B-CELL EPITOPE PREDICTION IN CYSTIC FIBROSIS LUNG MICROBIAL COMMUNITY

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JYOTI SHARMA

2k19/MSCBIO/24

Under the supervision of

DR. SMITA RASTOGI VERMA

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road Delhi-110042
May, 2021

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)

Bawana Road Delhi-110042

CANDIDATE’S DECLARATION

I, Jyoti Sharma, 2k19/MSCBIO/24, student of M.Sc. (Biotechnology), hereby declare that the project dissertation titled “B-cell epitope prediction in cystic fibrosis lung microbial community” which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Masters of Science, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

Place: Delhi **JYOTI SHARMA**

Date: 29th May, 2021 **(2k19/MSCBIO/24)**

DEPARTMENT OF BIOTECHNOLOGY

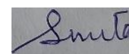
DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road Delhi-110042

CERTIFICATE

I hereby certify that the Project Dissertation titled “B-cell epitope prediction in cystic fibrosis lung microbial community” which is submitted by Jyoti Sharma, 2k19/MSCBIO/24, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Masters of Science, is a record of the project work carried out by her under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.



Place: Delhi

Date: 29th May, 2021

DR. SMITA RASTOGI VERMA

SUPERVISOR

Assistant Professor

Department of Biotechnology

Delhi Technological University

ABSTRACT

Human were considered as sterile for a long time period but, as the science advanced in Biology, a lot of techniques have been discovered that figured them out. Human body consisting of 100 trillion cells is inhabited by many bacteria and other microbes all over, for example, over skin, beneath the skin, gut, oral axis, and in lungs. All the microbial community is collectively called microbiome. They play role as the essential components of immunity, influence metabolism, and also modulate drug interactions. However, under disease conditions, as the normal physiology of body changes, dysbiosis of microbiota occurs and the advantageous normal microbes get replaced by the competent infectious ones. Human body thus acts as host to them. The present study aims at analysis of normal microbiota in human oral-gut axis and lungs, followed by the dysbiosis in lung microbiota in cystic fibrosis. This dysbiosis causes infection leading to a high mortality rate in cystic fibrosis patients. Most of these infectious agents in CF are drug resistant, so it has become difficult to treat them. Hence, a need has arisen to search for alternative strategies to treat cystic fibrosis. These infectious microbial community in lungs can be targeted on basis of their surface structures. This thesis aims to predict the B-cell epitope in three major microbes implicated in high mortality rate in CF. B-cell epitopes were predicted using major bioinformatics tool, IEDB, Uniprot, PDB, Bepipred, Discotope, Phyre^2 and Firstglance Jmol. Using data from the tools, antigen specific antibody can be synthesized. As, antibody binds the specific antigen, a higher chance is that the infection rate can be slowed or eliminated using other drugs along with the antibody cocktail.

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Jyotisharma

Place: Delhi **JYOTI SHARMA**

Date: 29th May, 2021 **2k19/MSCBIO/24**

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CHAPTER 1

INTRODUCTION

Cystic fibrosis (CF) is a autosomal recessive disorder of mucus gland, it is genetically inherited, and only inherited by the offspring if both parents have defected genes. Mutation in the gene responsible for the disease is in protein CFTR gene, which is an ion pump. It pumps Cl ions out of cell in mucus glands and reabsorbs Cl ions in sweat glands. A number of mutations have been identified in CF, but the common one in most of the populations with CF is at the position number 508, in CFTR gene; the deletion of three nucleotides coding phenylalanine. Due to this deletion, CFTR protein is not able to take a complete and correct conformation, and loses its function. The protein is not correctly folded in ER and undergoes impaired post-translational processing (protein does not undergo glycosylation), and is retained in ER and degraded rather secretion towards the cell membrane. Normally, CFTR pumps Cl ions into the mucus, present around the mucus cells, these Cl ions attract water molecule, which makes it thin. In CF patients, as the CFTR is non-functional, the mucus is thick which causes difficulty in breathing, non-motility of cilia preventing the clearing of airways pathways, lung becomes home to infectious microbial community, and dysbiosis occurs. Microbes harbouring the lungs of CF patients are predominantly *Stenotrophomonas maltophilia, Stenotrophomonas, Burkholderia* cepacia complex, *Haemophilus influenza, Staphylococcus aureus, Pseudomaonas aeruginosa,* Methicillin-resistant *Staphylococcus aureus* (MRSA)*, Mycobacterium abscessus, Achromobacter spp., Streptococcus milleri, and Aspergillus fumigatus*. The high mortality rate in CF is not due to disease, rather it is due to the infection caused by different classes of bacteria, viruses and fungi. Most of them have developed resistance due to the development of MDR transporters on the cell membrane or formation of biofilms. So, to increase the lifetime of a CF patient, it becomes necessary to target infectious agents individually. By targeting the Epitopes for B-cell and T-cell on the surface, creating monoclonal antibody (Ab) (idiotype), the rate of infection can be slowed down, along with the other drugs, physiotherapy and exercise.

B-cell epitope identification is important step in the development of vaccines based on epitope, therapeutic Abs, and diagnostic tools. Epitope based Abs, are most promising class of biopharmaceuticals. Categorization and analysis (*in silico*) of identified epitopes, have important role in the production of epitope-based Abs.

For the prediction of B-cell epitope, a number of *in silico* tools are available which are based on sequence / structural data. Prediction of B-cell epitopes provides the ability for the identification of correct structure, and replace the Ag in the immunization process, serodiagnosis and Ab production. Computational prediction and epitope mapping added more insights into the process of recognition and development of Ag- Ab complexes, which is helpful to localize B-cell epitope more precisely.

T-cell epitope prediction main aim is to identify peptides the shortest, within an ag that are able to rouse either CD4 or CD8 cells. Calculation of discrete peptides with Ags and T-cell epitopes aims in the identification of those peptides that are immunogenic. Most of the *in silico* methods to generate T-cell epitopes are similar to epitopes of B-cell, but T-cell epitope processing and its binding and formation of complex is still a problem. So, currently Abs are mostly generated for B-cell epitope. In the present work, experiments (*in silico*) which is for the calculation of B and T cell epitopes, for the database which is already existing, with the help of tools available online and performed.

CHAPTER 2
LITERATURE REVIEW

Cystic fibrosis is known as fatal, chronic and progressive genetic disorder of human body's mucus and sweat gland, and it majorly affects respiratory and digestive systems [Steven et al., 2000]. It is an autosomal recessive disease. The most common symptoms observed are excessive loss of salts upon sweating and the mucus accumulation majorly in lungs and intestine. Along by these major problems, the other medical problems consider are, following sinusitis, nasal polyps, pneumothorax, liver disease, inflammation in pancreas, diabetes, also occur with CF [Steven et al., 2000]. This disorder is more common in white people, Americans, Europeans, and Canadians; and less common in Africans and Asians.

In 1985, it was proved that CF locus was linked to DNA polymorphism, and the gene for CF is positioned on the longest arm of human chromosome 7 [Davies et al., 2007]. Later, progressive fragments of DNA associated with CF gene were found. From all the linkage-disequilibrium data collected it is implied that the CF chromosome in a large proportion of cases arose from mutational events**.** Mutations in the gene encoding CFTR pump, it comprises of 27 exons and is situated on chromosome 7 [Davies et al., 2007]. The most common mutation observed in CF is the deletion of phenylalanine codon 508 (phe508del, until recently known as ΔF508). This occurs in about 70% of patients [Davies et al., 2007]. Although CFTR is expressed in many of the internal organ, but most effected organs are Intestine, lungs, and reproductive tract [Coffey et al., 2019]. Pulmonary CF is the most lethal. Air epithelia in defective cases have high rates of sodium adsorption which dehydrates the airways and impairs mucus transport. As the mucus gets more and more viscous the mucociliary clearance mechanism (MCC) becomes unable to clear the microbial infection. The second major organ impacted is the gastrointestinal tract [Coffey et al., 2019]. Dysfunction of the CFTR also results in an altered intestinal condition which is a reduced concentration of Bicarbonate and low ph in intestinal, delayed intestinal transit, and an impaired innate immunity and the problem continues from childhood to adulthood [Coffey et al., 2019].

2.1 Cystic fibrosis transmembrane conductance regulator (CFTR)

Protein encoded by CFTR gene is Pump which is C-AMP regulated (Cyclic-AMP) which is situated in the exocrine epithelial cells of the apical membrane. This pump functions to regulate the ion flow along the trans-epithelial layer, which is important for maintaining the proper ionic composition. [Rafeeq et al., 2017]. It is also involved in the other process involved in the regulation of the bicarbonate, sodium channels, and the pH of the intracellular organelles and the cell. In a normal human, the isotonic secretions travel from the acinus of the sweat gland to the skin surface, epithelial cell lining of the duct reabsorbs NaCl resulting in hypertonic solution [Rafeeq et al., 2017].

CFTR is basically phosphorylation regulated, ATP-dependent Cl-channel. It belongs to the family of ATP-binding cassette (ABC transporter) family. This pump has two NBD (Nucleotide binding domain), along with two membrane spanning domain [Cotton et al., 1996]. In the ABC family the cell uses ATP and pump out solutes uphill against the concentration gradients, as ATP bind transporter show conformational changes, that leads to hydrolysis of ATP, and rearrangements in the transmembrane domains, which allow substrate to move alternately exposed sides of the membrane. [Miller, 2010]. CFTR has two remarkable distinctions, one that it is only ion channel in ABC family and other is that it is the only ATP gated channel [Hwang and Kirk, 2013]. CFTR pumps out chloride and bicarbonate ion. As, transport domains have two NBD and MBD, the cycle began with the appropriate substrate binding to MBD [Vallières, 2014]. After, this ligation of ATP to NBD, there is subsequent dimerization, binding of ATP provides energy for the release of bound substrate. Later, ATP is hydrolyzed, NBD destabilised, ADP and Pi released and protein gets in original space [Vallières, 2014].

2.2 Mutations in CF

Genes for CF are present on chromosome 7. Most of the common mutation found in CFTR is at position 508 for phenylalanine, present in NBD1(N-terminal cytoplasmic) [Thibodeau et al., 2010]. A single Aa deletion leads to results in dramatic reduction of mature CFTR protein. The undeveloped state of a protein is recognized by the cell, it is arrested in the intermediate conformational and targeted for the degradation process by ubiquitination [Thibodeau et al., 2010]. Five major classes of mutations have been reported in CFTR gene [Burney and Davies, 2012]. These are summarized in Table 1.

Table 1. Mutations classes in CFTR gene in CF patients

Class of mutation	Effect due to mutation	Problem caused due to mutation
Class 1	Failure to reach the membrane due to incorrect folding	Protein production mutations
Class 2	Commonest Caucasian defect is Phe508Del	Mutations lead to protein processing
Class 3	Defects in gating	Gating mutations
Class 4	Conductance	Transfer mutations
Class 5	Abnormally low channel numbers	Insufficient protein mutations

Mutated CFTR becomes impermeable to chloride ions and alters the surface of airways in CF patients, making it a chronic infection [Steven et al., 2000]. The Patch clamp technique is used to observe single-channel activity that suggests the defect in the regulation of the chloride-channels [Steven et al., 2000]. The associated other responses like neutrophilic inflammatory responses, pulmonary failure can be the cause of death in CF [LiPuma, 2010].

2.3 Microbes and human body

The human body has 10^{13} cells and harbours 10^{14} numbers of microflora. This is normal microflora and is relatively stable in the specific individual in a complete life. The role of this microflora is far beyond the local environment [Belkaid and Hand, 2015]. The study of the oral-lung and gut-lung axes has shown it to be essential for disease etiology and treatment. Dysbiosis of these microbes is associated with the alteration of an individual's immune response. This review discusses the role of lung microbiota and cross-talk between oral-lung and gut-lung axis in the case of CF [Belkaid and Hand, 2015]. Trillions of microbes are associated with humans and act as a major factor in their healthiness and disease. Human microbial community include Bacteria, virus, archaebacterial, and eukaryotic microbes. . The

vital part of human microbiota is explained via several mechanisms [Françoise and Héry-Arnaud, 2020]. The most important role of microbiota has the potential to increase the arte extraction rate of energy from the food, also with this it increase nutrient harvest and alter appetite signalling. Along with this it provide a barrier that protects the host from foreign pathogens via the prohibiting as well as by producing antimicrobial substances [Françoise and Héry-Arnaud, 2020]. Several bacterial communities that inhabit various organs, and tissues of the human body, for example, gut, skin, lung, etc. are also important for their role in maintaining tissue, organ and immune homeostasis**.** Microbiota is also known as for promoting a state of immune tolerance to prevent inflammatory response caused by inhalation of harmful substances and maintain homeostasis [Françoise and Héry-Arnaud, 2020]. Microbes in the human body is in a symbiotic relationship that is coevolved and coexisted [Beck et al., 2012]. Commensal bacteria colonize just after birth and grow as a community with the growth of the host. To characterize importance of microbes in health and disease it is important to understand their functions in a healthy individual. Germ-free animals, mostly mice, have been used for decades to find the relationship between the host and microbiota. The health condition of the host is greatly impacted by the diversity of resident microbes [Beck et al., 2012]. Microbiomes of oral cavity and gastrointestinal tract have been implicated in maintaining homeostasis with the immune system. Cross-talk between oral-lung and gastrointestinal tract-lung has been suggested through studying the role of oral and gut microbes in respiratory disease such as asthma [Kilian et al., 2016].

2.4 Microbiome of oral cavity and gastrointestinal tract

Microbes are important for human physiology and health. The strategy adopted for studying human microbes involve clinical, technical, analytical and translational steps [Dumas et al., 2018]. The details are presented in Table 2.

Table 2. Strategy adopted for studying human microbes

Steps	**Details**
Clinical	Procedural details, sample type obtained, sample processing
Technical	Characterization of bacterial, viral and fungal microbiota, Inter-kingdom microbe and host interactions
Analytical	Functional properties of the microbiome
Translational	Use of relevant animals or other models to understand mechanism of human microbe observation

Different microbial habitats such as teeth, cheeks, lips, hard palate are present in mouth/oral cavity [Dewhirst et al. 2010]. 13 phyla and 619 are reported in HOMD (Human oral microbiome Database) [Dewhirst et al. 2010]. Salivary components of the oral cavity, for example, secretory immunoglobin A (IgA are the primary nutritional source for the microorganisms. These, directly and indirectly, regulate the microbiome and are important for balanced microbiome growth [Dewhirst et al. 2010].

The role of salivary flow and microaspiration is the pathway to foster lung microbe. The composition of oral and lung microbe is almost similar, but some of the microbes are specific to the lungs, and they eliminate common bacterial such as *Prevotella* sp. [Gaeckle et al., 2020]. A healthy gut microbe shows a huge bacterial diversity and richness. The gastrointestinal tract shows the best host-associated microbiota that increases in diversity along with the gastrointestinal tract. Interpersonal variations in the gut microbiota arise due to genetics as well as prevailing environment including lifestyle, diet, pH, bile concentration, digestion retention time and host defense factors [Budden et al., 2016]. Considering all these disparities, the gut microbiota is dominated by four major bacterial phyla, with the lesser and, sporadic representation of other phyla, including Fusobacteria, verrumicrobia and spirochaetes [Cani, 2018]. In total 14 bacterial genera and 150 bacterial species are known to inhabit the gut. In addition to the bacterial species, gut microbiota also consists of phages, yeast, and fungi [Kho and Lal, 2018]. Gut microbiota is essential to the host digestive system and generates nutrients

by using a substrate that cannot be digested by the host alone. For example, digestion of xyloglucan found in dietary vegetable lettuce and onions is facilitated by microbial gene products. Genes for xyloglucan digestion have been reported in Bacteroides in 92% of individuals [Shreiner et al., 2015].

Variations in gut microbiota also have a profound influence on immune response and disease susceptibility. Microbiomes of the gastrointestinal tract always maintain homeostasis with the immune system. Microbial dysbiosis at the phylum level in the gastrointestinal tract causes metabolic disorders including IBD, asthma, obesity and, diabetes [Cani, 2018]. APC have a key feature, as their ability to protect the body against infection and maintaining immune tolerance. Example, Dendritic cell of Peyer's patches and dendritic cell of spleen triggered by same ag DC of Peyer's patches will produce interleukin-10 (IL-10) in higher amounts than the splenic DC [Wu and Wu, 2012]. In the case of B-cells, mostly IL-10 is found in Peyer's patches and the most secreted form is IgA. Major driving force for the production of secreted forms of IgA is gut microbiota. [Wu and Wu, 2012].

From the mucosal and systematic immune system revealed that the, systematic is independent and highly specialised, whereas mucosal immune system undergoes major changes after bacterial colonization [Lazar et al., 2018]. Immune receptors like toll-like receptors (TLRs) from lymphoid cells and membrane epithelial cells of small intestine are involved in the differential recognition and responsible for normal development [Lazar et al., 2018]. Receptors like NLRs nod like receptors, recognize various microbial specific mol and initiate the assembly of inflammasomes, it acts as a sensor damage- associated patterns [Lazar et al., 2018]. Many of the metabolites produced include Folate, indoles, secondary bile acids, SCFAs. [Hufnagl et al., 2020].

SCFAs are most investigated, which are produced by the anaerobic bacteria. Besides of serving an important energy source, SCFAs are critical for maintaining and regulating Immune system and its response [Hufnagl et al., 2020].

2.5 Lung microbiota

Lungs were considered sterile organs for a long time, but advanced technology enabled the detection of the microbial community of lung. Microbial sampling in the lungs is much more difficult as a consequence of low biomass and contamination from oral cavities [Dumas et al.,

2018]. Lung microbiota analysis is mainly based on metagenomics using RNA sequencing. The composition of bacteria differs in upper and lower respiratory tract showing niche-specific microbial colonization [Dumas et al., 2018]. It is observed that some bacterial community is shared between the oral cavity and the lungs, as it is needed in the lungs. The human major gut and lung also share similar microbiota composition at the phylum level but differ in the species composition [Dewhirst et al., 2010]. Lung microbes composition is eepnd upon factor that are immigration, microbial elimination and regional growth conditions [Mathieu et al., 2018]. Physiological factors including nutrient, oxygen tension, blood flow, local pH and temperature also influence bacterial population [Mathieu et al., 2018]. Selective elimination is the major determinant of the lung microbiota and a balance is maintained between elimination and migration [Wang et al., 2017]. The processes involving microbial immigration and elimination are listed in Table 3. However, this balance is disturbed in case of lung diseases. In most healthy population, environment is not conductive for bacterial community development.As a consequence, lungs have a comparatively low microbial biomass, however, they are exposed to continuous entry of microorganisms [Wang et al., 2017].

Table 3. Balance between microbial immigration and elimination maintains lung microbiota

Microbial immigration	**Microbial elimination**
Inhalation	Cough
Micro-aspiration	Immunity (innate adaptive host defense)
Mucosal dispersion	Mucociliary clearance

Dysbiosis of microbiomes in lungs affects metabolic, inflammatory or immune pathways [Mao et al., 2018]. Healthy lung microbes belong to the phylum Bacteroidetes, Actinobacteria and Firmicutes. In case of respiratory infections microbes found in more abundance are Proteobacteria [Hufnagl et al., 2020]. This dysbiosis activates inflammatory pathways that contribute to hyperbronchial responsiveness and bronchoconstriction. Outer environment factor can affect Lung microbiota both positively and negatively [Hufnagl et al., 2020]. Antibiotics, antiulcer medications severely impaired gut and leads to a dysfunctional microbial community. Dysbiosis result in the lack of microbes which ultimately leads to dysregulation of the gut-lung cross talk, resulting in hypersensitivity and hyperactivity [Hufnagl et al., 2020].

In case of asthma, patients have lower microbial diversity but some of the species that increase in the number include phylum Proteobacteria and *H. influenzae* [Hufnagl et al., 2020]. Lack of microbial diversity clues to weakening of the immune system (pulmonary) resulting in the host vulnerable to lung disorder [Hufnagl et al., 2020].

2.6 Cross talk between gut-lung axis

Microbes play a very important role in maintaining and regulating immune response in addition to maintaining host immune homeostasis. Lung microorganisms are important for the homoeostasis of lung immune response and its dysbiosis could invoke host immune response.

The healthy hallmark gut and lung axis is the rich microbe diversity. An adjacent connection is observed in between the lung and gut microbe and the two affect each other's composition [Enaud et al., 2020]. Although the site-specific microbiota provides local immunization, a long-reaching impact of gut microbe is observed on pulmonary immune system [Enaud et al., 2020]. The intact bacteria, their metabolites and their fragments can translocate crossways of the intestinal barrier via the mesenteric lymphatic system, and are associated with Respiratory disease [Enaud et al., 2020]. Thus, bidirectional cross-talking amongst gut and lung, creates a relation that dysbiosis in one organ affects the other [Enaud et al., 2020]. A mechanism is followed by gut microbe to influence the immune response and inflammation in the lungs includes T-cells subsets, TLRs, inflammatory cytokines and surfactants [Enaud et al., 2020].

In a pre-clinical model, infection due to influenza has been shown to trigger an increased proportion of Enterobacteriaceae and decreased abundance of Lactococci and Lactobacilli [Enaud et al., 2020]. Lymphoid cells of innate adaptive system shows tissue repair have been shown to recruit from gut to lung in response the interleukin 25 (IL-25) [Enaud et al., 2020]. The glucopyranosyl lipid adjuvant (GLA) immunization results from the complex interaction between the gut-lung axis microbe with a local and long leaving effect of the immune system [Enaud et al., 2020]. The immunization of gut depends on the lung APCs (dendritic, macrophages and B-cells) and influenza infections that modulates the microbiota of gut[Zhang et al.,2020]. The nasal inoculation by salmonella provides a specific immunization that depends on lung dendritic cells [Zhang et al.,2020]. In people suffering from chronic obstructive pulmonary disorder (COPD), change in gut microbiota is observed that is associated with change in the modulation of innate immunity and for the systematic immunization [Zhang et al., 2020]. SCFAs, one of the most widely recognised metabolites

produced by gut microbiota, are produced through fermentation and involved in lung-gut axis [Cait et al., 2017]. SCFAs generated by gut microbes control immune system and participate in the increase in allergic reactions, as example, in modulating the immune system in case of asthma [Cait et al., 2017]. SCFAs also act as the lung's signalling molecules APCs to increase the rate of inflammatory and allergic reactions [Cait et al., 2017]. SCFAs act as a potent anti-inflammation molecule and release of cytokines molecule induce apoptosis [Vaughan et al., 2019]. In asthma patients, a shift is observed in the metabolite production from SCFAs toward the production of carbohydrates, amino acids and lipids. Asthma is majorly due to Th2 cell respone. (Vaughan et al., 2019). SCFAs are also produced during cystic fibrosis, as the hypoxic environment airways allow the growth of the facultative anaerobic bacteria which produce SCFA through fermentation [Vaughan et al., 2019]. A progressive correlation between SCFA and sputum neutrophil count has been suggested. The amount of SCFA present in the airways affects granulocyte colony-stimulating factor, granulocyte-macrophage colony stimulating factor and IL-6. [LiPuma et al., 2010].

Segmented factor bacteria (SFB) are long-reaching immune effectors components, also commensal bacteria that colonize ileum of most animals, and involve in modulation of immune system [Enaud et al., 2020]. SFBs also regulate polarization ofCD4+ T-cell pathway, which work in response to fungal infections and lung autoimmune manifestations [Enaud et al., 2020].

2.7 Major microbiota in CF

High rate of mortality and morbidity in CF is due to the infection in the airways. [Zemanick and Hoffman, 2016]. Traditional CF respiratory pathogens show three major feature are a) Diverse in nature b) Polymicrobial in nature c) Continuous evolution. [Zemanick and Hoffman, 2016].

When Cf was recognized in 1938, it was linked with *S. aureus*. It was thought that *S. aureus* play a critical role in mortality rate [Hauser et al., 2011]. In 1950s, *Pseudomonas aeruginosa* is also added in Cf pathogen list, by 1970s, *Burkholderia cepacia* complex (BCC) is added and were recognized as a major pathogen, as they are associated with rapid declines in pulmonary function, bacteremia, and increased mortality [Hauser et al., 2011]. A pattern of contagion is observed in most of the patients, initially infection began with *S. aureus* and *H. influenzae* in new-born and ends with *P. aeruginosa* in adolescence. Bacteria colonising Lung in CF moderately controlled by antibiotics, with the exception of *Pseudomonas* and *Burkholderia*

species which leads to the contagion infection and high mortality [Hutchison and Govan, 1999]. As, the treatment become more effective and improved for most of the conventional pathogens, this leads to discovery of new pathogens [Surette, 2014]. It has been recognized that lower airways in pateints are colonized by more poly microbila community. [Surette, 2014]. A list of conventional and evolving pathogens in CF lung infections is given in Table 4.

Table 4. Pathogens in CF lung infections (Fungal pathogen)*

Conventional	**Emerging**
Pseudomonas aeruginosa	*Stenotrophomonas maltophilia*
Staphylococcus aureus *Staphylococcus aureus*	Methicillin-resistant *Staphylococcus aureus* (MRSA)
H. influenzae	Achromobacter spp
BCC (*Burkholdera cepacia* complex)	Mycobacterium abscessus
Stenotrophomonas	Streptococcus milleri / anginosus group
	*Aspergillus fumigatus**

Pseudomonas aeruginosa, Staphylococcus aureus and *BCC* are major bacterial classes [LiPuma et al., 2010], have an important in the life expectancy of the patient. *B. cepacia* known as versatile organism and a plant phytogen [Woodhead et al., 2001]. From past few decades it emerged as pathogen with devastating effects in the CF community. Its pulmonary colonisation accelerates the decline in the lung function. *B. cepacia* cause a rapid pneumonic illness "cepacia syndrome", also the *B. cepacia* is highly transmissible and resistant to most of the antibiotics [Woodhead et al., 2001].

B. cepacia show transcriptional reprogramming when the host immune system response, in case of antimicrobial therapy, in response to the nutrient availability and limitation of oxygen [Scoffone et al., 2017]. *B. cepacia* is due to the overexpression of the efflux pumps, up to 5 family of transporters are involved which include major facilitator superfamily (MFC), ATP-binding cassette family, small multidrug resistance family (SMR), the resistance nodulation family (RND) [Zlosnik et al., 2015]. Virulence factor expressed by *B. cepacia* those intercat with host, that leads to greater mortality and morbidity associated with pathogens. [Woodhead

et al., 2001]. Endotoxin act as virulence factor, in *B. cepacia* infection. Isolated lipopolysaccharides (ILPs) of *B. cepacia* are nine times more virulent than *P. aeruginosa* [Woodhead et al., 2001].

Staphylococcus aureus, gram positive nonmotile, non-spore-forming, unencapsulated most common and the first recovered pathogen of Cf patient. The overall increase in rate of infection in *S. aureus* is because of the Methicillin-resistant *S. aureus* (MRSA) in the people a variant of original S. aureus [LiPuma et al., 2010]. Increase in frequency of infection with the MRSA, is for person of every age but highest frequency is for the teen-agers, aged between 11 to 17 years [Razvi et al., 2009]. MRSA is unique from other strains as they have either beta-lactams or carry *mec A* gene, which encode for the resistance [Zemanick and Hoffman, 2016]. Another variant of *S. aureus*, is SCVs (small colony variants), which are slow growing, antibiotic resistant. Patients with infection of SCVs are found to have a, lower lung infection, and show a faster rate in decline of lung functions [Hoffman et al., 2006]. SCVs are known for resistance to aminoglycoside and persistence in infections, as in CF [Hoffman et al., 2006]. *Pseudomonas aeruginosa*, is proven to be the responsible for chronic infection of lung, leads to ultimate high mortality rate [Lyczak et al., 2002]. Infection with the *P. aeruginosa* cause damage to epithelia surface, airway plugging, impairing way [Lyczak et al., 2002]. Chronic contagion with *P. aeruginosa* is major factor responsible for morbidity and mortality [Hutchison and Govan, 1999].

2.8 B-cell and T-cell epitope recognition

Development of pathogen specific memory that provides immunological function is a key characteristic of B-cell and T-cell epitopes. Their ability to recognize specific Ag, in pathogens via the specific receptors is effective and important function. Humoral immune response, respond to invading pathogen by Ag-Ab interactions. For every ag, there is an Ab, for the discrete regions known as ag determinants. Ability of B-cell to access the cluster of Aa, which are recognized by secreted form of Ab, and able to elicit the immune response.

B-cell receptors (membrane-bound immunoglobulins), recognize solvent-exposed Ags. Activation of B-cells leads a process known as differentiation of B-cells in effector cell (Ab secreting cells), secrete Ab, and memory cells. Function of Ab is to neutralize and destruct pathogens. Ag portion that bind to ab is B-cell epitope. It may consist of exposed solvent region in Ag.

Receptors of T-cell are specific that enables them to recognize when they are bound to MHC and displayed on APCs. Epitopes for T-cell are presented by Class 1 MHC and class 2 MHC that are further recognized by different class of T-cells, CD8 and CD4. CD8 are Cytotoxic T-cells and CD4 become Th (helper cells). Th cell amplify the immune response, differentiate in to 3 major classes: Th1, Th2 and Th3.

CHAPTER 3

MATERIALS AND METHODS

So far, we have discussed how the infection by microbes in cystic fibrosis cause the major problem increasing the mortality rate. Major bacteria of the lung implicated in CF were analysed for B-cell and T-cell epitopes by using various Bioinformatics tools. Three bacteria were selected on the basis of associated high mortality in CF. These include *Pseudomonas aeruginosa, Staphylococcus aureus* and *Burkholderia cepacian.*

3.1 Tools used in B-cell epitope, T-cell epitope and Ab prediction

Bioinformatics tools used in epitope and Ab prediction included Immune Epitope Database and Analysis Resource (IEDB), UniProt, Protein Data Bank (PDB), Bepipred, DiscoTope, Phyre^2, FirstGlance in Jmol.

3.1.1 Immune Epitope Database and Analysis Resource (IEDB)

IEDB is tool that assist in biomedical research, therapeutics, and vaccine design. It is freely available, and provide access to variety of epitope analysis and prediction tools. Tools in IEDB also validate methods of prediction to bind with class I and 2 MHC. It can be combined with other tools, for the better result. In addition to this secondary analysis tools are associated with IEDB to calculate relevant analytic variables, epitope conservation, and coverage of population. Data in IEDB is vast and experimentally derived. It is also linked to other pathogen-specific and immunological database. An extensive data of experimentally measured epitopes, and tools for their prediction is present along within IEDB.

3.1.2 UniProt

It is known as a resource for protein, their sequence and data annotation. It also consists of UniprotKB, UniprotRef, Uniparc. It is freely accessible and provide information of protein data sequence. Data in Uniprot is managed by consortium, it combines European Bioinformatics

institute (EBI), Swiss institute of Bioinformatics (SIB) and Protein Information Resource (PIR).

3.1.3 Protein Data Bank (PDB)

It is a data base for large molecules, consist of Primary structural database. It includes X-ray crystallography, NMR and Cryo-electron microscopy. Structured files can be viewed in free at open source computer programs, such as Jmol, VMD. Data stored in PDB is a reflection of ongoing research. PDB have structural information for proteins and nucleic acid. It also shows data for ribosome, for whole virus, oncogenes and drug targets**.**

3.1.4 Bepipred

It is a server for the prediction of B-cell epitopes from ag sequence. Its prediction method is based on Markov model and a propensity scale method for B-cell linear epitope.

3.1.5 DiscoTope

It is a tool for the prediction of discontinuous epitopes in 3D structure of protein in the PDB format. It combines spatial properties of proteins and surface localization with a propensity scale. It is based on Aa stat, and accessibility to surface epitopes determined by X-ray crystallography of antibody/antigen protein complexes. It is 1st method for discontinuous epitope prediction.

3.1.6 Phyre^2

It is web tool to predict and analyse the structure of protein, mutations and functions. Main focus of the tool is to provide simple and informative result. It use advanced homology based detection based method, and build 3D model, than predict ligand binding sites, and give analysed result. It can be used to check both 3D and 2d models. The server is available on at http://www.sbg.bio.ic.ac.uk/phyre2. It take 30 minutes to 2hours for one prediction.

3.1.7 FirstGlance in Jmol

It is web tool, free and open source for molecule visualization. It use Jmol, also a free open source for final result. It works in all windows and IOS. It provide tooltips extensively, and explanatory which appear automatically. The major macromolecules can be turned in to 3D structure are Protein, DNA, RNA, ligand and solvent. It reveals secondary structure, Aa and carboxy termini, salt bridges and cation-pi interactions. This is the most advanced tool available for students.

3.2 Steps involved in epitopes and Ab prediction

Step 1. Open the IEDB (IEDB.org) in browser

Step 2. Type the organism's name in the search bar of epitope source

Step 3. Select epitope type as linear

Step 4. In assay select T-cell, B-cell and MHC ligand

Step 5. In MHC restriction select any

Step 6. In host select Human (For this case)

Step 7. In disease section, add cystic fibrosis

Step 8. Press the search button

Step 9. If epitope data is present inside the software, it will appear in result

Step 10. Copy antigen and organism name on the UniProt search bar

Step 11. Select the FASTA sequence for the same organism and antigen

Step 12. Copy FASTA sequence in to the word, find the similar epitope in FASTA as mentioned for the antigen on IEDB

Step 13. If the epitope of the organism is present in the FASTA, then proceed for next step

Step 14. Go back to the IEDB home page

Step 15. Select B-cell epitope prediction tools

Step 16. Select prediction of linear epitopes from protein sequence

Step 17. Copy the epitope sequence selected earlier, on the tool and submit

Step 18. Select the longest peptide from the result

Step 19. The longest one is the B-cell epitope

Step 20. Next step is to predict the T-cell epitope for both class MHC1 and MHC2

Step 21. For T-cell prediction, go back to IEDB home page and select the T-cell epitope prediction tools

Step 22. Select the Tepi tool on the page

Step 23. Copy the longest peptide of B-cell on the page and press next

Step 24. Select human in host species and class 1 in allele

Step 25. Press the next button

Step 26. On next page select the specific alleles and press next

Step 27. Select apply default settings for low number of peptides, for the selected peptides to be included and press next

Step 28. For the selection of peptide and prediction, select IEDB recommended, for prediction method to use and select peptides based on predicted percentile rank for selection of predicted peptides

Step 29. Press submit

Step 30. Result will be in tabular form

Step 31. For class, MHC2, go on the T-cell epitope prediction and click on the tepi tool

Step 32. Select human for host, and class2 for allele class

Step 33. For specific alleles, select Predict for pre-selected panel of alleles

Step 34. Select apply default setting for low number of peptides, for peptides to be included in prediction

Step 35. For the selection of peptide and prediction, select IEDB recommended, for prediction method to use and select peptides based on predicted percentile rank for selection of predicted peptides

Step 36. Press next, and submit

Step 37. Result will be in tabular form

By using the same tools and steps, B-cell and T-cell epitopes are predicted for all the infectious microbiota in case of disease cystic fibrosis

Step 38. Add Ag name in PDB

Step 39. Find the PDB Id

Step 40. Copy this PDB Id in DiscoTope

Step 41. Result from DiscoTope predicts the discontinuous epitope

Step 42. Enter the PDB id in Bepipred, it will predict the linear epitope

Step 43. For the 3D structure of the ag, use tool Phyre^2

Step 44. Download this 3D structure, using First glance mol (Using jmol)

This tool uses sequence alignment method, for generation of a 3D generation

iedb - Bing | IEDB.org: Free epitope database | TepiTool results

https://www.iedb.org

The Immune Epitope Database (IEDB) is a freely available resource funded by NIAID. It catalogs experimental data on antibody and T cell epitopes studied in humans, non-human primates, and other animal species in the context of infectious disease, allergy, autoimmunity and transplantation. The IEDB also hosts tools to assist in the prediction and analysis of epitopes.

Learn More

Upcoming Events

AAAAI Virtual Booth	Feb 26-Mar 1, 2021
Virtual User Workshop	Oct 28-29 & Nov 3-4, 2021

* register here

Summary Metrics

Peptidic Epitopes	1,033,216
Non-Peptidic Epitopes	3,101
T Cell Assays	399,318
B Cell Assays	557,327
MHC Ligand Assays	3,110,951
Epitope Source Organisms	4,038
Restricting MHC Alleles	898
References	22,025

Epitope
- Any
- Linear peptide (Exact M; Ex: SIINFEKL)
- Discontinuous
- Non-peptidic

Assay
- T Cell
- B Cell
- MHC Ligand
- Ex: neutralization — Find
- Outcome: Positive, Negative

Epitope Source
- Organism: Pseudomonas aeruginosa — Find
- Antigen: Ex: core, capsid, myosin — Find

MHC Restriction
- Any
- Class I
- Class II
- Non-classical
- Ex: HLA-A*02:01 — Find

Host
- Any
- Human
- Mouse
- Non-human primate
- Ex: dog, camel — Find

Disease
- Any
- Infectious
- Allergic
- Autoimmune
- cystic fibrosis (ID:...) — Find

Reset | Search

T Cell Epitope Prediction

Scan an antigen sequence for amino acid patterns indicative of:
- MHC I Binding
- MHC II Binding
- MHC I Processing (Proteasome, TAP)
- MHC I Immunogenicity

B Cell Epitope Prediction

Predict linear B cell epitopes using:
- Antigen Sequence Properties

Predict discontinuous B cell epitopes using antigen structure via:
- Discotope
- ElliPro

Epitope Analysis Tools

Analyze epitope sets of:
- Population Coverage
- Conservation Across Antigens
- Clusters with Similar Sequences

Search for anything | ENG 10:49 23-05-2021

[A]

iedb - Bing | IEDB.org: View Results and Refine | TepiTool results

https://www.iedb.org/result_v3.php?cookie_id=085f95

Reset | Search

Filter Options: Default

Epitope
- Any
- Linear peptide (Length, Sequence)
- Discontinuous
- Non-peptidic
- 3D structure available
- Amino acid modification

Epitope Source
- Organism: Pseudomonas aeruginosa
- Antigen: Ex: core, capsid, myosin
- Include related structure

Epitopes (3) | Antigens (1) | Assays (12) | Receptors (0) | References (3)

Go To Records Starting At 1200 GO — Export Results

3 Records Found — Page 1 of 1 — 25 Per Page

Details	Epitope	Antigen	Organism	# References	# Assays
13799	EQAISALPDYASQPGKPPREDLK	Exotoxin A	Pseudomonas aeruginosa	1	5
136105	alpha-L-rhamnopyranose			1	3
225662	oligosaccharide			1	4

3 Records Found — Page 1 of 1 — 25 Per Page

Go To Records Starting At 1200 GO — Export Results

Search for anything | ENG 11:30 23-05-2021

[B]

iedb - Bing | IEDB.org: Free | T Cell Tools | TepiTool result | exotoxin a pse

Not secure | tools.iedb.org/main/tcell/

Overview | T Cell Tools | B Cell Tools | Analysis Tools | Tools-API | Usage | Download | Datasets | Contribute Tools | References

T Cell Epitope Prediction Tools

T Cell Epitopes - MHC Binding Prediction

These tools predict IC50 values for peptides binding to specific MHC molecules. Note that binding to MHC is necessary but not sufficient for recognition by T cells.

Peptide binding to MHC class I molecules

This tool will take in an amino acid sequence, or set of sequences and determine each subsequence's ability to bind to a specific MHC class I molecule.

Peptide binding to MHC class II molecules

This tool employs different methods to predict MHC Class II epitopes, including a consensus approach which combines NN-align, SMM-align and Combinatorial library methods.

TepiTool:

The Tepitool provides prediction of peptides binding to MHC class I and class II molecules. Tool is designed as a wizard with 6 ste as described below. Each field (except sequences and alleles) is filled with default recommended settings for prediction and selection of optimum peptides. The input parameters can be adjusted to suit your specific needs. You can go back to previous ste to change your selection before submission of the job. Once you submit the job (at the end of step-6), you will not be able to make any more changes and will have to start the prediction all over again with updated input parameters.

T Cell Epitopes - Processing Prediction

Search for anything | ENG | 12:10 | 23-05-2021

[C]

iedb - Bing | IEDB.org: Free | TepiTool | TepiTool result | exotoxin a pse

Not secure | tools.iedb.org/tepitool/

Steps 1 2 3 4 5 6

ALLELES - Specify alleles:

Alleles — Human - Class I

- Select from list of frequently occuring alleles (Frequency > 1%)
- Select from list of all available alleles
- Select from list of representative alleles from different HLA supertypes
- Use panel of 27 most frequent A & B alleles
- Upload allele file

Start Over | Back | Next

Current selections:	
No. of sequences	1
Host species	Human
Allele class	Class I
Selected alleles Reset alleles	1. A*01:01 2. A*02:01 3. A*02:03 4. A*02:06 5. A*03:01 6. A*11:01 7. A*23:01 8. A*24:02 9. A*26:01 10. A*30:01 11. A*30:02 12. A*31:01 13. A*32:01 14. A*33:01 15. A*68:01 16. A*68:02 17. B*07:02 18. B*08:01 19. B*15:01 20. B*35:01 21. B*40:01 22. B*44:02 23. B*44:03 24. B*51:01

Search for anything | ENG | 12:20 | 23-05-2021

[D]

Prediction results - concise (Download table):

Seq #	Peptide start	Peptide end	Peptide	Percentile rank	Allele
1	24	32	RLIGHPLPL	0.05	HLA-A*32:01
1	24	32	RLIGHPLPL	0.05	HLA-A*02:03
1	24	32	RLIGHPLPL	0.06	HLA-A*02:01
1	3	11	LPGFYRTGL	0.07	HLA-B*07:02
1	17	25	EAAGEVERL	0.08	HLA-A*68:02
1	24	32	RLIGHPLPL	0.11	HLA-A*02:06
1	22	30	VERLIGHPL	0.14	HLA-B*40:01
1	5	13	GFYRTGLTL	0.35	HLA-A*23:01
1	28	36	HPLPLRLDA	0.35	HLA-B*07:02
1	17	25	EAAGEVERL	0.4	HLA-A*26:01
1	24	32	RLIGHPLPL	0.41	HLA-B*15:01
1	24	32	RLIGHPLPL	0.47	HLA-B*08:01
1	25	33	LIGHPLPLR	0.49	HLA-A*31:01
1	5	13	GFYRTGLTL	0.54	HLA-A*24:02
1	24	32	RLIGHPLPL	0.55	HLA-A*30:01
1	26	34	IGHPLPLRL	0.57	HLA-B*51:01
1	39	47	GPEEEGGRL	0.6	HLA-B*07:02

[E]

Figure 1 [A to E]. Windows observed for B-cell and T-cell epitope prediction (Image reference: IEDB.org)

CHAPTER 4

RESULTS AND DISCUSSION

The epitope prediction results obtained for the selected microbes, namely, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Burkholderia cepacia* are presented here. Using, IEDB tool antigen and its epitope were predicted in interested bacteria. FASTA sequence for the same Ag was extracted from Uniprot. Longest peptide was selected using B-cell linear epitope predictor on IEDB home page. Using Bepipred, graph for the linear epitope was predicted. In Bepipred, yellow part indicated the probability to be a part of epitope during binding to Ab (Parameters such as hydrophilicity, flexibility, accessibility, turns, exposed surface, polarity and antigenic propensity are considered). Using DiscoTope, the region of Ab binding site on actual Ag on surface of Ag was predicted (only represented for chain A). In DiscoTope, green predictions were the positive one; peach were negative ones. 3D structure of Ag was created using Phyre^2 and animated version was created by using Firstglance in Jmol.

4.1 Epitopes and Ab prediction in *Pseudomonas aeruginosa*

Organism name: ***Pseudomonas aeruginosa***

Epitope: EQAISALPDYASQPGKPPREDLK

Antigen: Exotoxin A

FASTA result:

MHLTPHWIPLVASLGLLAGGSFASAAEEAFDLWNECAKACVLDLKDGVRSSRMSVDPAIA
DTNGQGVLHYSMVLEGGNDALKLAIDNALSITSDGLTIRLEGGVEPNKPVRYSYTRQARG
SWSLNWLVPIGHEKPSNIKVFIHELNAGNQLSHMSPIYTIEMGDELLAKLARDATFFVRA
HESNEMQPTLAISHAGVSVVMAQAQPRREKRWSEWASGKVLCLLDPLDGVYNYLAQQRCN
LDDTWEGKIYRVLAGNPAKHDLDIKPTVISHRLHFPEGGSLAALTAHQACHLPLETFTRH
RQPRGWEQLEQCGYPVQRLVALYLAARLSWNQVDQVIRNALASPGSGGDLGEAIREQPEQ
ARLALTLAAAESERFVRQGTGNDEAGAASADVVSLTCPVAAGECAGPADSGDALLERNYP
TGAEFLGDGGDISFSTRGTQNWTVERLLQAHRQLEERGYVFVGYHGTFLEAAQSIVFGGV
RARSQDLDAIWRGFYIAGDPALAYGYAQDQEPDARGRIRNGALLRVYVPRSSLPGFYRTG
LTLAAPEAAGEVERLIGHPLPLRLDAITGPEEEGGRLETILGWPLAERTVVIPSAIPTDP
RNVGGDLDPSSIPDKEQAISALPDYASQPGKPPREDLK

B-cell epitope

SSLPGFYRTGLTLAAPEAAGEVERLIGHPLPLRLDAITGPEEEGGRLET (49) (Longest peptide selected)

Bepipred result

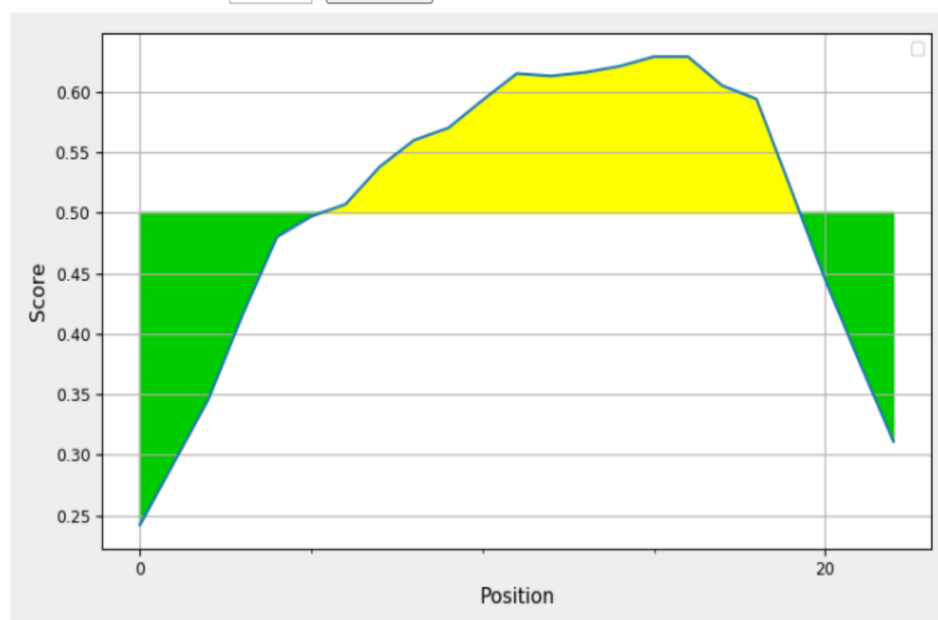

Figure 2. **Bepipred, linear epitope graph representation for antigen Exotoxin A (*Pseudomonas aeruginosa*) [X-axis represents residue positions in the sequence; Y-axis represents correspondent score]**

DiscoTope result

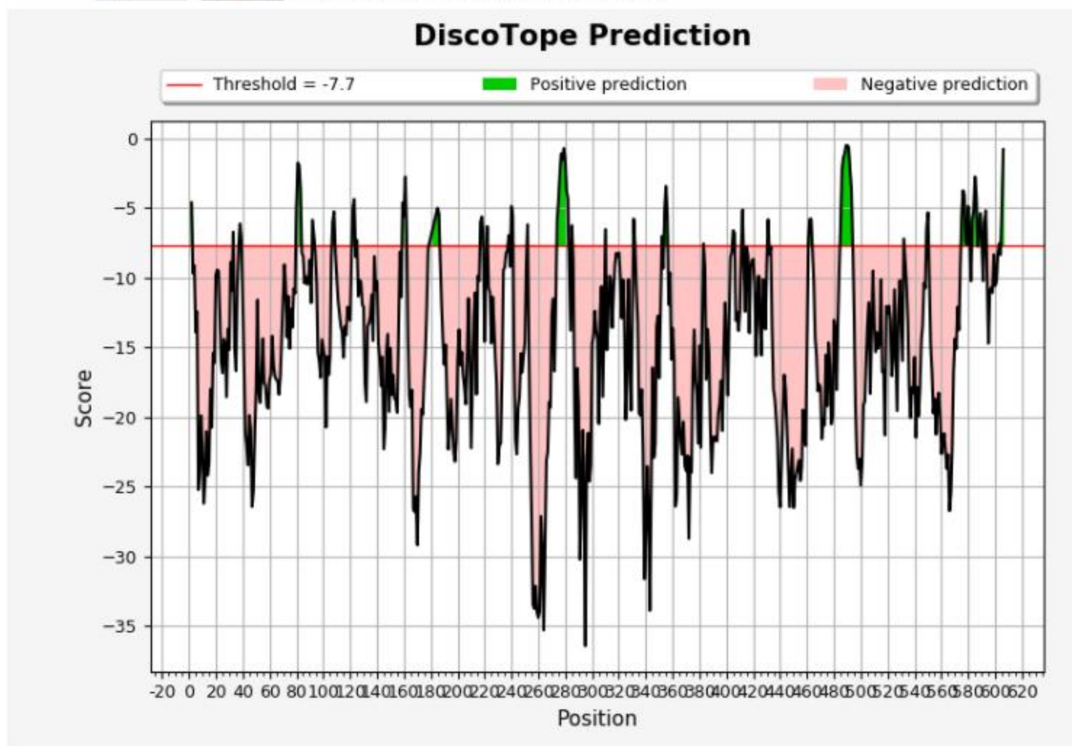

Figure 3. **DiscoTope structure based Ab prediction rule (*Pseudomonas aeruginosa*) (B-cell epitope from the discontinuous epitopes from 3D structures of proteins in PDB format)** Green predictions are the positive one; Peach are negative ones

Table 5. T cell epitope: MHC class 1 (*Pseudomonas aeruginosa*)

Seq #	**Peptide start**	**Peptide end**	**Peptide**	**Percentile rank**	**Allele**
1	24	32	RLIGHPLPL	0.05	HLA-A*32:01
1	24	32	RLIGHPLPL	0.05	HLA-A*02:03
1	24	32	RLIGHPLPL	0.06	HLA-A*02:01
1	3	11	LPGFYRTGL	0.07	HLA-B*07:02
1	17	25	EAAGEVERL	0.08	HLA-A*68:02
1	24	32	RLIGHPLPL	0.11	HLA-A*02:06
1	22	30	VERLIGHPL	0.14	HLA-B*40:01
1	5	13	GFYRTGLTL	0.35	HLA-A*23:01
1	28	36	HPLPLRLDA	0.35	HLA-B*07:02
1	17	25	EAAGEVERL	0.4	HLA-A*26:01
1	24	32	RLIGHPLPL	0.41	HLA-B*15:01
1	24	32	RLIGHPLPL	0.47	HLA-B*08:01
1	25	33	LIGHPLPLR	0.49	HLA-A*31:01
1	5	13	GFYRTGLTL	0.54	HLA-A*24:02
1	24	32	RLIGHPLPL	0.55	HLA-A*30:01
1	26	34	IGHPLPLRL	0.57	HLA-B*51:01
1	39	47	GPEEEGGRL	0.6	HLA-B*07:02
1	22	30	VERLIGHPL	0.66	HLA-B*44:02
1	22	30	VERLIGHPL	0.67	HLA-B*44:03
1	28	36	HPLPLRLDA	0.67	HLA-B*35:01
1	17	25	EAAGEVERL	0.68	HLA-B*53:01
1	24	32	RLIGHPLPL	0.7	HLA-B*07:02
1	3	11	LPGFYRTGL	0.74	HLA-B*08:01
1	20	28	GEVERLIGH	0.82	HLA-B*44:03
1	17	25	EAAGEVERL	0.82	HLA-B*35:01
1	26	34	IGHPLPLRL	0.82	HLA-B*08:01
1	25	33	LIGHPLPLR	0.88	HLA-A*33:01
1	25	33	LIGHPLPLR	0.89	HLA-A*03:01
1	20	28	GEVERLIGH	0.93	HLA-B*44:02
1	17	25	EAAGEVERL	0.95	HLA-B*51:01
1	25	33	LIGHPLPLR	0.96	HLA-A*68:01
1	25	33	LIGHPLPLR	0.98	HLA-A*11:01

Table 6. T-cell epitope: MHC class 2 (*Pseudomonas aeruginosa*)

Seq #	Peptide start	Peptide end	Peptide sequence	Consensus percentile rank	Allele
1	3	17	LPGFYRTGLTLAAPE	8.1	HLA-DPA1*03:01/DPB1*04:02
1	3	17	LPGFYRTGLTLAAPE	9.1	HLA-DQA1*01:02/DQB1*06:02
1	30	44	LPLRLDAITGPEEEG	0.46	HLA-DQA1*03:01/DQB1*03:02
1	3	17	LPGFYRTGLTLAAPE	4.4	HLA-DQA1*03:01/DQB1*03:02
1	11	25	LTLAAPEAAGEVERL	7.3	HLA-DQA1*03:01/DQB1*03:02
1	30	44	LPLRLDAITGPEEEG	3.1	HLA-DQA1*04:01/DQB1*04:02
1	3	17	LPGFYRTGLTLAAPE	6.6	HLA-DQA1*04:01/DQB1*04:02
1	11	25	LTLAAPEAAGEVERL	6.6	HLA-DQA1*04:01/DQB1*04:02
1	11	25	LTLAAPEAAGEVERL	6.6	HLA-DQA1*05:01/DQB1*03:01
1	3	17	LPGFYRTGLTLAAPE	8.1	HLA-DQA1*05:01/DQB1*03:01
1	20	34	GEVERLIGHPLPLRL	2.8	HLA-DRB1*01:01
1	3	17	LPGFYRTGLTLAAPE	8.2	HLA-DRB1*01:01
1	30	44	LPLRLDAITGPEEEG	4.5	HLA-DRB1*03:01
1	3	17	LPGFYRTGLTLAAPE	0.9	HLA-DRB1*04:01
1	3	17	LPGFYRTGLTLAAPE	7	HLA-DRB1*09:01
1	3	17	LPGFYRTGLTLAAPE	9.8	HLA-DRB1*11:01
1	20	34	GEVERLIGHPLPLRL	8.4	HLA-DRB1*12:01
1	20	34	GEVERLIGHPLPLRL	9.5	HLA-DRB1*13:02
1	30	44	LPLRLDAITGPEEEG	4.6	HLA-DRB3*01:01
1	3	17	LPGFYRTGLTLAAPE	8	HLA-DRB3*02:02

4.2 Epitope and Ab prediction in *Staphylococcus aureus*

Organism: ***Staphylococcus aureus***

Antigen: ABC transporter, ATP-binding protein, putative (UniProt: Q2FYP2)

Epitope: DRHFLNNVC

FASTA result:

>tr|Q2FYP2|Q2FYP2_STAA8 ABC transporter, ATP-binding protein, putative OS=*Staphylococcus aureus* (strain NCTC 8325 / PS 47) OX=93061 GN=SAOUHSC_01392 PE=4 SV=1

MLQVTDVSLRFGDRKLFEDVNIKFTEGNCYGLIGANGAGKSTFLKILSGELDSQTGHVSL
GKNERLAVLKQDHYAYEDERVLDVVIKGHERLYEVMKEKDEIYMKPDFSDEDGIRAAELE
GEFAEMNGWNAEADAANLLSGLGIDPTLHDKKMAELENNQKIKVLLAQSLFGEPDVLLLD
EPTNGLDIPAISWLEDFLINFDNTVIVVSHDRHFLNNVCTHIADLDFGKIKVYVGNYDFW
YQSSQLAQKMAQEQNKKKEEKMKELQDFIARFSANASKSKQATSRKKQLEKIELDDIQPS
SRRYPFVKFTPEREIGNDLLIVQNLSKTIDGEKVLDNISFTMNPNDKAILIGDSEIAKTT
LLKILAGEMEPDEGSYKWGVTTSLSYFPKDNSEFFEGVNMNLVDWLRQYAPEDEQTETFL
RGFLGRMLFSGEEVKKKASVLSGGEKVRCMLSKMMLSSANVLLLDEPTNHLDLESITAVN
DGLKSFKGSIIFTSYDFEFINTIANRVIDLNKQGGVSKEIPYEEYLQEIGVLK

B-cell epitope

ANASKSKQATSRKKQLEKIELDDIQPSSRRYPFVKFTPEREIGN

Bepipred result

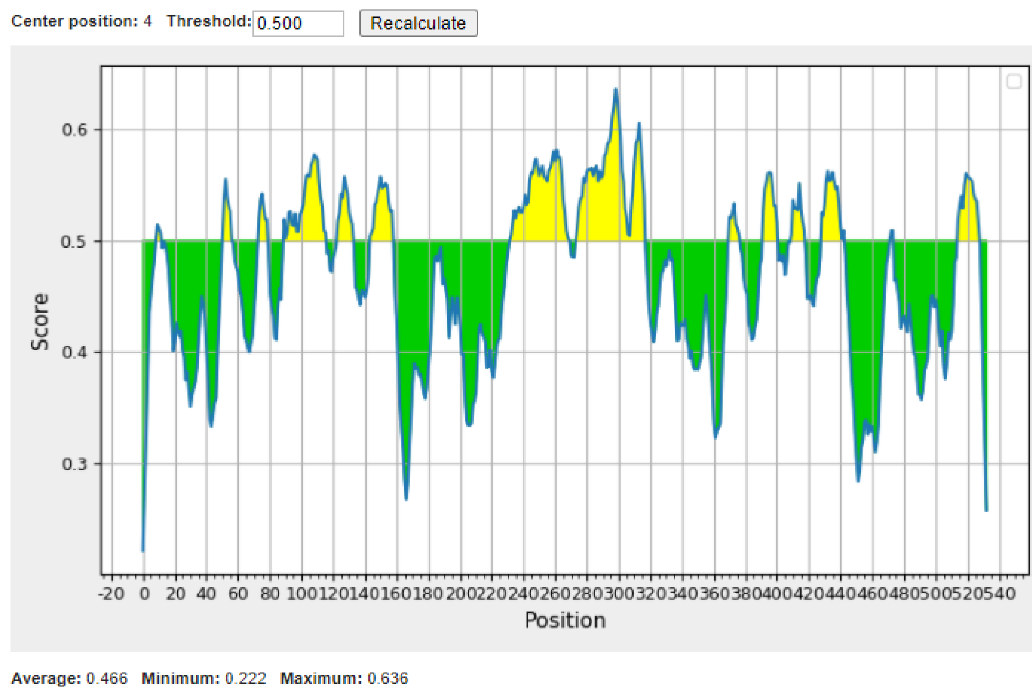

Figure 4. **Bepipred, linear epitope graph representation for antigen exotoxin A (*Staphylococcus aureus*) [X-axis represents residue positions in the sequence; Y-axis represents correspondent score]** Yellow part indicates the probability to be a part of epitope during binding to Ab (Parameters such as hydrophilicity, flexibility, accessibility, turns, exposed surface, polarity and antigenic propensity are considered).

DiscoTope result

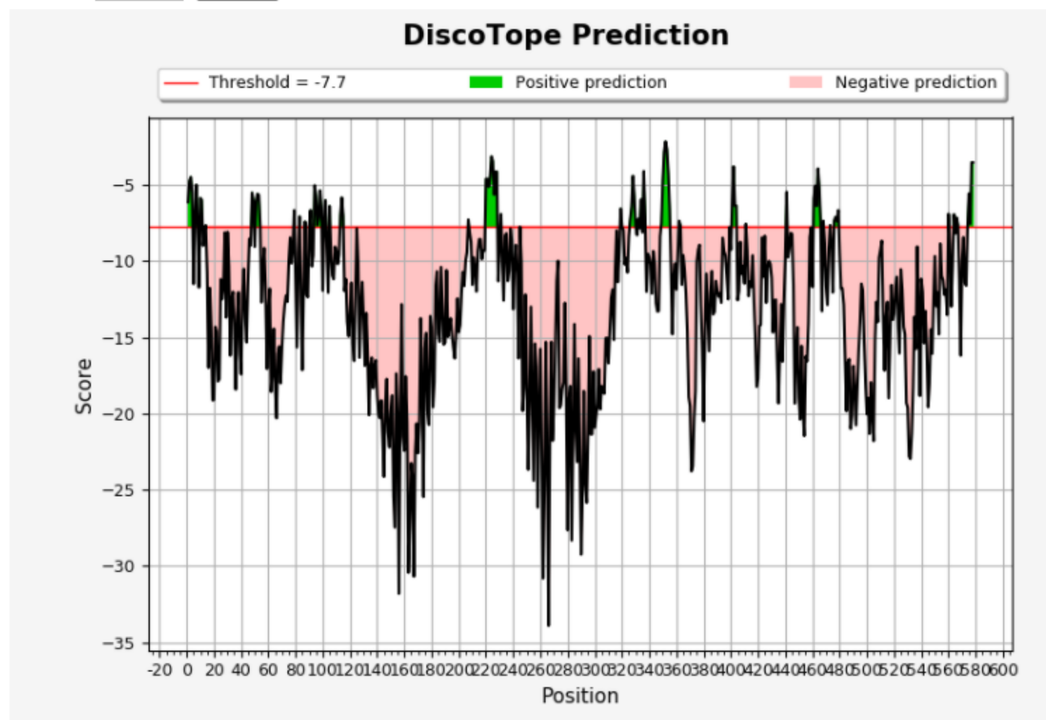

Figure 5. **DiscoTope structure based Ab prediction rule (*Staphylococcus aureus*) (B-cell epitope from the discontinuous epitopes from 3D structures of proteins in PDB format)** Green predictions are the positive one; Peach are negative ones

4.3 Epitope and Ab prediction in *Burkholderia cepacia*

Organism: *Burkholderia cepacia*

Ag: Peptidoglycan-associated protein

Epitope: SYSVKDEYQPLMQQHAQYLK

FASTA result:

>tr|B4EDC1|B4EDC1_BURCJ Peptidoglycan-associated protein OS=*Burkholderia cepacia* (strain ATCC BAA-245 / DSM 16553 / LMG 16656 / NCTC 13227 / J2315 / CF5610) OX=216591 GN=pal PE=1 SV=1

MMSNKARLALAVMMISALAACKSGVKLDDKANNAGAVSTQPSADNVAQVNVDPLNDPNS
PLAKRSIYFDFDSYSVKDEYQPLMQQHAQYLKSHPQRHVLIQGNTDERGTSEYNLALGQKR
AEAVRRAMALLGVNDSQMEAVSLGKEKPQATGHDEASWAQNRRADLVYQQ

B-cell epitope: KDEYQPLMQQ

Bepipred result

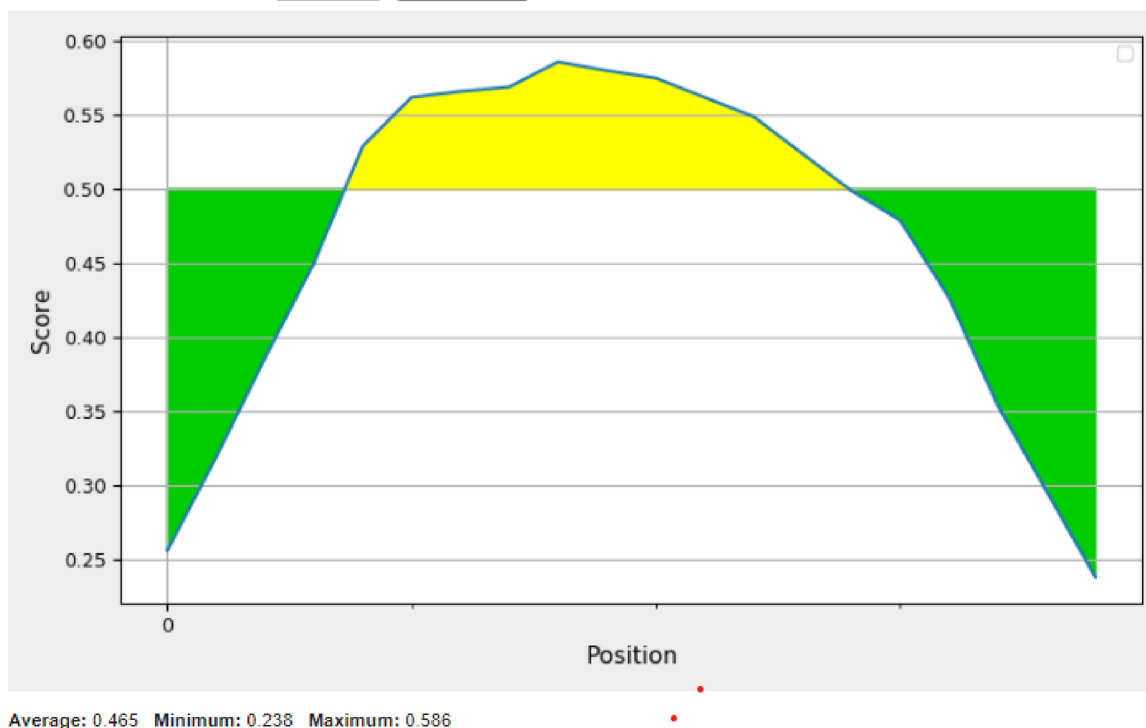

Figure 6. **Bepipred, linear epitope graph representation for antigen exotoxin A (*Burkholderia cepacia*) [X-axis represents residue positions in the sequence; Y-axis represents correspondent score].** Yellow part indicates the probability to be a part of epitope during binding to Ab (Parameters such as hydrophilicity, flexibility, accessibility, turns, exposed surface, polarity and antigenic propensity are considered)

DiscoTope result

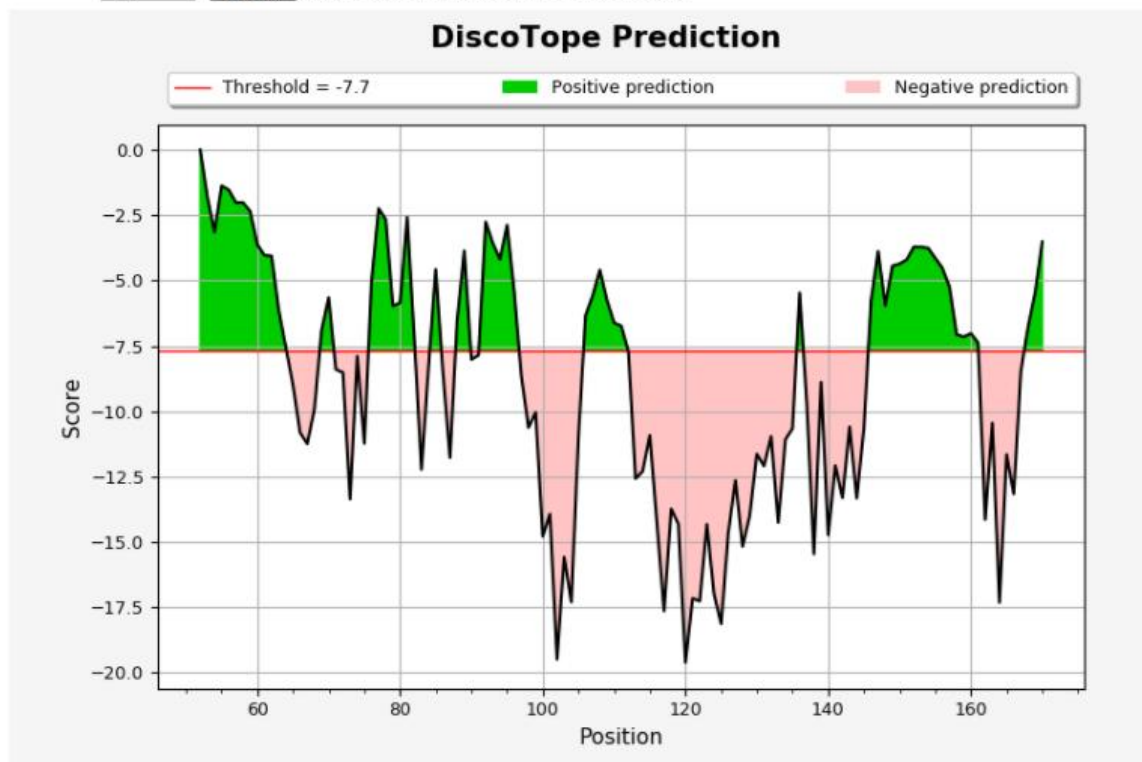

Figure 7. **DiscoTope structure based Ab prediction rule (*Burkholderia cepacia*) (B-cell epitope from the discontinuous epitopes from 3D structures of proteins in PDB format)**

4.4 Discussion and Conclusion

Human body harbours tremendous amounts of microorganisms ranging from the bacteria, archaea to virus and fungi. Increase in the evidence of the complex cross-talk between the gut and lung, and also between the gut and the host immunity proves the importance of the microbiota for the human body. Neither lungs nor the gut is sterile in nature and change in the diversity of the microbes lead to several disorders. Dysbiosis of the microbiota is associated with the development of the common respiratory diseases, such as asthma, respiratory infection, chronic obstructive pulmonary disorder, cystic fibrosis and lung cancer. The microbiota associated with cystic fibrosis patients might somehow be important for the pathophysiology and development of the disease. Owing to high mortality rate (deaths in 90% cases), it has become imperative to come up with new therapeutic techniques to treat the deadly disease, and microbial therapy can be a ray of hope. Modification and the improvement of the gut microbiota through diet, probiotics, will not only improve our understanding about the role of the gut microbiota in respiratory disorders but also provide effective and new therapeutic strategies to combat cystic fibrosis.

Most of the drugs used during the treatment of CF are anti-inflammatory, CFTR modulators, Mucolytics, Bronchodilators. Mortality rate in CF patients is higher due to bacterial infections. Most of the bacteria which cause severe infection in CF have evolved and become antibiotic resistant. *P. aeruginosa* shows resistance to variety of antibiotics [Pang et al., 2019]. *B. cepacia* modifies their LPs layer and provide resistance to drug penetration [Rhodes and Schweizer, 2016]. In addition, adaptive antibiotic resistance of *P. aeruginosa* is a recently characterized mechanism, which includes biofilm-mediated resistance and formation of multidrug-tolerant persister cells, and is responsible for recalcitrance and relapse of infections. As most of the bacteria that are fatal to CF patients, have become drug resistant, the main objective of epitope identification is to replace an antigen in the immunization, antibody production, and serodiagnosis. Epitope-based antibodies are currently the most promising class of biopharmaceuticals. Identification of B-cell epitopes is a fundamental step for the development of epitope-based vaccines, therapeutic antibodies, and diagnostic tools. Prediction of B-cell epitope gives information that these bacterial sp. can be targeted by generating specific Ab. Thus, by using 3D Ag structure, F(ab) region of antibodies can be generated for specifically targeting bacterial pathogens and hence reducing the mortality rate in CF patients. The analysis done in the current study, is restricted to only single B-cell epitope for three bacterial sp. *Pseudomonas aeruginosa, Staphylococcus aureus* and *B. cepacia*, which needs to be extended.

Moreover, though the 3D structure of Ag gives information about F(ab), this is not all needed for generation of Ab. Accurate identification of B-cell epitopes and data integration on large-scale, is still a major challenge for immunologist. Using the predicted epitopes on surface of infectious microbes, monoclonal Abs can be produced *in vitro* using tissue-culture based hybridoma technique. By, targeting the B-cell and T-cell epitopes on the surface, creating monoclonal antibody (Ab) (idiotype), the rate of infection can be slowed down, along with the other drugs, physiotherapy and exercise.

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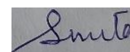

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