

COMPUTATIONAL IDENTIFICATION OF CELL SURFACE ANTIGENES FOR REVERSE VACCINOLOGY OF BRUCELLA ABORTUS

Shaikh Waseem

P G Department Of Bio Informatics, Shankarlal Khandelwal College Akola,
Sant Gadge Baba Amravati University Amravati Maharashtra, India

ABSTRACT

Brucella abortus is a gram-negative bacterium that is found in cattle populations. *Brucella abortus* are Gram-negative rod shaped bacteria that do not have flagella or pili, nor do they create capsule slime. They also do not produce spores. This heterotrophic bacteria carries out either aerobic or anaerobic respiration because it is a facultative bacterium. This means that the bacteria can grow with or without oxygen present. In view of vaccine development genome of *Brucella abortus* give us the opportunity for locating several surface antigen involving application. In my study bio program like signal 3.0, lipop1.0, TMHMM, Fuzz pro, BLASTP and HLA pred searched out highly conserved surface antigen as lipo protein and cell wall anchored protein which are involved in sub unit vaccine development.

1) INTRODUCTION

Brucella abortus is a gram-negative bacterium that is found in cattle populations (2). This intracellular parasite is a blood borne pathogen that causes premature abortion of a cattle fetus. What makes this bacterium so dangerous is that it is zoonotic, meaning it can be transferred from an animal to a human host and still remain pathogenic (1). In humans this disease cause both acute and chronic symptoms, but can be treated with antibiotics. Because of this economic effect on the cattle business and the disease potential in humans, the US has spent close to \$3.5 billion trying to vaccinate the cattle herds in the US (5). It is possible for *B. abortus* to be spread from wild populations of elk and bison into domestic cattle herds and this is why the US government continues to be vigilant in tracking potential cases within herds (8). In order to grow *Brucella abortus*, a very complex media is required, because it is a fastidious bacteria that requires most essential nutrients to be imported into the cell from the host (6). Although it is a fastidious bacteria, *Brucella abortus* does have "all major biosynthetic pathways" (3) available to it. In its primary host, cattle, the metabolic pathway for the breakdown of erythritol is one that is most desirable, it is even used "preferentially to glucose" (6). This is a possible factor in the bacteria's virulence because erythritol is found in bovine placenta.

Brucella abortus is an intracellular bacteria, which means that it does not replicate outside the host organism. This bacterium, as an intracellular pathogen, enters phagocytes, such as macrophages, in humans and in cows. It attaches to the endoplasmic

reticulum of these cells (3). These smooth bacteria enter macrophages and then live in compartments of vacuolar space along the ER. The few cells that make it to these vacuolar spaces down regulate apoptosis genes within the macrophage and therefore cause the cell to resist self-death and these pathogens become resistant within these cells of the immune system. These resistant bacteria are what go on to cause chronic disease in human hosts (9).

Materials and Methods:

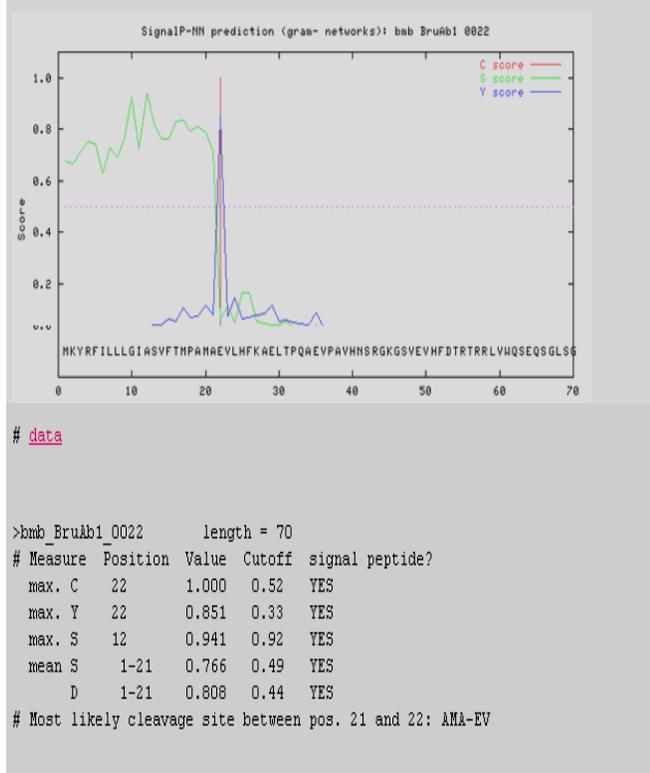
Data Collection: The primary information regarding the availability of protein sequences of *Brucella abortus* was gathered from the website: www.genome.jp/kegg/.

METHOD:-

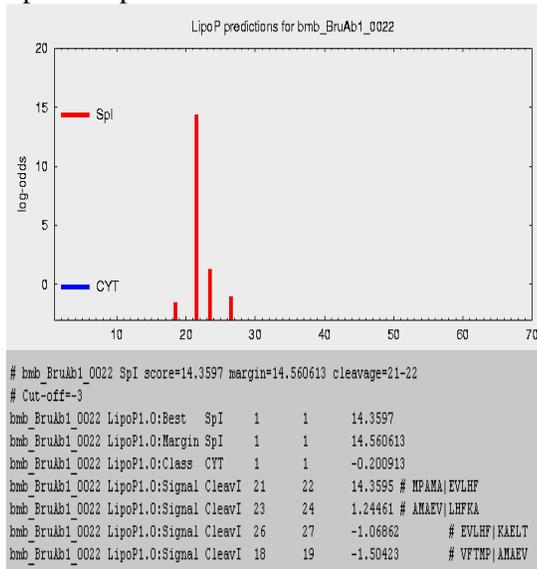
- 1) Identify the pathogen of selected disease
- 2) Retrieve the whole genome sequence of identified pathogen from KEGG
By using signal p3.0
- 3) Screen out the presence and location of signal peptide cleavage site in amino acid sequences
By using lipo p1.0
- 4) Find out the lipo protein signal peptide
By using TMHMM.
- 5) Find out ~~trans~~ membrane helices in protein by searching hydrophobic region
By using fuzz pro
- 6) Screen out the cell wall anchored protein
By using BLAST P
- 7) Screen out the highly conserved vaccine lead
By using HLA pred
- 8) find out HLA binding region from antigen sequence

OBSERVATION AND RESULTS:-

Signal P 3.0: In total 369 proteins of *BRUCELLA ABORTUS* program sorted only 125 proteins harboring signal sequences based on positive scores.

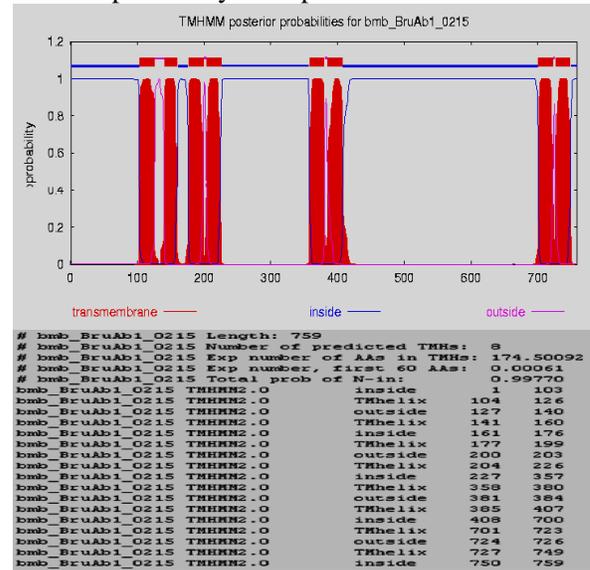


Lipo P 1.0 Out of 369 proteins of *BRUCELLA ABORTUS* screened for presence of lipoprotein, only 201 predicted to have defined signals, collectively for SpI and SpII



TMHMM By screening 369 proteins of *BRUCELLA ABORTUS*, algorithm predicted presence of trans membrane helices in the 227 proteins, which were

further screened for number of trans membrane helices spanned by each protein in the membrane



Fuzz Pro: In Fuzz Pro out of 369, only 1 protein predicted positive for the LPXTG pattern in which all have shown somewhat similar sequence patterns for the given LPXTG pattern as

```

Output file  outfile
# Program: fuzzpro
# Rundate: Fri 24 Jun 2011 06:14:46
# Commandline: fuzzpro
# -auto
#-sequence
/geninf/prog/www/htdocs/tools/emboss/output/48584
5/.sequence
# -pattern
"[LY]PX[TSA][GNAST]X(0,10){DEQNKRP}{DEQ
NKRP}{DEQNKRP}{DEQNKRP}{DEQNKRP}{D
EQNKRP}{DEQNKRP}{DEQNKRP}{DEQNKRP}
{DEQNKRP}X(0,15)[DEQNKRH]X(0,5)> "
# -outfile outfile
# -rformat2 seqtable
# Report_format: seqtable
# Report_file: outfile
# Sequence: from: 1 to: 759 # HitCount: 1
# Pattern_name Mismatch Pattern
# pattern1
[LY]PX[TSA][GNAST]X(0,10){DEQNKRP}{DEQNKR
P}{DEQNKRP}{DEQNKRP}{DEQNKRP}{DEQNKRP}
{DEQNKRP}{DEQNKRP}{DEQNKRP}{DEQNKRP}X(
0,15)[DEQNKRH]X(0,5)
Start End Pattern name Mismatch Sequence
721 759 pattern1
YPFTGTLSPVLAAGAMALSSIFVLSNALRLRRLRLA
VQ
# Total sequences: 1 # Total hit count: 1
    
```

BLAST P: Advanced BLASTP program with E-value threshold of 0.0001 helped to find out Streptococcus specific conserved vaccine leads obtained from four programs. BLASTP has reduced the vaccine lead number to acceptable total 104. These 104 leads were finally represented as vaccine candidates as they all qualified for conserved lipoproteins and cell wall anchored proteins which was required for vaccine success.

DISCUSSION

With the increased sensitivity of algorithms in bio programs along with available genome sequence information, bioinformatics helped to develop new methodology in the course of which the selection of vaccine candidates for *Brucella abortus* seems to be uncomplicated, and really this strategy enabled us to find out the 50 most probable *Brucella abortus* genome specific surface antigens. Whereas similar approach has developed vaccine for *Brucella abortus*. This indicates that selection of any often 50 vaccine leads may be a better choice as starting point for reverse vaccinology as per similar success recorded for lipoproteins and cell wall anchored proteins (8). The combined analysis by all five programs viz., LipoP, FuzzPro, TMHMM, SignalP and BLASTP, read out probable conserved vaccine leads as important cell surface antigens. Previous antigen success suggested that ABC transporter family and some lipoproteins involved in vaccine development program were better candidates, in that concern ABC transporter proteins were reported by us could be the initial leads which may be implemented prior to other in vaccine biology (9). The epitope prediction of antigens will allow us to develop subunit vaccine and in future may decide the success of the vaccine. The HLA Pred server allowed identification and prediction of peptides/regions from the antigenic sequence binding with HLA class I and/or class II alleles. The server identified the experimentally proven binders (available in MHCDB database) in query antigen sequence. The prediction of HLA binders (5 class I and 4 class II) in antigen sequence was based on quantitative matrices

This study may prove to be the better starting material for the reverse vaccinology of *Brucella abortus* and similar approach could be implemented for other organisms for searching probable vaccine leads.

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