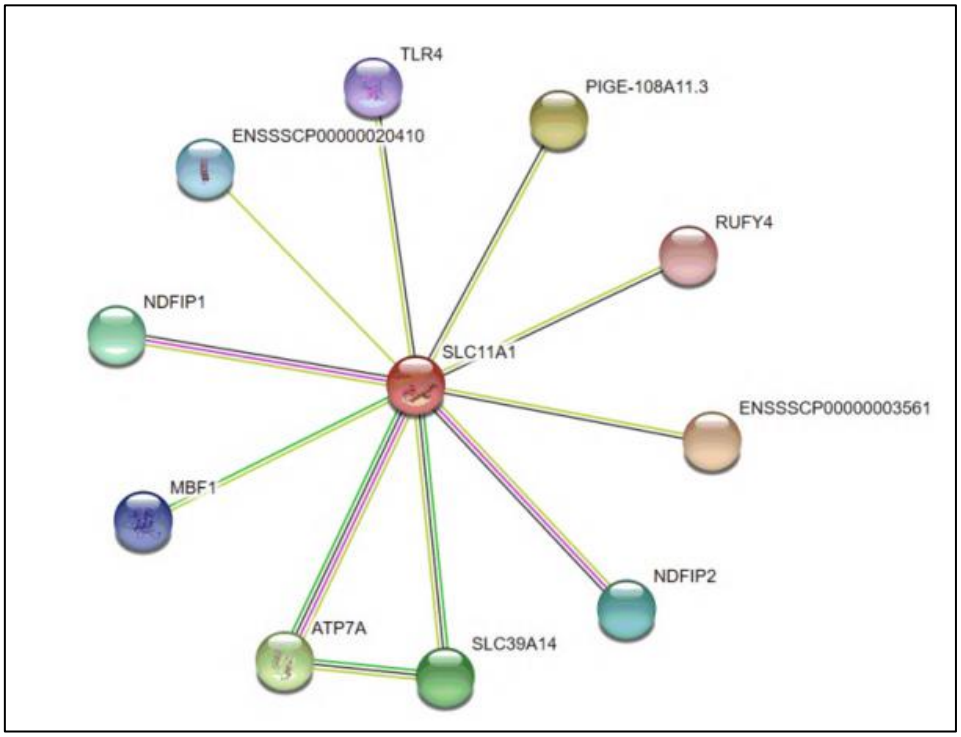

Fig 6: Interaction network analysis of the ABC transporter protein permease SapC protein in *Klebsiella pneumoniae*

4.3 Molecular phylogenetic analysis

Get the genus to which the sequence of *Klebsiella pneumoniae* belongs, then check the other genera under the family level to which it belongs, search the genus name directly in the website of NCBI, select the sequence of the model strain of the corresponding amplification region, get the exogenous bacteria, and construct a phylogenetic tree by the method of comparing the protein sequences and protein structures with the use of MEGA (Fig 7).

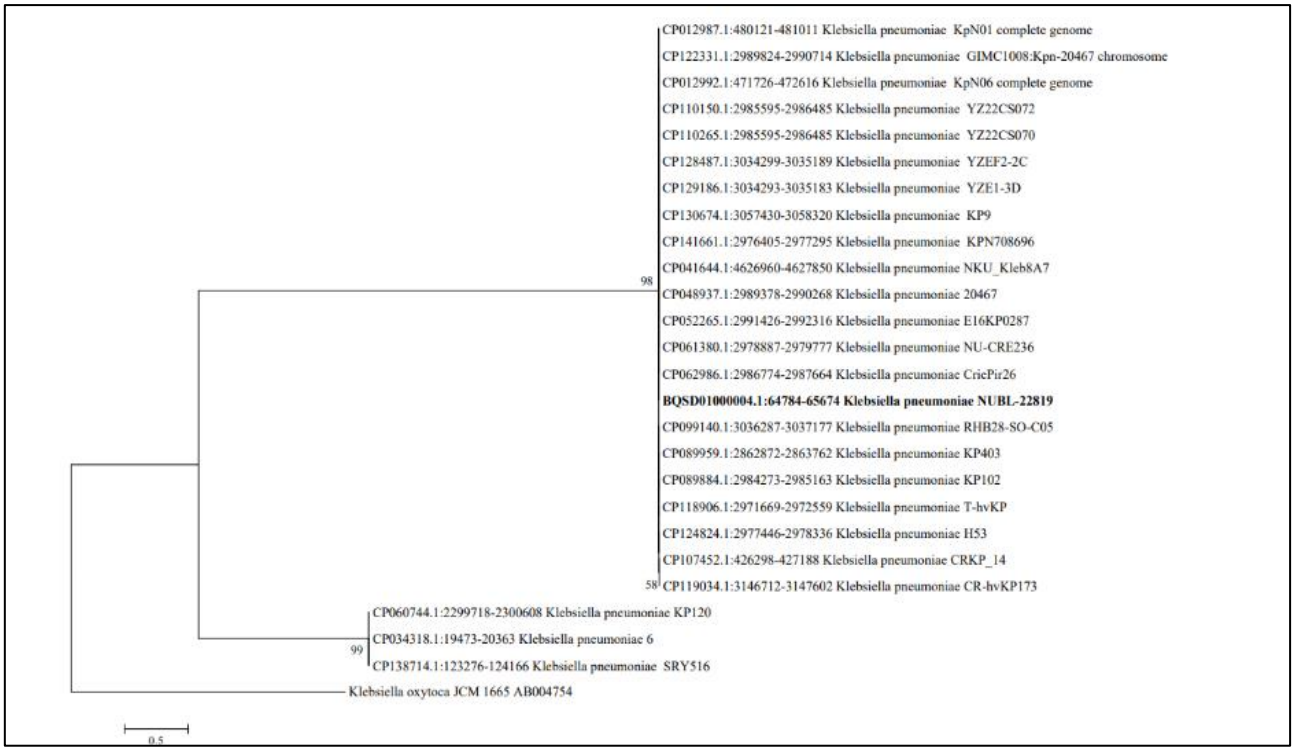

Fig 7: Phylogenetic tree

5. CONCLUSION

Klebsiella pneumoniae is a member of the genus Klebsiella of the family Enterobacteriaceae. In recent years, the widespread use of antibacterial drugs has led to an increasing number of drug-resistant strains of *Klebsiella pneumoniae*, resulting in the frequent occurrence of animal outbreaks caused by this bacterium, which not only causes serious economic losses to the farming industry, but also increases the risk of animal-to-human transmission substantially. Outer membrane proteins are the main components of Gram-negative bacteria, accounting for about 50% of the outer membrane structure, and are closely related to bacterial drug resistance, as well as excellent immunogenicity. The ABC transporter protein permease SapC is an important protein component of the extracellular membrane of *Klebsiella pneumoniae*, but few studies related to this protein have been reported.

In this paper, we analyzed the sequence (GKL76932) published by NCBI, and analyzed and predicted the gene of *Klebsiella pneumoniae* and analyzed and predicted the sequence and structure of the protein encoded by it by using various information analysis tools available in the websites of NCBI and ExPASy. It consists of three main parts: analysis of the gene sequence, protein sequence analysis and structure prediction, and molecular phylogenetic analysis. We successfully analyzed and predicted the sequence and structure of *Klebsiella pneumoniae* and its encoded protein in order to provide reference for the in-depth study of the biological properties of SapC, the ABC transporter protein permease of *Klebsiella pneumoniae*, the establishment of a rapid detection method for this bacterium, and the selection of targets for subunit and nucleic acid vaccines, etc., and to lay the foundation for further understanding and utilization of this gene.

According to the information in NCBI, *Klebsiella pneumoniae* was obtained as DNA, and the product was ABC transporter protein permease SapC. Using proteomics and analytical tools, it was concluded that the molecular formula of ABC transporter protein permease SapC of *Klebsiella pneumoniae* was 4546 relative molecular mass, the theoretical isoelectric point of 5.29, the total average hydrophilicity of 0.783, and it was mainly composed of leucine (17.6%), alanine (11.1%) and glycine (10.5%). The number of positively charged amino acid residues in the sequence was 16 (arginine + lysine), and the number of negatively charged amino acid residues was 22 (aspartic acid + glutamic acid), and the total number of atoms was 296. The extinction coefficient at A280 was 47,900, and the instability coefficient was 34.44, which was less than 40, indicating that the protein was stable in vitro. The half-life in mammals is 30 h, in yeast >20 h, and in

Escherichia coli >20 h, which meets the expression conditions of the prokaryotic expression system, and can induce the expression of *Klebsiella pneumoniae* ABC transporter protein permease SapC in vitro. The prediction of the hydrophilicity shows that the average hydrophilicity of the protein sequence is 0.783.The tryptophan (Ser) located at position 76 is the most hydrophilic, and the tryptophan (Ser) located at position 76 is the most hydrophilic. (The hydrophilicity of the protein was predicted to be 0.783, with tryptophan (Ser) at position 76 being the most hydrophilic, at -1.711, and alanine (ALa) at position 148 being the most hydrophobic, at 3.600. It was predicted to be a hydrophobic protein, which suggests that the protein may exist in bacterial lysate as an inclusion body, and the presence of the protein can be verified by the prokaryotic expression system of Escherichia coli at a later stage. The protein has two parts of intracellular domain and extracellular domain, containing transmembrane region, and the probability of having signal peptide is 0.167%, which may be an intracellular protein. The probability of having signal peptide is 0.167%, which may be an intracellular protein. It is suggested that this protein belongs to the non-secretory protein, and the non-classical pathway is required for this protein to enter the host cell to play a regulatory role. The results of subcellular localization prediction were analyzed, and it was predicted that this protein existed in the cytoplasm. The secondary structure of SapC protein, an ABC transporter protein permease from *Klebsiella pneumoniae*, was predicted by the GOR program, and the results showed that the secondary structure of the protein consisted of 74 α-helices (64.91%), 9 β-turns (7.89%), 21 irregular convolutions (18.42%), and 10 extended chains (8.77%). The results of tertiary structure prediction showed that the protein contained a large number of α-helices and irregular coils, which was consistent with the results of secondary structure prediction. Since α-helices, β-turns and irregular curls are easy to twist and fold, and are more likely to form structures that are chimeric with cell-surface receptors, the presence of more of the above structures in this protein suggests that more cellular antigenic epitopes may exist in this protein. In summary, this paper comprehensively analyzes the structure and properties of *Klebsiella pneumoniae* and its encoded proteins, and the experimental results obtained accumulate basic information for further research on the function of *Klebsiella pneumoniae* genes.

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