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Research Article

In silico identification of vaccine candidates against *Klebsiella oxytoca*

Sandipan Talukdar[1], Udeshna Bayan[1], Kandarpa Kr. Saikia*

Dept of Bioengineering & Technology, GUIST, Gauhati University, India

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ABSTRACT

Klebsiella oxytoca causes several diseases in immunocompromised as well as healthy individuals. Increasing resistance to a number of antibiotics makes treatment options limited. Prevention using vaccine could be an important solution to get rid of infections caused by *Klebsiella oxytoca*. In recent time, genome based approaches have contributed significantly in vaccine development. Our aim was to identify the most conserved and immunogenic antigens that can be considered as potential vaccine candidates. KEGG database was used to find out pathways unique to the bacteria. Subcellular localization of the protein sequences taken from the selected 36 pathways were predicted using PSORTb v3.0.2 and CELLO v2.5. Prediction of B cell epitope and the probability of the antigenicity were evaluated by using IEDB and Vaxijen respectively. BLASTp was done to find out the similarity of the selected proteins with the human proteome. Proteins failing to comply with the set parameters were filtered at each step. Finally, we identified 6 surface exposed proteins as potential vaccine candidates against *Klebsiella oxytoca*.

1. Introduction

Klebsiella oxytoca is a gram-negative, rod shaped, non- motile bacterium belonging to the family Enterobacteriaceae (Gorkiewicz, 2009). It is an opportunistic pathogen that causes nosocomial infections in hospitalized patients, including children and neonates (Podschun and Ullmann, 1998).It has now been established that *Klebsiella* is the second most frequent causative agent of gram-negative bacteremia after *E. coli* (Yinnon et al., 1996) and *Klebsiella oxytoca* is the second most frequent cause of bacteremia after *Klebsiella pneumonia* (Lin et al., 1997). *Klebsiella oxytoca* is also causative agent of antibiotic-associated hemorrhagic colitis (AAHC) (Hoffmann et al., 2010). The organism is capable of overcoming the innate immune defence. It has numbers of virulence factors such as adhesins, siderophores, capsular polysaccharides (CPLs) and cell surface lipopolysaccharides (LPSs). K antigen of the capsule plays an important role in pathogenicity (Smit et al., 1986). Although lipopolysaccharides (LPS) are able to activate complement but the capsular polysaccharide of *Klebsiella* covers the LPS (Podschun and Ullmann, 1998). LPS involve in the deposition of C3b onto LPS molecules at a position distant from the bacterial cell membrane that prevent the formation of lytic membrane attack complex (C5b–C9). As a result, the damage of the membrane as well as cell death do not take place (Podschun and Ullmann, 1998). *Klebsiella oxytoca* also produces numbers of adhesins that help the bacteria to adhere to the host cell which is a primary step for the bacteria to cause infections in the host cell. The bacteria attach to mucous or epithelial cells of the respiratory, intestinal and urogenital tracts through Type 1 pili and also bind to the mannose-containing trisaccharides of the host glycoproteins (Podschun and Ullmann, 1998). Type 3 pili is mannose resistant and agglutinate only tennin treated erythrocytes (Podschun and Ullmann, 1998). Resistance of *Klebsiella* spp. to current antibiotics like penicillins especially ampicillin and carbenicillin, cephalosporinases and carbapenemases and the oxyimino β-lactams such as cefotaxime, ceftazidime and the monobactam, aztreonam is increasing (Decre et al., 2004; Wu et al., 1991). Hence effective vaccination could be a better strategy in management of this pathogen. Reverse vaccinology (RV) could be a method of choice for identification of a potential vaccine candidate (Rappuoli, 2000). The method involves the screening of genomic information of the organism using computational tools. RV overcomes the problems in the conventional method of vaccine development by computationally predicting potential surface- exposed proteins from the genomic data (Rappuoli, 2000). Surface exposed proteins are mainly for the design of peptide vaccines, which can be recognized by the immune system to evoke immunity. The main components of peptide vaccines show B- and/or T- epitope activity which determine specificity of the immune response. Here, we have used

* Corresponding author.
E-mail address: kksaikia@gmail.com (K.K. Saikia).
[1] Equal contribution by these authors.

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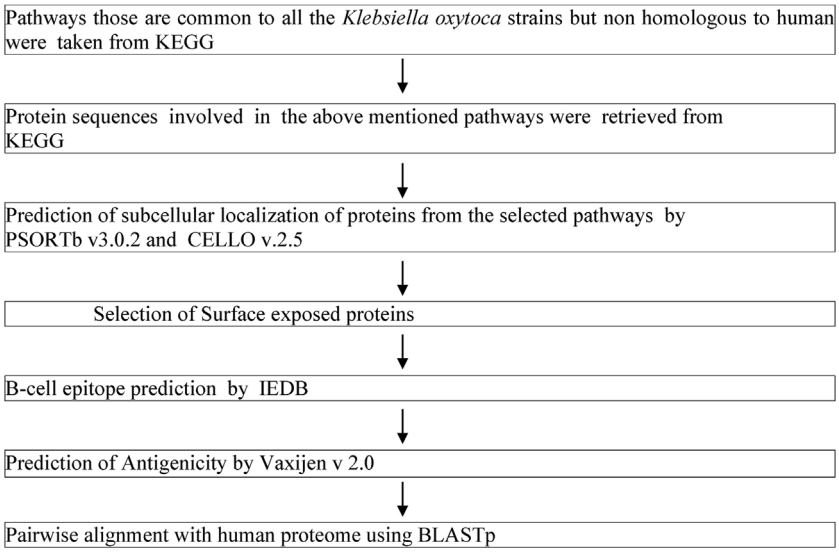
computational methods for the identification of the surface exposed proteins of *Klebsiella oxytoca* which could be potent vaccine candidates. The effectiveness of the proposed vaccine candidates lies in the fact that they are the conserved surface proteins found across the strains of *Klebsiella oxytoca.* These proteins may prove to be useful for vaccination against any strain of *Klebsiella oxytoca.*



2. Materials and methodology

2.1. Pathway retrieval

Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database of metabolic pathways (Kanehisa et al., 2008). Using KEGG databases, pathways of *Klebsiella oxytoca* and human were extracted. All the pathways were manually screened and the pathways which are not present in human, but unique to *Klebsiella oxytoca* were selected. Proteins from the common pathways were taken.

2.2. Subcellular localization prediction

The subcellular localizations of proteins obtained from the selected pathways were evaluated using PSORTb v3.0.2 (Yu et al., 2010) and CELLO v.2.5 (Yu et al., 2004) software tools. Both give the position of protein on different localization such as cytoplasm, cytoplasmic membrane, periplasm, outer membrane and extracellular space of the cell.

2.3. Prediction of B-cell epitope (*IEDB*)

The Immune Epitope Database (IEDB) is a free resource that gives information of B cell epitope. We used BepiPred method on IEDB to predict B cell epitope (Larsen et al., 2006).The surface exposed proteins obtained from both PSORTb v3.0.2 and CELLO v.2.5 were screened for B cell epitope prediction. The "average antigenic score" and the "maximum antigenic score" for the peptides were considered as the selection criteria for the best antigenic proteins. The threshold antigenic score was considered to be 0.35.

2.4. Prediction of antigenicity by Vaxijen

The surface exposed proteins were also analyzed for the prediction of vaccine candidates using Vaxijen v 2.0. The threshold antigenic score was considered to be 0.500 (Doytchinova and Flower, 2007).

2.5. Pair wise alignment with human proteome

The proteins from *Klebsiella oxytoca* selected for the vaccine candidates may cause autoimmunity if they have similarity with the human proteome. The protein sequences were aligned with the proteome of *Homo sapiens* (taxid 9606) using BLASTp. In BLASTp, the proteins with less than 35% similarity were considered as sufficiently distant to the human proteome and such proteins will not cause any problems of autoimmunity (McGinnis and Madden, 2004).

3. Results

3.1. Pathway retrieval

Only 36 pathways were found to be common to all the strains of *Klebsiella oxytoca* but not present in human. The *Klebsiella oxytoca* strains are listed in Table 1 and the selected pathways are listed in Table 2.

3.2. Sub cellular localization prediction

We retrieved 626 proteins from the 36 pathways. The numbers of surface exposed proteins obtained from PSORTb v 3.0.2 analysis

Table 1
List of *Klebsiella oxytoca* strains.

S. NO.	Organism
1	*Klebsiella oxytoca* KCTC 1686 [Kox]
2	*Klebsiella oxytoca* E718 [Koe]
3	*Klebsiella oxytoca* HKOPL1 [Koy]
4	*Klebsiella oxytoca* KONIH1 [Kok]
5	*Klebsiella oxytoca* M1 [Kom]