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Estimation of Inhibitory Action of Postbiotics Against Neonates *Streptococcus agalactiae*

by

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Every challenging work needs self-efforts as well as the guidance of elders.I dedicate this work to ALLAH Almighty, Hazrat Muhammad (PBUH) and to my parents and my sibling espaceily to Dr.Shahzad Ahmed Gorsi and my Uncle Shabir Ahmed (Late) who have been a constant source of motivation, whose guideline, encourgement and efforts makes me able to achieve such success and to my teachers whose support and motivation always a source of inspiration.

CERTIFICATE OF APPROVAL

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Abstract

Current life style, stress and medication have extremely increased the incidence of various diseases in humans. Bacterial infections account for a major cause of deaths throughout developing world. It is estimated that 409000 maternal and fetal cases and 147000 stillbirth have been occur due to *Streptococcus agalactiae* infections every year. Although many antibiotics have been used for the treatment of Streptococcus agalactiae diseases but excessive use of antibiotics to treat them led to the emergence of drug resistance strain. Bacterial infections such as neonatal sepsis, bacterimia and skin and soft tissue infections espacailly with multidrug resistance opportunistic pathogen such as *Streptococcus agalactiae* are hard to treat due to their resistance profile. Currently, there are no effective drug against these bacteria.Worldwide, researchers are looking for therapeutic agents that can cure the neonatal infectious diseases. Bioactive compounds of *bifidobacteria* were used as postbiotics in current research. It is known for its pharmacological properties for example anti-inflammatory, antioxidative, antiviral, internal body hemeostasis, immunologicals and anti-carcinogenic activities.

Bifidobacteria have rich source of bioactive compounds such as short chain fatty acid which have biological activities in humans. Identifying the natural, microbial based postbiotics drugs are essential for the treatment of neonatal GBS diseases. The present study was undertaken to evaluate protein-ligands intractions of all *S.agalactiea* protein with natural compounds from *bifidobacteria* in order to identify potential inhibitors of respective bacteria. Nine ligands from *bifidobacteria* were selected which act as potentail inhibitors of *S.agalactiae* e.g., Acetate, Propionate, Butyrate, Formate, Lactate, Isobutyrate, Valerate, Caproate and Octanoic acid. These bioactive compounds were taken as ligands and docked with *S.agalactiae* proteins such as CylE and C5a peptidase. The 3D structure of compounds and target proteins was docked. The best ligand was selected on the basis of docking score, absorption, distribution, excretion, toxicity screening and Lipinski rule of 5. By considering all these parameters Octanoic acid was seen obeying all drug-like properties with docking score -5.6 against CylE protein.It

fullfil standard criteria and selected as lead compound. So, it is concluded here that Octanoic acid can prove itself as anti-bacterial agent in future drug discovery.

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Abbreviations

ADMET: Absorption Distribution Metabolism Excretion & Toxicity

AI: Aliphatic Index

BibA: GBS immunogenic bacterial adhesin

β-H/C: β-hemolysin/cytolysin (β-H/C)

BBB: Blood Brain Barrier

CB Dock: Cavity-detection guided blind Docking

CAMP: Christie Atkins Munch Peterson

CADD: Computer Aided Drug Designing

CNS : Central Nervous System

CPS : Sialic acid rich Capsular Polysaccharide

CRC : Colorectal cancer

CASTp : Computer atlas of Surface Topology of proteins

CYP2D6 : Cytochrome P450 2D6

DCs : Dendritic Cells

EOS: Early onset diseases

Fbs : Fibrinogen-binding proteins

FDA : Food and Drug Administration

FIF : Fermented Infant Formula

GRAVY : Grand average of hydropathicity

GBS : Group B *streptococcus*

GIT : Gastrointestinal tract

HIV : Immunodeficiency virus

HMO: Human milk oligosaccharide

HRB : Human-Residential *Bifidobacteria*

HBA : Hydrogen Bond Acceptor

HBD : Hydrogen Bond Doner

hERG : Human Ether-a-go-go-Related gene

IBD : Inflammatory bowel disease

II : Instability Index

Lmb : Laminin-binding protein

LOS : Late onset disease

MW : Moleculer weight

NR: Total number of negatively charged residues (Asp+Glu)

NSAIDs : Non-Steroidal Anti-inflammatory Drug

NASH : Non-alcoholic steatohepatitis (NASH)

PDB : Protein data bank

Pi : Theoretical pI

PR : Total number of positively charged residues (Asp +Glu)

ScpB : Group B *Streptococcal* C5a peptidase

SCFA : Short Chain Fatty Acids

UV rays : Ultraviolent radiation

VDss : Volume of Distribution at steady state

VLBW: Very low body weight

WHO: World Health Organization

Chapter 1

Introduction

1.1 Introduction

Probiotics refers to live microbes when administrated in adequate quantity provided health benifits to their host. A natural probiotic should be of life forms, nontoxic and free of vectors capable of transmitting antibiotic resistance as well as pathogenicity [1] .

Beneficial strains which can be used as probiotic sources are most commonly found in the genera *Bifidobacterium* and *Lactobacillus* and some of these strains have substantial anti-inflammatory effects. Probiotic therapy has piqued researchers attention in infectious, inflammatory, and allergy diseases in humans [2] .

Lactic acid bacteria including *lactobacillus, bifidobacterial* and *Bacillus* strains are isolated from several vegetable and citrus fruit contain functional probiotic products. Fruit contains abundant of vitamins, dietary fiber, antioxidants, lack dairy allergies and minerals that's why it considered as a carrier for probiotics. The probiotics *L. paraplantarum* and *S. cerevisiae*, for example, were obtained from conventional fermented products in Korea and showed antioxidant and immunostimulatory activity suggesting that they may utilize in pharmaceutical products, functional food and pharmaceutical products [3] . Prebiotics are a collection of nutritionally enriched compounds grouped together with the ability to improve

and support the growth and maintain particular healthy gut microflora [4] . Gibson and Roberfroid 1995 coined the term "synbiotics" to describe as a mixture of probiotics and prebiotics that work together synergistically [5]. Human ingestion of synbiotics is said to provide the following health benefits:

1. Increased *lactobacilli* and *bifidobacterial* counts as well as a balanced gut microbiome.
2. Improve liver function of cirrhotic individuals.
3. Increased immunomodulatory capacity.
4. Prevent bacterial translocation and lower nosocomial infection rates in surgical patients and so forth [6] .

Inactivated microbial (probiotic or non-probiotic) complete cells called paraprobiotics that provide benefits to consumers when given in adequate amount [7]. Standard and emerging technologies for the production of Paraprobiotics include such as thermal processes, irradiation, UV rays, high pressure and ultrasound, these techniques are used for deactivation of bacteria for safety purposes. Paraprobiotics have been show to regulate anti-inflammatory and positive immune responses in animals and humans. Non-viable microbial cells may show increased safety i.e. lower threat of sepsis and antibiotic resistance as well as provide technological and real benefits such as extend shelf life [8]. Postbiotics are potentially viable by products of bacterial metabolism or by products of microorganisms that are biologically active in the host. In cell-free supernatants, postbiotics comprises of vitamins, secretory proteins, enzymes, short chain fatty acids, amino acids, peptides, organic acids and other useful biological products or probiotic secreted components. Inactivated or dead probiotics are referred to as non-viable probiotics. Although heat treatment is commonly used technique but other methods such as chemicals (such as formalin), UV irradiation and sonication treatment can also be used to kill living bacteria. However, the inactivation processes, their effects on cellular structural elements and their influence on biological activities are different [9].

Postbiotics may have antibacterial, antioxidant and immunomodulatory activities according to scientific evidence. These characteristics can influence physiological, immunological, neurohormone biological, regulatory and metabolic processes as well as the homeostasis of microbiota and metabolic and signaling pathways of host. Probiotics have been showing benefits on health but postbiotics cells may have a safety advantage over probiotics by decreasing the threat of microbial translocation, infection and inflammatory reaction which have been observed in some probiotics in individual with imbalanced or weaken immune system. Postbiotics have potential to be used as fermented functional foods, microbial free food supplements and preventive medications in the treatment of a variety of disorders [10]. Furthermore, Pique and coworkers in 2019 findings from a recent literature review show that postbiotics have significant pharmacodynamic advantages over living bacteria, as listed under

1. There is no bacterial translocation from the intestine to blood in sick and weak people.
2. There is no concern of acquiring and transmitting resistance antibiotics genes.
3. Extraction, standarzation, transportation and storage are easy.
4. The loss of vitality caused by cell lysis can have additional productful benefits. Each liberated molecule interacts more effectively from the damaged cells to epithelial cells directly [9] .

Microbiota present in the gut provides protection against pathogen colonization or gut-related opportunistic pathobiont. Role of gut microbiota is important in newborns because infection is a leading cause of death in newborns. Passive immunity which is developed due to uptake of protective maternal antibodies is essential to protect children whose immune systems are still developing. The rate of infection is enhanced dramatically among preterm whom birth weight is less than 1500 g. There are two types of sepsis in neonates one of them is Early onset sepsis (EOS) which occurs within three to five days after birth while the other one is

late onset sepsis (LOS) which occurs between seven to ten days. Group *B streptococcus* (GBS) and *E. coli* both are prevalent pathogens in newborn infection especially in neonates or very low birth weight (VLBW) babies. Commensal skin or gut bacteria when proliferate throughout the body, resist against host killing and eventually cause harmful sepsis. Antibiotics administered to preterm children or pregnant women will establish EOS and may destabilize the gut microbiota and allowing opportunistic pathobiont to dominate, proliferate and eventually resulting to LOS [11] . Infection in newborns usually occurs during passing via an affected birth canal. The rate of vertical transmission after vaginal delivery was 67% and it was 8% after cesarean delivery in recent Chinese research. C.trachomatis exposed to infants whose having 20%-50% conjunctivitis risk and 5%-20% risk of pneumonia [12] . There are various diseases occur due to gut microbiota imbalance on of them is gut dysbiosis disorder in which gut microbiota balance is disturb resulting in a harmful consequence. Dysbiosis is characterized by a decline of beneficial microbial flux and an increase of harmful microorganisms. Dysbiosis is triggered by pro-inflammatory effects and immunological dysfunction which has been associated with a wide range of diseases including non-alcoholic steatohepatitis (NASH).

Dysbiosis is considered to have a role in the progression of inflammatory bowel disease (IBD), systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, type I diabetes and other immune-mediated diseases [13] . *Archaea*, Gram-positive, and Gram-negative bacteria all have S-layers which contain pores and have a thickness of 5–25 nm [14] . Surface layer protein have ability to inhibit inflammatory cytokines, induce the secretion of anti-inflammatory cytokines and also enhance function of gastrointestinal barrier [15] .

Anaerobic bacteria required a decreased oxygen level for their growth and inhibit their growth on solid surface media contain 10% CO2, in air 18% oxygen [16] . Anaerobic bacteria predominate on normal skin and mucous membranes. Anaerobic bacteria infections are widespread and can be serious or life-threatening. They emerge from areas where they are normal flora (endogenous) [17] . The presence of anaerobic genera and species such as *Robinsoniella peoriensis, Oscillibacter ruminantium, Solobacterium moorei* and *Ruminococcus gnavus* are spore-forming,

Gram-positive and rod-shaped bacteria cause bacteremia and other dangerous illnesses. Anaerobic meningitis has been related the diseases of the head and neck in children including otitis media, sinusitis, mastoiditis and dental or brain abscesses. Anaerobic bacteria are present in abdominal and pelvic aggregates as well as meningitis in infants on rare occasions [18] .

Streptococcus agalactiae is an anaerobic catalase-negative, beta-hemolytic group of facultative bacteria. Group B *Streptococcus* is a gram-positive bacteria contain a variety of immune resistance phenotype and is a major cause of many dangerous disorders including newborn septicemia, pneumonia, meningitis and infections from orthopedic devices. The GBS has the ability to effect urogenital tract of pregnant women (15 to 35%) by allowing virus to infect their preterm child. *Streptococcus agalactiae* is also related to deadly conditions such necrotizing fasciitis and toxic shock syndrome. GBS encodes wide range of virulence factors which lead to its pathogenicity. Single virulence factor is insufficient to initiate a streptococcal infection instead required the combined action of many pathogenicity factors [19].

GBS infections in newborns is classified into two parts: early onset (EO) and late onset (LO) illness. GBS creates a number of virulence determinants, all of which contribute to the pathogenicity of the GBS. Group *B streptococcus* encodes two significant virulent factors that are capsular polysaccharide and toxins such as pore-forming toxins. GBS generates a lot of virulence determinants, all of which promote to the pathogenicity. GBS encodes two important virulence factors one of them is pore-forming toxins while the other one is sialic acid-rich capsular polysaccharide (CPS). Common virulence factors isolated from GBS has ability to encodes cyl (E) (encoding β-hemolysin/cytolysin) with 90.5% frequency.

Another virulence factor scp (B) encodes invasion of C5a peptidase activity at the rate 75%. Rib is a surface protein of Alp family which create resistant to protease effect (rib) with a frequency of 62.1%, bca encode beta subunit of the C protein with a frequency of 43.4% respectively. *S. agalactiae* (GBS) has an increased resistance profile to aminoglycoside antibiotics such as sulphazotrim , tetracycline and ampicillin as well as fluoroquinolone drugs [20]. The GBS has the ability to

effect urogenital tract of pregnant women (15 to 35%) by allowing virus to infect their preterm child.

Bifidobacteria is one of the first bacteria to colonized a newborn's gastrointestinal tract and it's one of the most common bacteria found in the gut of lactating babies. During the first week of life of breast lactating neonates comprise microbiota of the obligate anaerobes *Bifidobacterium and Bacteroides*. Human milk has been identified as a source of commensal microorganisms that can colonized the newborn intestine.

Bifidobacteria is first microorganism that is transfer from mother to neonates and then colonize in their intestine immediately after delivery in vaginally born infants. Cesarean section (C-section) has been linked to a reduced population of *bifidobacterial* in the newborn gastrointestinal as compare to viginally born neonates. During the delivery process the birth canal is a major source of maternal bacteria particularly *bifidobacterial*. The development of gut microbiota done shortly after birth and effected by different factor such as mode of delivery and neonate feeding etc [21]. *Bifidobacterium* members were among the initial microorganisms which colonize in the human intestinal microbiota and they are considered to provide health advantages to their hosts. *Bifidobacteria* have been used as active compounds in several functional foods due to their potential health-promoting characteristics. Tissier 1899 was first to identify *bifidobacterial* from the faeces of breast-feed babies and *bifidobacterial* obtained from variety of biological environments including the oral cavity, sewage, insect guts, the gastrointestinal track of numerous animals and water kefir now a day. Gastrointestinal illnesses such as inflammatory bowel diseases (IBD), colorectal cancer, diarrhea and lactose intolerance can be treated by using *bifidobacterial*. Rotavirus which causes sporadic diarrhea in neonates, was also treated with these *bifidobacterial* [22]. Postbiotics and paraprobiotics during in vitro and in vivo investigations show anti-inflammatory, immunomodulatory, anti-proliferative, antioxidant, and antibacterial properties. Health-promoting effects of postbiotics seen in clinical trials but signaling pathways and mechanism of action involved have not yet to be completely explained, despite the scientific evidence [7] .

1.2 Hypothesis

Postbiotics include any material released by or created through the metabolic activities of the microbe that has a direct or indirect beneficial effect on the host. Virulence factors of GBS such as cyl(E) (encoding cytolysin -hemolysin) and C5a peptidase cause several infections in virginally born neonates such as septicemia, meningitis and other infection. Identification of postbiotics from *bifidobacterial* and their metabolites may be excellent sarvior to inhibit the genes of virulence and drug development against GBS and may help viginally born neonates to develop initial gut microbiota shortly after birth.

1.3 Problem Statement

The neonates GBS is causing more than one disease in neonates and approximately 409000 cases have been reported every year in vaginally born babies. Current treatment method poses multiple side effects i.e., sepsis disease, intestinal translocation and transmission of antibiotic resistance. Use of specific postbiotics as an anti-adhesion factor could be helpful in avoidance of both problems.

1.4 Aim & Objectives

To identify effective postbiotics to suppress virulent factors of group *B streptococcus*. The objectives of research were:

1. Identification of gut normal flora of neonates.
2. To identify virulence factors of *Streptococcus agalactiae* in preterm vaginally born neonates.
3. Identification bioactive compounds of *Bifidobacteria* as postbiotics metabolites. Identification of lead compound as postbiotics drug candidate in suppression of virulence factors.

Chapter 2

Review of Literature

2.1 Gut Microbiota in Neonates and Adults

The gut microbiota is a complex and abundant assemblage of bacteria found in the human gastrointestinal tract. Nutrition, immune system and the host's defense are all have physiological roles of the gut microbiota [23] . The neonatal period following from birth is critical for the development of early life microbiota which promotes long-term development of the microbial population.

The newborn baby gut has a simpler bacterial, fungal and viral population as compared to adult gut. As describe in in-silico prediction of anti-plasmodial activity of spices. The newborn's acquired microbiota from mother through vertical transmission and provide the first and most essential take part to the development of the infant microbiota [24].

2.1.1 Factors Involved in the Gut Microbiota Development in Neonates

Mode of delivery, feeding type, pregnancy, older siblings, use of antibacterial agents, maternal vagina, gut microbiota in diet and potable water are all factors in the establishment of the gut microbiota from the neonatal period through

early infancy. The mode of delivery and feeding type are two factors that have the most effect [25] .

2.1.2 Mode of Delivery

The microbial population which newborns are exposed during birth depends on the mode of birth. For instance, infants born vaginally are exposed to the bacteria that have residence in the mother's birth canal. Infants born via vaginal delivery have a microbiota that is similar to their own mother as compared to other mothers. On the other hand, neonates born via C-section and their mother microbiota appear to significantly overlap and their microbiota is different. Babies born via C-section had lower levels of anaerobes (such as *Bacteroidetes*), a less diversified microbiota, delayed colonisation of the microbial community. [26] .

2.1.3 Feeding Type

Preterm children with extremely low birth weights and very preterm newborns who are breastfed have more gut microbial alpha-diversity than their 20–30-day-old formula-fed infants which demonstrates the importance of breast milk in promoting infant health.

Bifidobacteria, a probiotic in the human gut can colonize in the gut of breast-fed full-term newborns more easily than the gut of formula-fed infants and its lowers the risk of necrotizing enterocolitis, type I diabetes and Crohn's diseases [27] .

2.1.4 Environmental Factors that Affect Microbiome Development in Neonatal Gut

The colonization and diversity of GI microbiota in neonates are influenced by exposure to various extra-uterine factors during early gut development. It is believed that babies born through C-section are more vulnerable to environmental influences, such as the hospital environment, staff, and surgical instruments [26] .

2.1.5 Diet

The gut microbiota is a proliferative and active collection of microorganisms found in the human gastrointestinal (GIT) tract has beneficial effect during homeostasis and disease on host. Various factors contribute in the establishment of the human gut microorganisms during infancy. Diet is considered one of the most influencing factors in the development of microbiota of gut through whole life. Intestinal bacteria play a critical role in immunological and metabolic homeostasis as well as pathogen defense. Dysbiosis (abnormal gut bacterial composition) has been linked to the pathophysiology of a variety of inflammatory illnesses and infections [28] .

2.1.6 Colonization of Gut Microbiota in Neonates

There are approximately 500 to 1000 species of microbiota present in the adult gastrointestinal tract be more than human cells by at least 10:1 [29] . The microbiota of infants is made up of billions of microbial cells that live in the gastrointestinal track having the highest microbial diversity and abundance. Despite some debate, new research supports the idea that preterm gut colonization occurs during and immediately after birth and influence by a variety of neonates variables including delivery route, feeding type, gestational age, composition of mother gut microbiota, antibiotic treatment and stress [30] . Vaginally born neonates contain gut microbiota composed by *Bacteroides, Bifidobacterium, parabacteroides and Escherichia/Shigella* specie as compared to CS born neonates.

Infants delivered via CS have been shown to have poor bacterial diversity and abundance later in life as well as postponed *Bacteroidetes* colonization [31] . Postbiotics are especially beneficial in high-risk populations of preterm newborns whose intestinal barrier is damage, immune system is still developing and clinical circumstances are frequently severe. In infants particularly in neonates postbiotics have no effect on the formation and physiological alteration of the microbiota of intestine at different stages of neonates. From above observation scientists rediscovered the functional benefits of fermented foods such as FIFs (fermented

infant formulae) is especially for the pediatric population. Postbiotics have a favorable effect in a group of healthy newborns.

L. paracasei CBA L74 fermented with milk of cow and fermented food produced by *L. paracasei* CBA L74 influenced the microbiota in a positive way [30] . The majority of in vitro investigations have explored potential benefits of postbiotics in a group of healthy term newborns. The *L. paracasei* CBA L74 fermented food was shown to have a good effect on establishing a healthy microflora [32] .

2.2 Postbiotics

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have stated that probiotics may cause adverse effects and have safety concerns due to the usage of living microorganisms despite of health benefits. Potential of inactivated forms or metabolites of live microbes can minimize safety concerns and reduce infection risk in those whose with enhanced intestinal permeability and weakened immune systems [33].

Criteria for determining whether a product qualifies as a postbiotics [34].

- Progenitor microorganisms should have molecular characteristics.
- Genome sequence should be fully annotated to capable correct identification and screening for safety concern of potential genes.
- Procedure and the matrix for inactivation should have fully described.
- Evidence of high- quality trial that showed beneficial health impact on host.
- Inactivation has occurred confirm it.
- Postbiotics preparation for the target host have no safety issue.
- Postbiotics Lipid Metabolism
- Postbiotics Function in Neonates.

2.2.1 Mechanism of Action of Postbiotics

Postbiotics which can be a heterogeneous group of substances that facilitate a health impact in the target host through various processes. Most research has been conducted in vitro on postbiotics but the processes by which the effect of postbiotics at a distance are not well define [32, 33] .

2.2.2 Modulation of Resident Microbiota

Postbiotics might adversely impact the microbiota by carrying quorum sensing and quorum quenching molecules. Some microbe members carrying lactic acid to produce SCFAs and butyrate both of which have positive functions. If postbiotics produce adhesions (such as fimbriae and lectin) that stay stable after processing. Postbiotics can fight with resident microbes for attachment sites [32].

The infant's gut microbiota grows shortly after birth which enhance its gut barrier functions and immune function during the initial phase of life due to symbiotic relationship among the beneficial colonizing bacteria, the epithelium of intestine and the related lymphoid system [32] .

2.2.3 Immunomodulation

Postbiotics such as Pili and protein p40/p75 which have been generated by *Lactobacilli* seem to have immunomodulatory effects by acting on the intestinal barrier, increasing assemblage, S-layer protein, bacteriocins and factor proteins as well as having antagonistic action against pathogens. Certain bacterial species and strains seems to have different immunostimulant effects due to change in cell wall components such lipoteichoic acid and peptidoglycan. The mechanism by which these bacteria conduct their immunomodulating activities has been proposed as enhancing Th1-associated cytokine, lowering Th2-related cytokines, activating dendritic cells (DCs), decreasing Th17, increase Treg expression and moving macrophages to M2 subtype or improves anti-tumor activity [33, 35] .

2.2.4 Enhancement of Epithelial Barrier Function

Various probiotics have been proposed to decrease colonic inflammation and repair integrity of gut barrier. Probiotics soluble protein improved epithelial restoration by increasing mucin synthesis and tight junction protein expression. *Lactobacillus rhamnosus, Lactobacillus plantarum and Escherichia coli Nissle* 1917 are among the probiotic strains which stabilize the expression of tight junction proteins (claudin-1, occludin, ZO-1, ZO-2), promoting mucin synthesis, decreasing inflammation and increasing epithelial restoration have all been proven to enhance gut barrier function. Probiotics may have a positive impact on CRC patients by repairing epithelial integrity [35] .

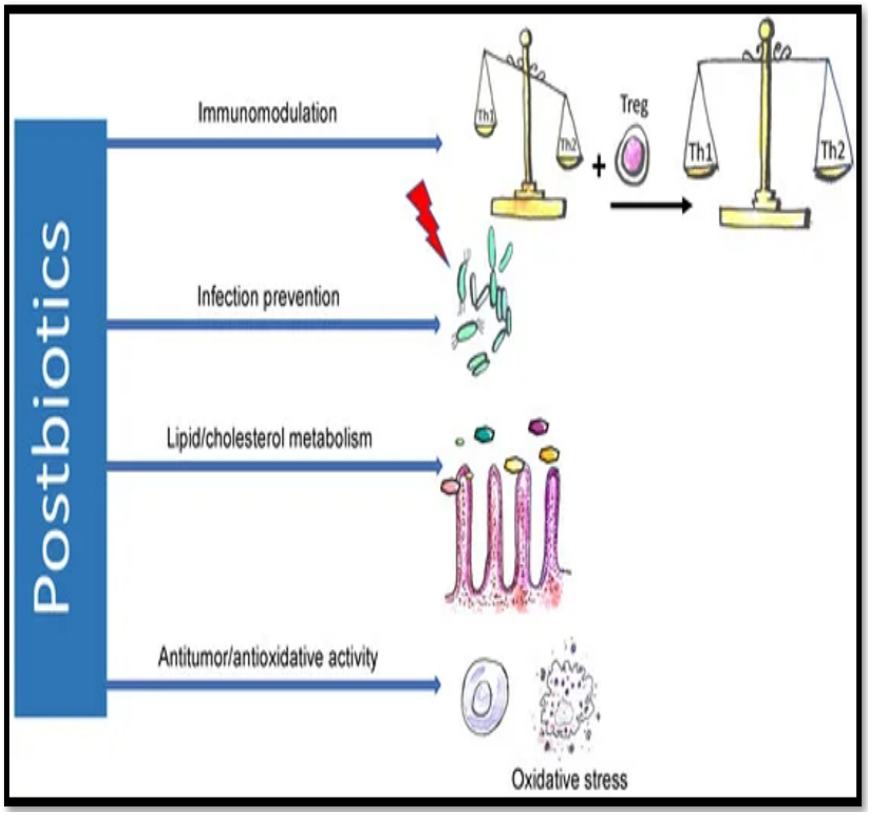


FIGURE 2.1: Mechanism of action of postbiotics [36].

2.2.5 Postbiotics Lipid Metabolism

Postbiotics have a positive impact on lipid metabolism by stimulating mechanisms which have ability to generate beta-oxidation of fatty acids and lipolysis

in adipocytes. As illustrated in figure 2.1 how postbiotics appear to work as an anti-obesity agent by hepatic insulin resistance and activating transcription factors that control inflammation in adipose tissue and glucose intolerance

2.2.6 Postbiotics Function in Neonates

Postbiotics performs various function in neonates such as antiinflammatory, immunomodulatory, antioxidative and antitumor which promote their health and prevent from many diseases such as diarrhea, bloating and improve gut barrier function.

2.2.6.1 Anti-Inflammatory

Bifidobacteria longum is a probiotic obtained from a healthy breast-feed neonates that has been found to induce anti-inflammatory benefits in various models including decreased γ-IFN and α-TNF-production and increased IL-10 production in human peripheral blood mononuclear cells. Furthermore, there was a decrease in pro-inflammatory markers and enhance IL-10 production in monocyte-derived dendritic cells as well as an increase in zonulin expression. Postbiotics as shown in fig 2.2 can decrease inflammation, interact with lymphocyte sites, regulate IgA immunity and synthesis at local level and promote beneficial bacterial strains [30, 37].

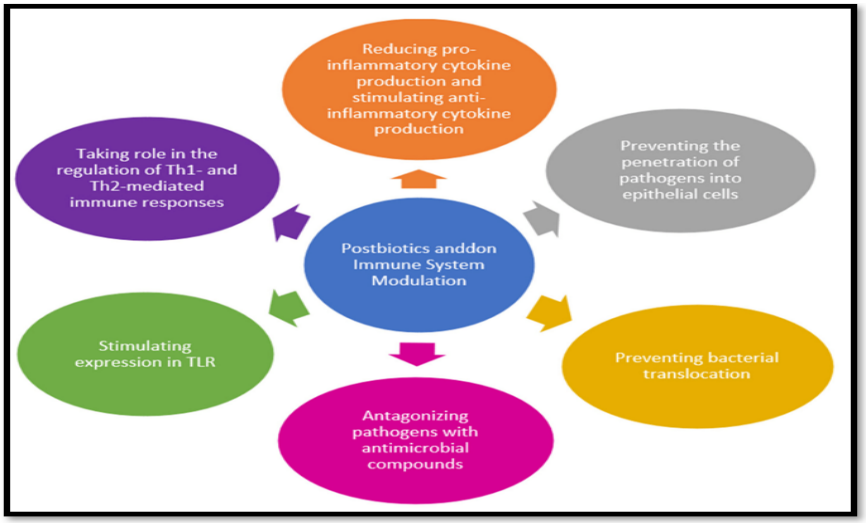


FIGURE 2.2: Benefits of postbiotics [36].

2.2.6.2 Antioxidative

Antioxidants are a kind of molecule that by neutralizing free radicals can protect the host from oxidative damage. Probiotics are a type of functional food that has health benefits for consumers particularly in terms of antioxidative activities. Probiotics may also enhance antioxidative activity by increasing expression of antioxidant-related genes and produce antioxidative enzymes [38] .

2.2.6.3 Antitumor

Through caspase-mediated apoptosis postbiotics cause considerable cytotoxicity in cancer cells. In breast and colorectal cancer (CRC) cells, heat-killed *L. plantarum* I-UL4, *L. brevis* and *L. paracasei* display strong selective cytotoxicity by reducing proliferation and inducing apoptosis. *B. bifidum* cell free supernatants are efficient in destroying cancer cells and are significantly linked with the treatment of gastrointestinal cancer [39] .

2.2.6.4 Antimicrobial Effect

Antibacterial (pathogenic and spoiler bacteria) properties of postbiotics agents help to prevent infectious illnesses and food spoilage. These chemicals prevent proliferation of harmful microbes in the gut and prevent disorders like irritable bowel syndrome and inflammatory bowel sickness. Postbiotics are identifiable living organisms that influence the microbial flora of the body and inhibit the replacement of microbial infection in the gut wall [40] .

2.3 *Bifidobacteria*

Bifidobacteria are common and abundant bacterial species found in mammalian guts. Members of the phylum Actinobacteria's genus *Bifidobacterium* are gram-positive, anaerobic, saccharolytic bacteria known as *Bifidobacteria*. In both human

and mouse models, the formation of metabolites like vitamins and antioxidants, development of the immune system and protection against specific gut disorders including enterocolitis and severe diarrhea all have been linked to their presence in the gut. Human milk oligosaccharides (HMOs) generated from breast milk are vital for being break down in infants by particular species of *bifidobacteria*. Infant health is aided by the fermentation of HMOs, which also discourages the colonization of possible dangerous microorganisms. Additionally *bifidobacteria* are excellent in breaking down and fermenting carbohydrates [41] . A person's subsequent health is significantly influenced by the formation of a healthy gut microbiota throughout the early stages of human life. Although it has been suggested, not everyone agrees that microbes start colonizing the infant while it is still in the uterus. Microbiota that resides in mother vagina then have get access to and interact with the infant, at least throughout the natural delivery process. This maternal vaginal and faecal microbiome contains rod-shaped, gram-positive *Bifidobacteria* species. The following bacteria are frequently found in the gut: *B. dentium, B. adolescentis, B. catenulatum B. longum, B. bifidum B. angulatum, B. pseudocatenulatum, B. breve, and B. pseudolongum* [42] . Human milk contains higher percentage of non-digestible oligosaccharides which boost colonic fermentation by *bifidobacteria* which produces organic and short-chain fatty acids and lowers stool pH as compared to formula fed. This creates unfavorable environment for the growth of harmful bacteria which may probably decrease chances of infections. This initial colonization has been shown to be significantly affected by the delivery method in particular with vaginally delivered newborns showing a greater number of *bifidobacteria.* than those born by cesarean section [43] .

TABLE 2.1: List of Microbiota found in neonates [44].

Sr.	**Name of Microorganism**
1	*Escherichia coli*
2	*Enterococcus spp*
3	*α-hemolytic streptococci*
4	*Staphilococcus spp*
5	*Bacteroides*

TABLE 2.1: List of Microbiota found in neonates [44].

Sr.	Name of Microorganism
6	*Bifidobacterium and*
7	*Clostridium spp*
8	*Enterobacteriacae*
9	*Streptococcia*

2.3.1 Classification of *Bifidobacteria*

Bifidobacteria could be categorized into two major groups based on their residential origins:

2.3.1.1 Human-Residential *Bifidobacteria* (HRB)

Bifidobacteria species that naturally found in the human gastrointestinal system is known as Human-Residential *Bifidobacteria* (HRB).

Infant-type HRB which includes *B. breve, B. longum subsp. infantis, B. longum subsp. longum and B. bifidum*, is distinguished from adult-type HRB which includes *B. catenulatum, B.longum subsp.longum and B. pseudocatenulatum* are abundant species present in the adults intestine [45]

2.3.1.2 Non-Human-Residential *Bifidobacteria* (non-HRB)

Non-HRB *Bifidobacterial* species naturally occur in animals and environment. *B. animalis subsp. animalis, B. animalis subsp. lactis, B. thermophilum and B. pseudolongum* are included in non- HRB category and some of these species exhibit a specific ecological adaption to a particular animal gut.

In the faeces of rabbits non-HRB species were often discovered such as *B. magnum* and *B. cuniculi* as well as *B. pullorum* and *B. gallinarum* were present in the intestines of chickens and *B. longum* subsp. suis in the faeces of piglets [45] .

TABLE 2.2: Hierarchical classification of *Bifidobacteruim* [46]

SerialNo	**Domain**	**Eukarya**
1	Kingdom	Bacteria
2	Phylum	Terrabacteria
3	Class	Actinobacteria
4	Order	Actinomycetia
5	Family	Bifidobacteriaceae
6	Genus	*Bifidobacterium*

2.3.2 Probiotic Potentials of *Bifidobacterium*

In the early life microbiome, *Bifidobacterium* species start to colonize and develop as soon as a baby is born. Giving *Bifidobacterium* probiotics has been associated with a significantly reduced risk of NEC and late-onset sepsis in neonates in ICU and have no safety issues. Combination of *Bifidobacteria* and *Lactobacillis* with probiotics can successfully prevent NEC in very premature babies [47] . Gastrointestinal problems like gastroenteritis, enterocolitis, irritable bowel syndrome (IBS), diarrhea, food allergies and intolerances are frequently brought on when pathogens pass through the barrier created by *bifidobacteria* probiotics. *Bifidobacteria* has been demonstrated to be successful in treating symptoms of constipation, stomach pain, flatulence and bloating by rebalancing the gut flora and lowering aberrant bacterial fermentation of dietary residues. Consumption of specific strains of *B. Bifidum, B. lactis BB12, B94 L. rhamnosus GG, L. acidophilus La-5 and B. longum* BB-536 have reported to decrease symptoms of diarrhea in adults and infants.

Bifidobacteria has been shown to have probiotic effects against pouchitis, ulcerative colitis and Crohn's disease. Additional health benefits include the treatment of *Helicobacter pylori* infections and the preventative measures of colorectal cancer [48]. It has been demonstrated that a few probiotic *bifidobacteria* strains have immunomodulating and anti-inflammatory effects on the human immune system. Probiotic *Bifidobacteria* can provide protection against intestinal damage caused

by autophagy. Some *bifidobacterial* species may inhibit coronavirus replication by decreasing ER stress-related autophagy via an impact on IL-17 [49] .

2.3.3 Pharmacological Properties of *Bifidobacteria*

Probiotics like *Lactobacillus* and *Bifidobacterium* can help the host to restore their health by eliminating infections and regulating immune responses in intestinal epithelial cells. Probiotics strains are become popular due to their ability to regulate immunological responses, particularly in the lower and upper respiratory tracts. Probiotics regulate allergic reactions and protect the body from viral and bacterial infections. Probiotics treat allergies by healing the digestive tract by reducing inflammation, strengthening the gut lining, and balancing the immune system [50].

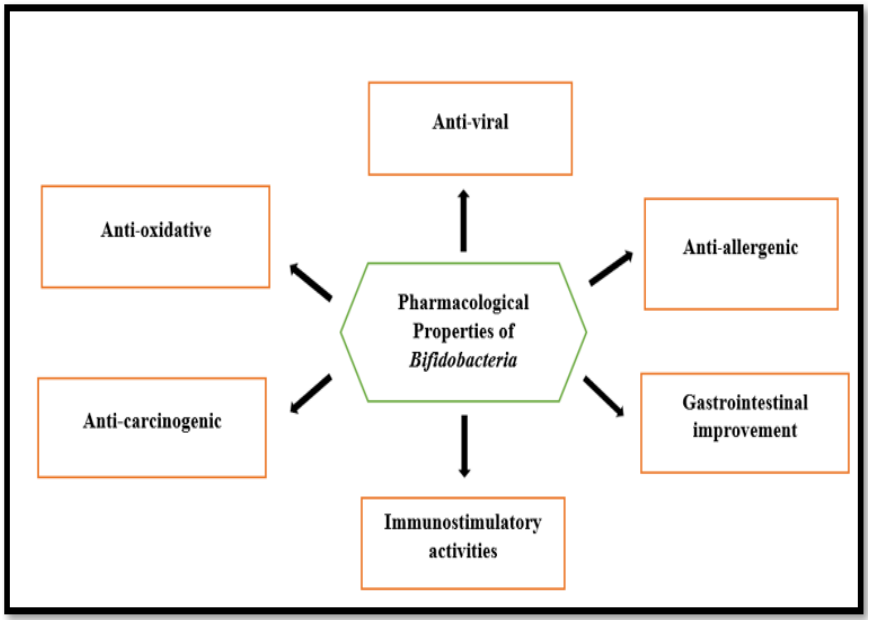


FIGURE 2.3: Pharmacological properties of *Bifidobacteria* [50].

2.3.4 Bioactive Compounds of *Bifidobacteria*

Probiotic bacteria can produce a wide range of beneficial metabolites for human health. Among these bioactive compounds made by probiotic bifidobacteria include bacteriocins, metabolic enzymes, amino acids, peptides, short chain fatty

acids, vitamins, antioxidants, anti-inflammatory, immune-modulating compounds and exopolysaccharides. These substances work together to enhance gut physiological function and improve overall health [51] .

2.3.5 Short Chain Fatty Acid (SCFA)

Bifidobacteria have capacity to release compounds that provide benefits to host health. Short-chain fatty acids are byproduct of fermentation of indigestible polysaccharides, such as HMOs. SCFAs that are most important to human health are acetate, butyrate, propionate and formate because they predominate in the colon. These compounds play a number of roles in maintaining human health including intestinal pH, gut barrier integrity and pathogen suppression. However because they feed colonocytes they are particularly important for a child's development [52] .

SCFAs produced by the intestinal microbiota are vital for development and homeostasis of central nervous system, the immune system, gastrointestinal system and host's metabolism. SCFA metabolites play important role in complex gut-brain interactions that may aid in the creation of new therapeutic targets for CNS disease treatment. SCFAs have potential to directly and indirectly affect CNS functions which in turn affect behavior and cognitive function. Due to their effects on the development and maintenance of healthy brain function these metabolites also have the potential to be used as dietary treatments for a range of psychological conditions [53] .

2.3.5.1 Acetate

Acetate has beneficial properties that are important for newborn health in addition to its role as a master regulator in the gut. Acetate can be produced from H_2 and CO_2 by acetogens and dissimilatory sulfate-reducing bacteria as well as from CH_2 and H_2S. Acetate perform various important activities including energy production, microbial ecosystem control and waste management [54]. If an infant's

digestive system generates too much gas which can result in bloating, they may feel significant discomfort. Therefore, the production of acetate might help with relief [55] .

2.3.5.2 Propionate

Propionate play important role in children who have high levels of propionate and butyrate in their faeces at the age of one are less likely to later develop asthma and food allergies according to epidemiological studies. Additionally, these substances work to strengthen the intestinal barrier. Propionate and butyrate are the two SCFAs that are most common in the intestines which account for 95% of the lumen of the gut. Propionate is a SCFA that lowers cholesterol and possesses anti-inflammatory, anti-cancer and reduced fat storage properties. It is a product of fermentation caused by bacteria in the large intestine [56].

2.3.5.3 Butyrate

Butyrate is essential in controlling immunological responses because it suppresses inflammatory reactions and serves as the main energy source for gut epithelial cells. Butyrate also nourishes intestinal epithelial cells and promotes the production of mucin which may change bacterial adhesion and enhance the strength of tight junctions. Therefore, it would maintain the integrity of the gut barrier requires the production of SCFA [57] . One of the SCFAs believed to be essential for preserving epithelial homeostasis is butyrate. Butyrate-containing fluids or SCFA mixtures have been used in the treatment of active IBD [58].

2.3.5.4 Formate

Formate is an important one-carbon donor for the synthesis of cytosolic 10- formyl-tetra hydrofolate and its subsequent transformed to more reduced folates. Formate contributes to supply of one-carbon groups for a variety of crucial processes including the production of methyl groups and purines and thymidylates. Numerous

mechanisms can produce formate in the cytosol and mitochondria but formate is mostly produced in mitochondria by the enzyme 10-formyl-tetrahydrofolate synthetase (MTHFD1L) when combines with 10-formyl-THF.The folate cycle offers crucial one-carbon units especially in the early stages of development. It has been known for more than 40 years that in pregnant women low folate status is a significant risk factor for aberrant neural tube closure. The concentration of formate in the amniotic fluid and fetal circulation was significantly higher than that of maternal circulation. Rat prenatal and neonatal development need formate as a one-carbon precursor more frequently. Increased expression of genes related to the formation and use of formate as well as elevated amounts of serine and glycine precursors in the prenatal and neonatal rats' plasma [59].

2.3.5.5 Lactate

Some microbiota species such as lactic acid bacteria, *bifidobacteria* and *proteobacteria* produce lactate. The effects of lactate are beneficial to the immune system. It can act as a mediator to produce both pro and anti-inflammatory cytokines. In addition, lactate improve brain function and also help to reduce intestinal inflammation. They also decrease depressive symptoms of depression [60].

2.3.5.6 Isobutyrate

Isobutyrate is a short-chain fatty acid which is produced by bacteria as a byproduct of the breakdown of valine, which is obtained through the proteolysis of both dietary protein and endogenous substances like pancreatic enzyme that are not digested in the upper gastrointestinal tract [61] .

2.3.5.7 Valerate

Valerate is a SCFA primarily made up of proteins or amino acids and supports intestinal barrier function. Valerate reduced paracellular permeability while raising TEER to its maximum at 2 mM. It performs function of the intestinal barrier

similarly to butyrate. Valerate was shown to activate AMPK and generate tight junctions (TJs) but not produce expression of TJs-related proteins. The importance of valerate in protective intestinal health facts to a previously unknown function for valerate and its precursor amino acids. Valerate has a role in protection of intestinal homeostasis and also show potential interactions with other SCFAs [62].

2.3.5.8 Caproate

Caproate is also known as hexanoic acid, is a short-chain fatty acid anion that is the conjugate base of hexanoic acid. It functions as a metabolite for both people and plants. It is a 6:0 fatty acid anion and a straight-chain saturated short-chain fatty acid anion. It is a conjugate base of hexanoic acid [63] .

2.3.5.9 Octanoic Acid

Octanoic acid is a straight-chain saturated fatty acid produced by replacing hydrogens in a terminal methyl group with a carboxy group. Octanoic acid is also known as caprylic acid. It serves as an antibacterial agent, an *Escherichia coli* metabolite and a human metabolite. It has a straight chain and is a medium chain saturated fatty acid. It is the conjugate acid of an octanoate [64].

2.4 *Streptococcus agalactiae*

Streptococcus agalactiae is also referred as Group *B streptococcus* (GBS) is a non-motile, gram-positive and facultative anaerobic bacterium. The gastrointestinal tract is normal reservoir of GBS in the body and here typically asymptomatic vaginal colonization first appeared. According to an estimate from World Health Organization infections are thought to be responsible for a million newborn deaths and a million stillbirths annually. GBS infection continues to have a substantial global impact, affecting 320,000 newborns, 9,000 of whom will die, as recorded

in 2015 in developed and developing countries equally. Globally it was estimated 40,9000 cases reported of GBS maternal or infant cases and approximately 1,47000 annually stillbirth and infants' death has been reported. The most prevalent germ to infect infants is *S. agalactiae* which is a significant contributor to stillbirths. GBS is present in the mother's vaginal tract at the time of birth or before the rupture of the membranes find out whether a baby is at risk for an offensive illness. Additionally, evidence have shown risk of vertical transmission, vaginal GBS load and the likelihood of serious disease in newborns. GBS must adhere to the vaginal epithelium in order to successfully colonize the vagina. It has been demonstrated that GBS adheres effectively to human exfoliated vaginal epithelial cells, with the strongest adhesion occur at an acidic pH which is typical of the human vagina's normal state. The limiting of bacterial cell-surface-related proteins, for example, pili to have extracellular framework components including laminin, fibrinogen and fibronectin which thus communicate with have cell-anchor proteins like integrins and works with high affinity contacts of GBS. GBS can infect the baby by eating or inhaling amniotic liquid in after ascending infection through ruptured or intact membranes. As an alternative, the newborn might get GBS after going through the delivery process [65] .

2.4.1 Types of Group *B Streptococcus*

The GBS can be categorized into two types on the basis of causing infection in the neonates.

- Early onset GBS
- Late onset GBS

2.4.1.1 Early Onset Group B *Streptococcus* (EOGBS)

Although the majority of newborns colonized with GBS approximately less than 98% (>98%) at birth are asymptomatic and approximately 1.1% develop EOD.

EOGBS shortly develop after birth within 6 days. Other risk factors associated with EOD include maternal bacteriuria, maternal fever, increased susceptibility of the second birth in twin pregnancies and preterm labor [66] .

2.4.1.2 Late Onset Group B *Streptococcus* (LOGBS)

LOGBS developed within 7–90 days of life of neonates. LOGBS can be caused by mucosal colonization of GBS during labor, delivery, or the postpartum period and from mother. Early research suggests that up to 40% of newborns who had colonization of GBS at delivery still shows colonisation of intestine at 12 weeks of age. The pathogenesis of LOGBS involves many stages including GBS adhesion to mucosal surfaces, penetration of the epithelium and finally bloodstream invasion. GBS adherence to mucosal surfaces, invasion of the epithelium and ultimately bloodstream invasion are all steps involve in the pathophysiology of LOGBS. Factors which facilitate the transition from intestinal colonization to invasive GBS illness or may mediate chronic intestinal colonization remain unknown. LOD risk is 4.4 times higher in babies born to HIV-positive mothers [67] .

2.4.2 Pathogenesis and Virulent Factors Associated with Group B *Streptococcus*

Pathogenic bacteria have capacity to produce a wide range of virulence factors and is important characteristics of these virulence factors is in sustaining its pathophysiology Pathogenic bacteria have ability to solve medical problems and have capacity to spread disease is made possible by the formation and secretion of virulence factors. GBS produces a number of virulence factors that strongly highlight its pathogenicity similar to its other pathogenic sibling.

Pore-forming toxins and sialic acid-rich capsular polysaccharide (CPS) are two significant virulence factors encoded by GBS. Important virulence determinants in GBS infections include pore-forming toxins and CPS in addition to adhesion factors, evasion factors and other virulence factors that have shown resistance

to antimicrobial peptides and other conventional therapeutics. Adhesion factors facilitate the binding to cells or extracellular matrix. Evasion factors regulate neutrophil recruitment [68] .

2.4.2.1 Pore Forming Toxins

Pore-forming toxins of GBS enable the invasion in the host after which they survive and proliferate throughout the body. Two highly defined pore-forming toxins are namely Christie Atkins Munch Peterson (CAMP) factor and β-hemolysin/cytolysin (β-H/C) were discovered in GBS. The secreted toxins of β-hemolysin/cytolysin (β-H/C) is predominant among the most significant GBS virulence factors due to its wide variety of host targets cells. More than 99% of GBS strains express the pore-forming toxin known as β-H/C, which is also linked to the phenotype of orange pigmentation. This toxin made a recognizable hemolysis ring around the colonies of GBS on blood agar plates.

The cylE gene is essential and sufficient for β-H/C activity. GBS cause invasive disease syndromes like pneumonia, joint issues, skin and soft tissues infections, sepsis, bacteremia and urosepsis in adults as well as cause pneumonia, meningitis and bacteremia in newborns. The toxins have a non-specific binding for the lipid bilayer of cell membranes that helps them to invade into tissue barrier and cause inflammatory injury. Preterm and neonates with low weight have the more risks for bacteremia and pneumonia caused by GBS because dipalmotyl phophatidylcholine (DPPC), the principal component of surfactant that is rich in lipids, sequesters and inhibits β-H/C [69] .

2.4.2.2 Sialic Acid-Rich Capsular Polysaccharide (CPS)

GBS uses a variety of virulence factors to colonize and spread diseases and one of them is capsular polysaccharide (CPS). Human Ten GBS serotypes have been classified (Ia, Ib and II–IX), according to recognized capsular polysaccharides that are considered to be a major virulence factor in invasive disease caused by GBS

and the current candidate capsular polysaccharide conjugate vaccines target only a subset of these [70] . A worldwide study that has provided serotype data such as in Americas (96%), Europe (93%), the Western Pacific (89%) and Africa (91.8%) the five most prevalent serotypes, Ia, Ib, II, III and V accounted for more than 85% of serotypes. Out of the 10 serotypes Ia, Ib, II, III and V account for 98% of the colonizing isolates that have been found over the world. Serotype III which is linked to invasive disease and makes about 25% of cases, is less common in various South American and Asian nations whereas serotypes VI through IX are more prevalent in Asia [71] . Sialic acid-rich capsular polysaccharide (CPS) is one of the important virulence factors involved in stability and survival of GBS within the host. CPS is essential for immune evasion. Additionally, it delays neutrophil's ability to perform their phagocytic role, obstructs complement-dependent defense pathways and promotes bacterial incorporation and survival in dendritic cells [72]. Postbiotics performs various function in neonates.

2.4.2.3 Adhesion & Evasion Factors

GBS have ability to colonize, persist, translocate and invade into host barriers as an opportunistic bacterium that have important role in vaginal and intestinal physiologic flora, GBS must be able to adhere to host cells and extracellular membrane. Functionally characterized adhesins that mediate adherence and invasion of GBS within the host include, fibrinogen-binding proteins (Fbs), streptococcal fibronectin-binding protein A (SfbA), fibrinogen-binding proteins (Fbs), laminin-binding protein (Lmb), GBS immunogenic bacterial adhesin (BibA) and the group B streptococcal C5a peptidase (ScpB). Surface protruding structures contain multiple gene such as pili play a critical role in GBS colonization, persistence, biofilm formation and invasion of the central nervous system [73].

2.4.2.4 Adhesion of Virulence Factors of *Streptococcus agalactiae*

GBS vaginal colonization increases chances of infections during pregnancy. Bacteria trafficking from the vagina helps to the spread of GBS infections during

pregnancy which ultimately results in bacterial invasion into fetus, amniotic fluid and placental membranes (chorion and amnion). Number of virulence factors expressed by GBS which enable vaginal penetration, host cell adhesion and invasion and either activate or repress inflammatory responses. These factors also raise the chances of prenatal harm, ascending infection or premature delivery. *Streptococcus agalactiae* adhere to epithelial cells to colonize in mother's vaginal and rectal tracts and retention in the newborn's lungs. SrtA aids in S. agalactiae's adhesion to fibronectin and cultured epithelial cell [74]. C5a peptidase may have a number of roles in the adherence of GBS. By increasing the expression of the surface complement receptor 3 on neutrophils and adhere to lungs epithelial cells and leads to opsonophagocytic [75].

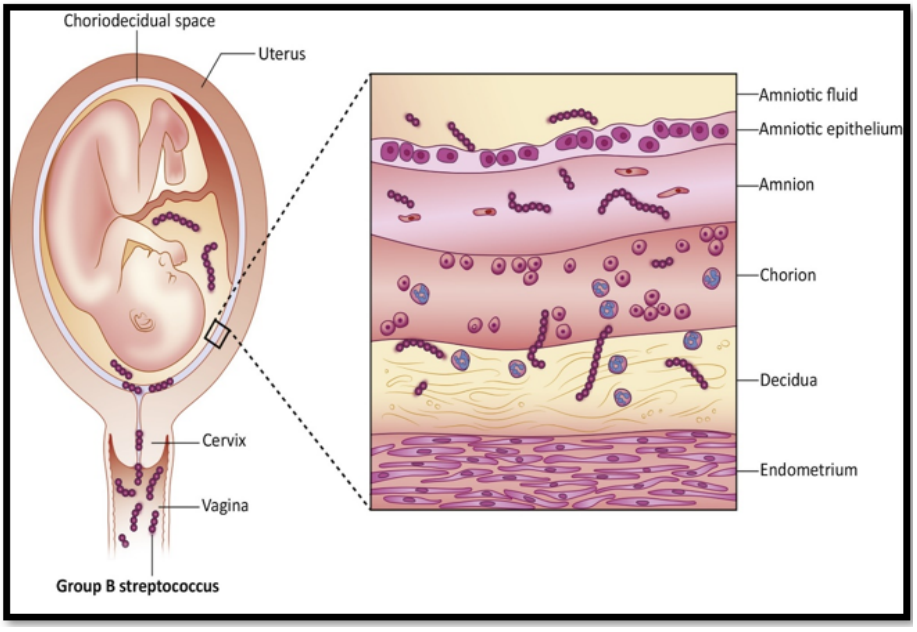


FIGURE 2.4: *Streptococcus agalactiae* adhesion to viginally born neonates [76].

GBS interactions with host extracellular matrix elements (ECMs) appear to be a mediator of adhesion and invasion and these interactions may also encourage GBS resistance to mechanical clearance, evasion of immune surveillance and paracellular transmigration. β-hemolytic GBS bacteria have hemolytic ability which is crucial for infection and immune evasion. GBS hemolytic function is regulated by ornithine rhamnolipid pigment which is produced by the genes of the cyl operon. The CovR/S two-component system adversely regulates the transcription of cyl genes as a result of generation of the hemolytic pigment. Deletion of covR/S

causes GBS to become hyperpigmented and hyper hemolytic. On the other hand, when the cylE gene which encodes a N-acyltransferase which is required for pigment synthesis is when deleted GBS becomes nonpigmented and nonhemolytic. According to Whidbey et al., the hemolytic pigment causes human amniotic epithelial cells to lose their barrier function and facilitates GBS penetration of the chorioamniotic membranes of the human placenta [77].

2.4.3 Prevalence and Risk Factors

Several risk factors associated with EOS GBS include maternal GBS carriage (especially with heavy colonization), previous infants with GBS disease, maternal fever, continued membrane rupture, preterm delivery and low maternal levels of anti-capsular polysaccharide antibody to the colonizing GBS serotype are risk factors for early-onset neonatal GBS disease. Infants born preterm have much worst outcomes than those born at term and are at significantly increased (3–30-fold) risk of GBS illness. However, even though preterm is a risk factor for GBS disease but newborns account more than 70% risk of GBS disease [74] .

2.4.4 Antibiotic Usage and Resistance

The use of antibiotics has significantly impacted human health and has played a vital role in medical science. Their advancement has enhanced life expectancy, decreased infant mortality and given medical professionals an essential tool for invasive surgery and the treatment of bacterial infections.

TABLE 2.3: Antibiotics along with Mechanism of Resistance and Target Bacteria [77].

Antibiotics	**Mechanism of resistance**	**Target bacteria**
Penciline	Reduced access to PBPs, Efflux pump	Gram +ive
Tetracycline	Resistance genes associated	Broad Spectrum
Fluoroquinolones	Presence of mutations within the	Broad Spectrum
Aminoglycosides	Bifunctional aminoglycoside	Gram +ive

TABLE 2.3: Antibiotics along with Mechanism of Resistance and Target Bacteria [77].

Antibiotics	**Mechanism of resistance**	**Target bacteria**
Erythromycin	Efflux pump, Ribosomal Modification	Gram +ive
Azithromycin	Efflux pump, drug inactivation	Gram +ive
Glycoprotein	Synthesis of peptidoglycan	Gram +ive

But the prevalence of antibiotic resistance is known as a serious public health issue. Antibiotic resistance in GBS strains is a primary cause of newborn illness globally. According to estimates from all across the world, 18% of pregnant women carry GBS in their genitourinary tracts [75]. Since all GBS isolates are thought to be consistently sensitive to all b-lactams.Penicillin, especially penicillin G, is the first line medication for intrapartum antibiotic prophylaxis and for treating *S. agalactiae* infections in either newborns or adults. Except for the introduction of extremely uncommon isolates with a lower susceptibility to penicillin, most b-lactams and GBS clinical isolates are still fully sensitive to penicillin on a global scale. For the prevention or treatment of *S. agalactiae* infections, penicillin G and ampicillin are the preferred medications while erythromycin or clindamycin are suggested options for individuals who are allergic to b-lactams [76] . Additionally, combination of medicines that provide synergistic results are a viable approach that might aid in preventing the future development of antibiotic resistance. Natural antimicrobials may prove to be effective alternatives to antibiotics. Additionally, bacteriophage are used for treatment of GBS infections during pregnancy is a strategy that might be very helpful in lowering the number of GBS infections [78].

2.5 Target Virulence Factor

There are 2 different types of proteins which are used as virulence factors for molecular docking process such as β-hemolysin/cytolysin also known as CylE and

C5a peptidase.

2.5.1 β-hemolysin/cytolysin

Streptococcus agalactiae virulence factor β-hemolysin/cytolysin (β-H/C) facilitates Streptococcus translocation across the epithelial barrier. In addition to encouraging bacterial invasion across epithelial and endothelial barriers, including the blood-brain barrier, β-H/C triggers cytolysis in eukaryotic cells. Independent of bacterial ascent, β-H/C causes premature birth and placental inflammation in mice. Pneumonia, sepsis, and neonatal meningitis are only a few examples of the different in vivo models where β-H/C-deficient GBS exhibit decreased pathogenicity [79] . The GBS β-hemolysin/cytolysin (β-h/c) is a virulence factor that might be involved in this process of pathogenicity. The majority of GBS clinical isolates generate this toxin that is surface-associated. When cloned in Escherichia coli, the cylE gene from the GBS chromosome is enough to impart β-h/c hemolysis and is necessary for production of β-h/c. Additionally, sub cytolytic doses of β-h/c can promote GBS penetration in lung epithelial cells and cause the neutrophil chemokine interleukin-8 to be released (IL-8). An important component of human surfactant, the phospholipid dipalmotyl phosphatidylcholine (DPPC) blocks GBS β-h/c mediated cytotoxicity, invasion, and IL-8 activation suggesting a possible connection between premature, surfactant-deficient neonates' increased susceptibility to sepsis and lung damage from GBS infection. The β-h/c toxin is capacity of the pathogen to reach the lung barrier, create bacteremia and cause substantial mortality in GBS-induced pulmonary dysfunction [80] .

2.5.2 C5a Peptidase

Streptococcal C5a peptidase B is commonly known as C5a peptidase is a 120 kDa GBS surface protease, adhesin and invasin produced by all GBS serotypes. Group B streptococcus (GBS) is a prevalent cause of serious illness in infants. The human complement (C3) and complement-derived chemotactic factors are

cleaved and subsequently rendered inactive by the highly conserved surface-bound serine protease known as C5a. Despite decades of research, C5a peptidase has been unsuccessfully targeted for vaccine development due of the significant roles it plays in pathogenicity and notably increase its survival in the host [81] .

2.6 Molecular Docking

Molecular docking is a technique for structure-based drug design that simulates molecular interaction and forecasts the binding mechanism and affinity between receptors and ligands. This method has been extensively employed in the field of drug designing research in recent years. In addition to making, it easy for researchers to buy, manufacture and finish further pharmacological experiments using the compounds database to screen possible pharmacophores also significantly increases efficiency and lowers research costs. The development of reverse molecular docking technology could also considerably increase the ability to forecast therapeutic targets and understand the associated molecular mechanisms for drug design [82] .

Moleculer docking is a technique used to estimate the strength of a bond between a ligand and a target protein through a special scoring function and to determine the correct structure of the ligand with in the target binding sites. It also help in recognition of new small compound, revealing the essential properties such as high interaction between binding with target protein having reasonable absorption, distribution, metabolism, excretion and toxicity which help in the selection of a lead for the target [83] . The target protein was CylE and C5a peptidase and ligands were acetate, propionate, butyrate, formate, lactate, isobutyrate, valerate, caproate and octanoic acid selected for the molecular docking process for current research.

Chapter 3

Research Methodology

3.1 Methodology Flowchart

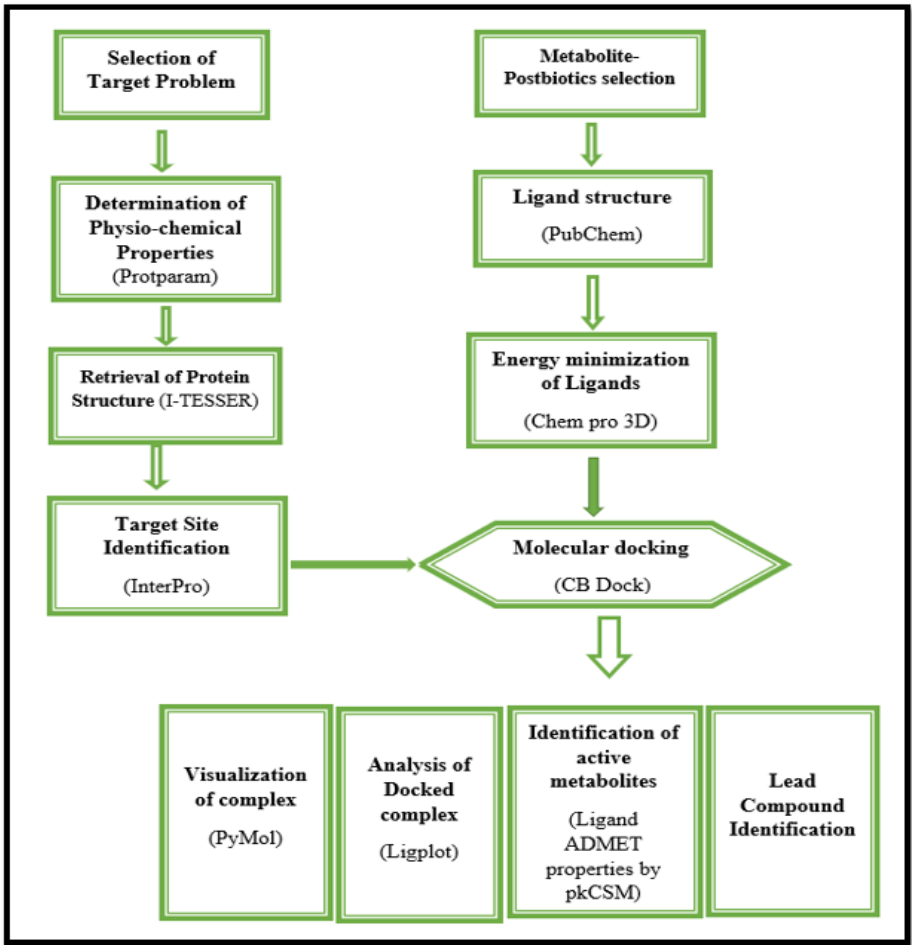


FIGURE 3.1: The flowchart of research methodology.

3.2 Selection of Problem

Neonates are commonly thought to be more susceptible to pathogens because of neonatal immaturity, immune tolerance or immune deviation, a developmentally controlled transitional state. Group B *streptococcus* (GBS) is one of the most important pathogens in neonatal infections that affect preterm infant babies during early days of life.

It commonly causes meningitis and sepsis in neonates after few days of birth. The pore-forming toxin of GBS β-hemolysin/cytolysin also known as CylE encoding cylE gene is a key virulence factor inside host phagocytic cells as 90.5% frequency rate and C5a peptidase is an adhesion factor with a 75% frequency rate demonstrated in several in vivo models [73], [84].

3.3 Target Virulence Factor Selection

Group B Streptococcus (GBS) is a common commensal bacterium in adults but it is also an important cause of invasive bacterial infections in neonates in developing countries. The β- hemolysin/cytolysin (β-h/c) is linked with production of an orange-to-red pigment which is a major virulence factor which is used for GBS diagnosis [85]. C5a peptidase is a surface-localized serine protease which cleaves human C5a (a component of the human complement system)and thus leading to reduce neutrophil chemotaxis as well as opsonophagocytic killing [86].

3.4 Primary Sequence Retrieval

The UniProtKB (`http://www.uniprot.org/proteomes`) provides complete genome sequence of more than 84 thousand species. Primary sequence of target virulence factors (CylE and C5a peptidase) were protein in nature were retrieved in FASTA format from protein sequence database UniProt under accession number A0A0H1WEH5 and Q53637 with residues length of 667 and 1150 respectively [84].

3.5 Analysis of Physiochemical Properties of Protein

The ProtParam (http://web.expasy.org/protparam/) tool of ExPASy was used for the analysis of the proteins physiochemical properties obtained from our protein sequence [87]. Protparam was used to predict these properties of CylE and C5a peptidase. These properties including molecular weight, aliphatic index, isoelectric point (pI), Extinction coefficients and GRAVY (Grand Average of Hydropathy) etc. were analyzed through this tool [88].

3.6 3D Structure Prediction of Virulence Factor

I-TASSER is a hierarchical protocol used for the prediction structure of proteins and annotating their functions on the bases of their structures. After generating full-length atomic structural models based on multiple threading alignments, iterative structural assembly simulations and refinement at atomic level, I-TASSER refines the structure at atomic level using the amino acid sequence of targeted proteins.

On the basis of structure and sequence profile comparison of known protein function databases are used to understand the biological functions of the protein such as ligand-binding sites, enzyme commission numbers and gene ontology terms [89]. The I TESSER server also predict the 3D structure of protein. On the basis of C-score we can select the best 3D structure of proteins.

3.7 Structure Analysis by Use of PyMoL

PyMoL is an open-source molecular graphics tool allows for three-dimension (3D) visualization of proteins, small molecules, nucleic acids, electron densities, surfaces and trajectories. Additionally, it can edit molecules, perform ray tracing and create

movies. This Python-based software is developed with many Pythons plugin tools to enhance its utility and facilitate the drug design process. After downloading the protein structure the extra constitutions attached to the protein needs to be removed such as water molecules and ligands by the using of a PyMol [90].

3.8 Functional Domain Identification of Targeted Virulence Factor

The InterPro database (http://www.ebi.ac.uk/interpro/) is a database used for protein sequences to classify them into families, predict important domains and sites [91].

3.9 Retrieval of Chemical Structure of Postbiotic Ligands

The PubChem database (https://pubchem.ncbi.nlm.nih.gov) contains information about chemical substances and their biological activities. Chemical compounds used as ligands were taken from the PubChem database. The ligands were selected on bases of their inhibitory properties. These includes the acetate, propionate, butyrate, formate, lactate, Isobutyrate, valerate, caproate and octanoic acid.

The chemical structures of all these ligands were retrieved from PubChem database. The selected ligands(acetate, propionate, butyrate, formate, lactate, Isobutyrate, valerate, caproate and octanoic acid) were refines through Chem Draw Ultra version12.0.2 software [92]. The interaction of active pockets of the ligands and the protein were calculated for the interpretation of docking results. Two types of interactions were studied

- one is hydrogen bonding while other one is hydrophobic bonding interaction.

3.10 Energy Minimization of Ligands

Energy minimization of ligands were carried out by the chem pro software (chem 3D v 12.0.2). This is an essential step in the preparation of ligands for docking because unstable ligands will show unreliable vina score in docking results.

3.11 Molecular Docking of Target Virulence Factor

Moleculer docking is a major tool in structural molecular biology and computer-assisted drug designing. Molecular docking is used to predict the dominant binding mode(s) of a ligand with a protein having a known three-dimensional structure is the aim of ligand-protein docking. Acetate, propionate, butyrate, formate, lactate, isobutyrate, valerate, caproate and octanoic acid are the ligands for the target virulence factors CylE and C5a peptide.

CB Dock is an online docking server which automatically identifies binding sites and used to perform docking. It can simplify docking procedure and improve accuracy by predicting target protein binding sites [93].

3.11.1 Process of Molecular Docking

The first step in performing the docking process is to prepare the target protein and ligands files. Firstly, the target protein file was compiled following few steps.PDB file of target protein was given as input into CB dock as an input file. After those amendments the target file was saved in pdbqt.

After compilation of protein file, the ligands files were also prepared by following the same procedure and saved in PDB format. Both prepared files was given as input in CB dock online server which automatically gives vina score, cavity size and grid map [94]. The were studied and Molecular Docking help in find the binding of the ligands.

3.11.2 Active Site Identification

CASTp 3.0 is online server used to provide reliable and comprehensive identifications and quantifications of protein topography. CASTp provide topographic features of biological assemblies along with enhanced visualization of protein structures and pockets and give more comprehensible structural and annotated data such as information on secondary structure, functional sites, variant sites and other annotations of protein residues [95]. It also provides imprints of the negative volumes of pockets, cavities and channels.

3.12 Analysis of Docking Complex

Interactions between proteins and ligands are key role in biological processes in living system. Molecular recognition depends heavily on protein-ligand interactions, which include noncovalent bonding including salt bridges, hydrophobic forces, aromatic stacking, hydrogen bonds and hydrogen bonds. One of the latest techniques used to study protein-ligand interactions is LIGPLOT which display the 3D representation in molecular viewers. Ligplot Plus is a software that generates superimposed interactive 2D protein-ligand interaction diagrams for a few complexes. Additionally, when the two structures are overlapped it shows conserved [96].

3.13 Lead Compound Identification

After the detailed analysis of protein ligand interaction, docking score and toxicity properties studies most active inhibitor was identified. The selected compound was our lead compound [97].

Chapter 4

Results and Discussions

4.1 Structure Modeling

The structure modeling have following steps.

4.1.1 Primary Sequence Retrieval

Primary sequence of target virulence factors (CylE and C5a peptidase) that are protein in nature were taken in FASTA format from UniProt databases (http://www.uniprot.org) under accession number A0A0H1WEH5 and Q53637 with 667 and 1150 residues length respectively. These virulence factors were selected on the basis of pathogenicity and virulence causing factors.
>tr—A0A0H1WEH5—A0A0H1WEH5-STRAG CylE protein OS=*Streptococcus agalactiae* OX=1311 GN=cylE PE=4 SV=1

MKDDNKLKISEASLEDYSEVVHLFNRNHVYQFPDGSPLTVDDLDLTL
EVTHLFLLKNHGVLIGTSAFFKFITYGCLDWNSSFSGFLLISKSRGQA
LYKTILKKITKLKFSNIYTEISNYNKPSLALSKLNGFKEDKTYEDILHC
RSHLPKILNTFRISNYYGKTYDISTFQIMEEIENPLEEETEIRTVSDEEI
AEDSASLPYYLKMSLFQMEIARLDNRYVLQVDFLSEQVKRVRVKTGK
ANLTRAHPSLTLSRFANYYYIQATVETLYGNIDVQLERRKKYRDATIC

TFQGYDLLISPNGSLIFEKQKRKILEDSFLIFSQPLDKKLVVKEEENHITI
YQGALIEKIMTFTSDEEITCVYKCNQKAKEMFPKLLKQTFKLHCQEQL.

>tr—Q53637—Q53637-STRAG C5a peptidase OS=*Streptococcus agalactiae* OX=1311 GN=scpB PE=3 SV=1

MRKKQKLPFDKLAIALMSTSILLNAQSDIKANTVTEDTATEQTVETPQ
EAPSSKETKTPQTPSDGETVADDANDLAPQAPAKTADTPATSKATIRD
QVKTLQEKAGKGAGTVVAVIDAGFDKNHEAWRLTDKTKARYQSEDL
EHGITYGEWVNDKVAYYHDYSKDGKTAVDQEHGTHVSILSGNAPSET
LEGAMPAQLLLMRVEIVNGLADYARNYAQAIRDAINLGAKVINMSFGN
ANLPDETKKAFDYAKSKGVSIVTSAGNDSSFGGKTRLPLDHPDYGVV
ADSTLTVASYSPDKQLTETVRVKTADQQDKEMPVLSTNRFEPNKAYD
GTKEDDFKDVKGKIALIERGDIDFKDKIAKAKKAGAVGVLIYDNQDK
NVDQMPAAFISRKDGLLLKDNPQKTITFNATPKVLPTASGTKLSRFK
KPEQDGSGQTPDKKTETKPEKDSSGQTPGKTPQKGQPSRTLEKRSA
KASTRDQLPTTNDKDTNRLHLLKLVMTTFFLLVAHIFKTKRQKETKK.

4.1.2 Physicochemical Characterization of CylE and C5a Peptidase

Protein physicochemical properties are essential for to be efficient, safe and long-lasting in a biological system. Expasy's ProtParam determine physico-chemical parameters of proteins such as molecular weight, protein content, extinction coefficient, instability index, aliphatic index, atomic composition, theoretical pI, grand average of hydropathicity (GRAVY) to understand stability, activity and nature of protein.

Numerous standalone and web-based programmes are available to calculate the physico-chemical characteristics of proteins. Expasy's web-based AACompIdent programme uses the composition of amino acids to identify protein [97]. The isoelectric point (pI) would be useful when solubility is lowest and mobility is zero in an electro focusing system. Proteins were compact and stable at pI. The value

at isoelectric point (pI 7) should be less than 7. In a test tube protein stability may be estimated by using the instability index. A protein is considered to be stable if its instability index is less than 40 and if it is greater than 40 the protein may be unstable. The proportional volume of a protein occupied by aliphatic side chains (A, V, I, and L) is known as the aliphatic index (AI) and it is considered to enhance the thermal stability of globular proteins. The total hydropathy of all the amino acids divided by the total number of residues in the sequence provides the grand average hydropathy (GRAVY) value for a particular protein. The lower value of GRAVY indicates that proteins may interact with water molecules more efficiently. All these parameters which were selected for this research work were taken according to the previous research work [98]. Table 4.1 & 4.2 shows the physio-chemical properties of CylE and C5a peptidase.

TABLE 4.1: Physiochemical properties of CylE that was determined through ProtParam.

Target Virulence Factor	**CylE**
M.wt	78338.70
PI	8.61
NR	86
PR	94
Ext.Co1	77185
EX.Co2	76560
Instability index	46.67
Alphatic index	87.84
GRAVY	-0.49

TABLE 4.2: Physiochemical properties of C5a peptidase.

Target Virulence Factor	**C5a Peptidase**
M.wt	126310.00
PI	5.79
NR	164

TABLE 4.2: Physiochemical properties of C5a peptidase.

Target Virulence Factor	**C5a Peptidase**
PR	149
Ext.Co1	0.836
Instability index	37.18
Alphatic index	68.01
GRAVY	-0.701

M.wt indicates molecular weight, PI stands for theoretical isoelectric point, NR indicates the total number of negatively charged residues (Asp + Glu), PR for total number of positively charged residues (Arg + Lys), Ext.Co1 indicates extinction coefficients when assuming all pairs of Cys residues from cystines, Ext.Co2 for extinction coefficients when assuming all Cys residues are reduced and GRAVY indicates grand average of hydropathicity. The PI value of CylE was higher than 7 which indicates that it was basic in nature and C5a peptidase PI value was less than 7 which was acidic in nature.

4.1.3 3D Structure Prediction of Proteins

I-TESSER server stands for Iterative Threading ASSEmbly Refinement is an online tool for automated protein 3D structure prediction and structure-based function annotation. I-TASSER uses different threading alignment techniques to initially identify structural templates from the PDB. Following that repeated stimulations of fragment assembly are used to build full-length structural models. The projected structural models are compared to known proteins in the function databases to generate the functional insights. Although the server has been extensively utilized for several scientific and biomedical research. It predicts secondary structure region from amino acid sequences such as the alpha helix, beta sheet and coil. I-TASSER offers a confidence score (C-score) to determine accuracy of the model [99]. I TESSER team mail complete results of job id with 5 best possible models of selective protein and on the basis of C score the best 3D structure model

was selected. The 3D structure of CylE and C5a peptidase were taken in PDB file under job ids respectively.

1. S697942
2. S698144

The proteins structures were prepared and visualized in PyMol by removing the water molecules and other small molecules such as ligands if existed. The energy minimization for structure was performed to get the stable conformation by preventing overlap and saved the modified file in PDB format.

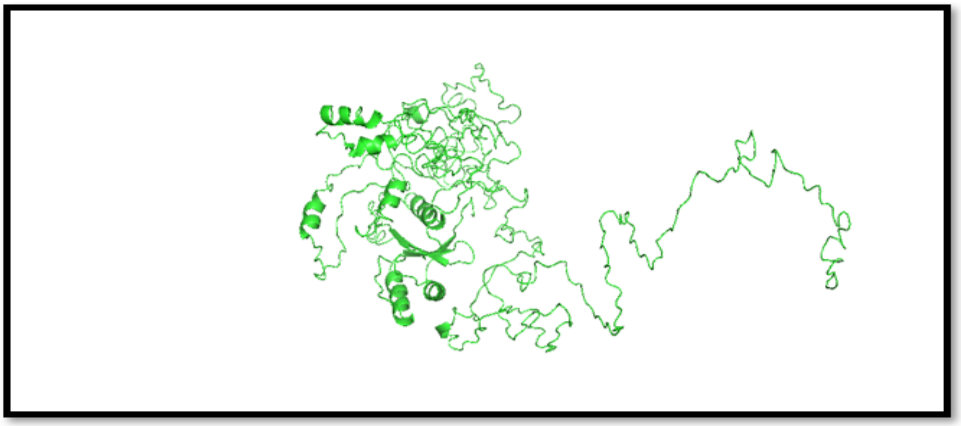


FIGURE 4.1: 3D Structure of virulence factor CylE.

Figure 4.1 representing the three-dimensional structure of virulence factor called β-hemolysin/cytolysin which is also known as CylE protein present in GBS and have a stronger role in pathogenesis in viginally born neonates.

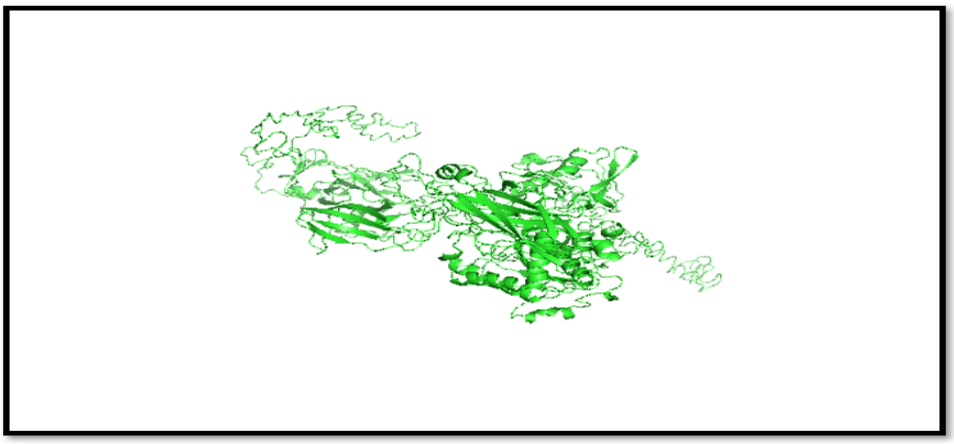


FIGURE 4.2: 3D Structure of C5a peptidase.

3D structure of C5a peptidase virulence factor of *Streptococcus agalactiae* which is encoded by Scp B as shown in figure 4.2. It is an adhesion/evasion factor that was contribute in the infectious diseases of neonates.

4.1.4 Protein Functional Domains Identification

The functional domain is the active part of protein that is involved in the interaction of proteins with other substances. Protein can contain more than one active domain that performs different functions. Functional domain of proteins along with the residue length were shown in figure 4.3 & 4.4 respectively.

The InterPro database (`http://www.ebi.ac.uk/interpro/`) categorized protein sequences into families, identifies functionally significant domains and conserved regions. InterPro Scan underlying tool that enables protein and nucleic acid sequences to be checked against InterPro's signatures. Multiple databases include signatures which are prediction models that define protein families, domains and locations. In order to create a complete database for protein categorization, InterPro combines signatures indicating similar families, domains or sites with details like descriptions, literature references and Gene Ontology (GO) [100]. β hemolysin/cytolysin which is also known as CylE is a 667 a.a long protein consisting of one domain known as Acyl-CoA-acyltransferase starting from residue 8 and ending at 149 respectively as shown in figure 4.3.

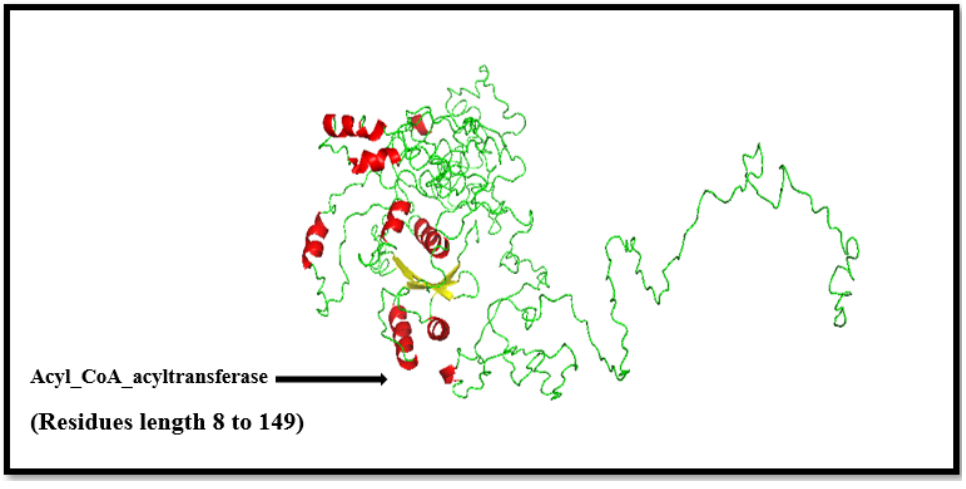


FIGURE 4.3: Functional domain of CylE protein with residues length.

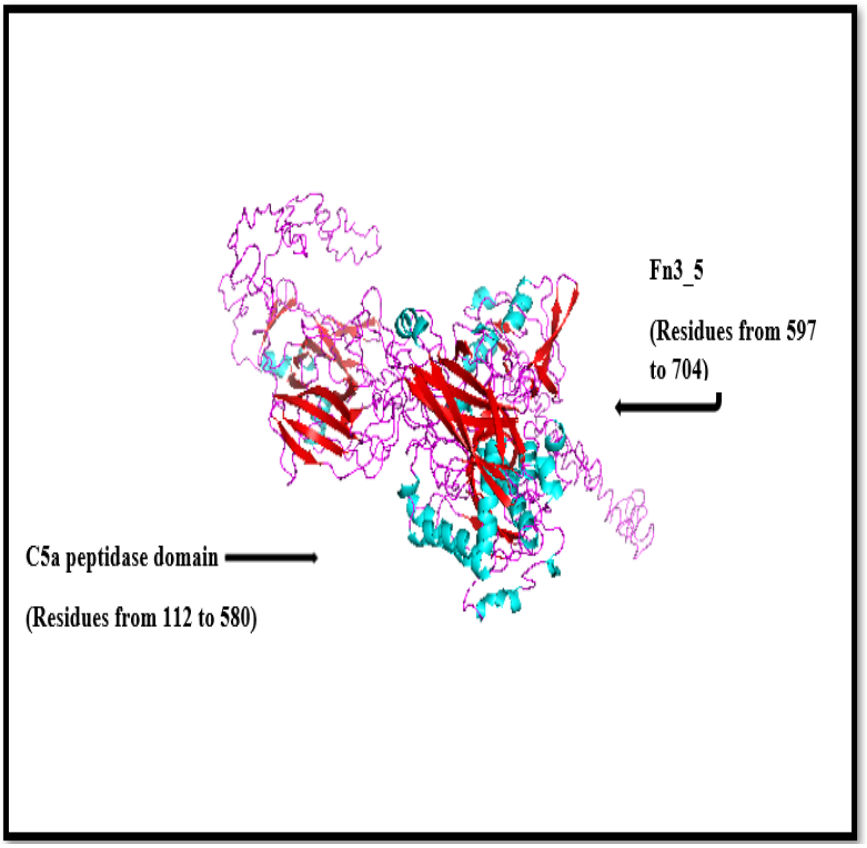


FIGURE 4.4: Functional domains of C5a peptidase with residues length.

Above figure 4.4 representing C5a peptidase with a sequence of 1150 a.a consist of two domains. One is C5A peptidase domain started at residue 112 and ending at 580 while other one is Fn3-5 domain whose residues started at 597 and end at 704 resides respectively.

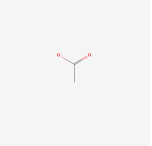
4.2 Ligands Selection

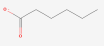
Protein data bank contains a large amount of protein-ligand complex especially for the target protein. Therefore, the selection of ligands is based on the best resolution of the structure, co- crystal ligand bound to the protein structure and best binding affinity. Conformational selection is defined as a process in which a ligand selectively binds to one (or a subset) of these conformers, stabilizing it and increasing its relative population with respect to the total protein population and eventually resulting in the final observed complex. The concepts of ligand-induced

shifts in protein conformational dynamics can be usefully exploited in the context of drug designing and discovery perspectives [101].

PubChem is top public repository for bioassay data by supporting a variety of bioactivity data formats, steadily improving the database architecture, streamlining data submission and adding powerful search, retrieval, analysis and download options. Ligands 3D structures were downloaded from PubChem in SDF formate. After selection of ligands their energy were minimized by chem pro software (chem 3D v 12.0.2) [102]. If we need to select a single protein-ligand complex for structural based drug designing, we need to look at the ligand should be present in the active site. The ligands should bind well to the receptor. Able to adjust the function of proteins and can be able to use as a drug molecules or lead compound. So, on the basis of these properties we can select best ligands. Bioactive metabolites of *Bifidobacteria* which act as inhibitors were selected as ligands for the present study and represented in table 4.3. The selected ligands were acetate, propionate, butyrate, formate, lactate, Isobutyrate, valerate, caproate and octanoic acid, this selection is on the basis of Lipinski rule of five. According to the Lipinski rule of five, a drug-like compound should have a molecular weight (MW) less than 500g/-mol, a log p value should be less than 5 representing its hydrophobicity, hydrogen bond donors (HBDs) is less than 5 and number of hydrogen bond acceptor (HBA) sites should be less than 10 [103]. When a compound violates two or more Lipinski criteria, it is declared poorly absorbed. A compound is termed a drug if it follows three or more rules. So that's why below selected ligands in table 4.3 follow the Lipinski rule of five for the current research work. Selected ligands with molecular formula, molecular weight and structure are represented in the table 4.3.

TABLE 4.3: Ligands and their related properties

S. No	**Ligands Name**	**Molecular formula**	**Molecular weight**	**Structure**
1	Acetate	$C_2H_2O_2^-$	59.04 g/mol	

2	Propionate	$C_3H_5O_2$	73.07 g/mol	
3	Butyrate	$C_4H_7O_2$	87.1 g/mol	
4	Formate	CHO_2	45.017 g/mol	
5	Lactate	$C_3H_5O_3$ $^-$	89.07 g/mol	
6	Isobutyrate	$C_4H_8O_2$	88.11 g/mol	
7	Valerate	$C_5H_9O_2$ $^-$	101.12 g/mol	
8	Caproate	$C_6H_{11}O_2$ $^-$	115.15 g/mol	
9	Octanoic acid	$C_8H_{16}O_2$	185.226 g/mol	

4.3 Molecular Docking

Molecular docking method determined the activity of small molecules (ligands) in a target protein's binding site. Docking against homology-modeled targets also becomes possible for unknown protein structure. Molecular docking has been used more frequently in the drug designing process.

The input for docking is the target proteins and ligands in three-dimensional (3D) structures. Through a specialized scoring function is used to determine the proper

structure of the ligand within the target binding site and measure the strength of the binding between the ligands and the target proteins. A docking score indicates the capacity for binding which approximately represents the sum of all these interactions. A lead chemical for drug design is found by searching for the ligand at the binding site in a six-dimensional rotational or translational space. It also aids in the identification of novel small molecules by identifying crucial characteristics including strong interactions with target proteins and appropriate distribution, metabolism and excretion that help in the selection of lead compound for the target [104]. The docking was performed using target proteins such as β h/c (CylE) and C5a peptidase and ligands were acetate, propionate, butyrate, formate, lactate, Isobutyrate, valerate, caproate and octanoic acid. Ligands with best binding score values with target proteins were represented in table 4.4 & 4.5. We created the user-friendly blind docking web server CB-Dock in order to automatically predict binding modes without information of binding sites. CB-Dock predicts binding sites, calculates the centers and sizes of a given protein using a novel curvature-based cavity detection approach and docks with the well-known docking programme Auto dock Vina [105].

CB dock gives five best interacting conformation of each ligand molecule. These confirmations were arranged based on the binding affinity and then finest confirmation selection was done on the basis of highest affinity score of protein ligand interaction. After docking process, the docked structures were selected for further analysis. On the basis of docking score, cavity size Grid map and binding energy one can select best docking structure. Table 4.4 & 4.5 predicting the docked results of proteins and ligands.

TABLE 4.4: CB dock results along with ligand names, binding score, cavity size and grid map.

S. No	Ligands Name	Binding Score	Cavity Size	Grid Map
1	Acetate	-3.2	10465	33

2	Propionate	-3.7	1107	17
3	Butyrate	-4.3	1107	17
4	Formate	-2.6	3667	18
5	Lactate	-4.2	1261	19
6	Isobutyrate	-4.3	10465	33
7	Valerate	-4.9	1107	17
8	Caproate	-5.4	1107	17
9	Octanoic acid	-5.6	1107	19

TABLE 4.5: CB dock results with ligand name, minimum energy and maximum energy values.

S. No	**Ligands Name**	**Minimum Energy**	**Maximum Energy**
1	Acetate	0.00	1.6E+00
2	Propionate	0.00	1.6E+00
3	Butyrate	0.00	1.6E+00
4	Formate	0.00	1.6E+00
5	Lactate	0.00	1.6E+00
6	Isobutyrate	0.00	1.6E+00
7	Valerate	0.00	1.6E+00
8	Caproate	0.00	1.6E+00
9	Octanoic acid	0.00	1.6E+00

4.4 Active Site Identification

The CASTp server was used to estimate the active sites in the modelled proteins. Active sites of proteins were identified. With the knowledge gained from this study, it will be possible to identify, design and develop medications that are particularly effective against the virulence factors CylE and C5a peptidase in order to reduce the loss caused by GBS in newborns in developing countries as shown 4.6 [106].

TABLE 4.6: Area and volume of binding pocket of CylE obtained by CASTp.

Pocket ID	**Areas (SA)**	**Volume(SA)**
1	1668.51	6583.147
2	674.301	3517.278
3	1611.87	734.213
4	186.738	287.437
5	321.126	224.356
6	235.715	129.653
7	181.077	119.38
8	119.343	54.153
9	99.258	49.905
10	73.691	34.767
11	56.478	31.98
12	63.021	20.18
13	61	16.348
14	27.709	10.87
15	24.673	8.871
16	43.586	6.405
17	8.251	3.436
18	6.608	0.938
19	4.983	0.693
20	3.539	0.265
21	1.339	0.069
22	0.769	0.068

Table 4.6 showing the binding pocket IDs along with area and volume of virulence factor CylE. It represents that there were twenty-two pockets available for protein CylE. The largest binding pocket has surface area 186.738 whereas its volume is 287.437 while the smallest binding pocket has surface area 0.769 and volume 0.068 respectively.

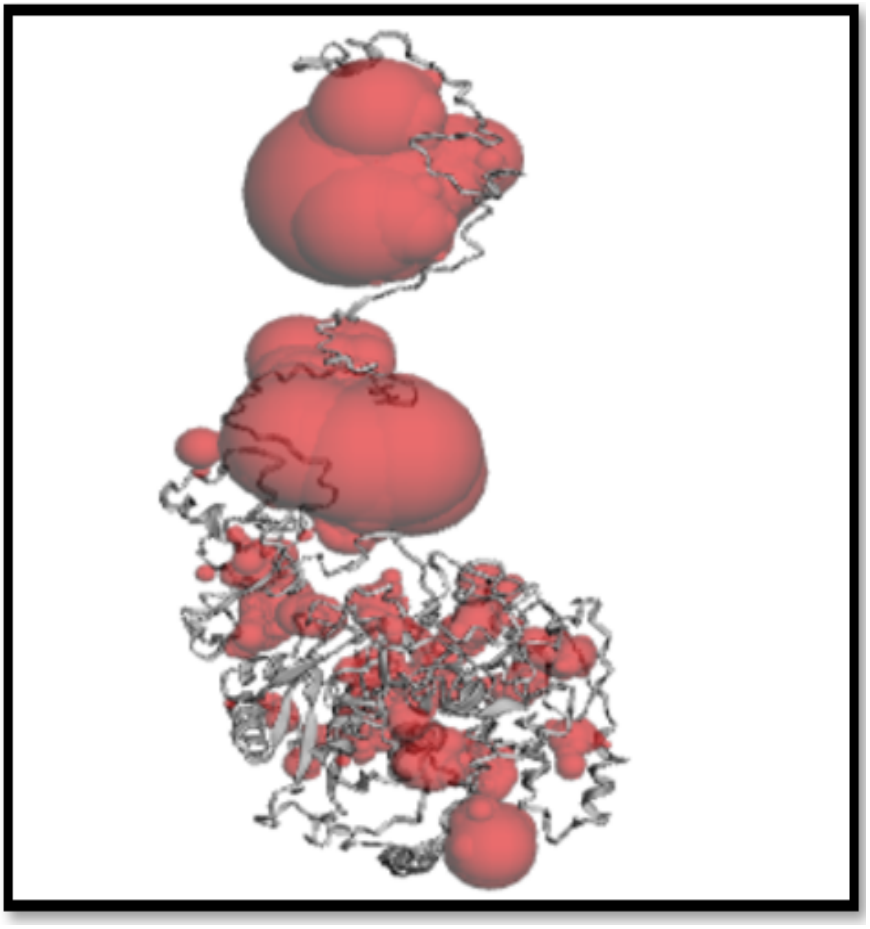


FIGURE 4.5: Structure of CylE protein showing available pockets for ligands.

Figure 4.5 representing CylE protein structure. Red color showing the available binding pocket for protein. Binding pocket is a region where ligands can bind. The number of pockets along with size and volume is already shown in table 4.6.

TABLE 4.7: Area and volume of binding pocket of C5a peptidase obtained by CASTp.

Pocket ID	**Areas (SA)**	**Volume(SA)**
1	7011.27	23365.9
2	238.433	395.41

TABLE 4.7: Area and volume of binding pocket of C5a peptidase obtained by CASTp.

Pocket ID	**Areas (SA)**	**Volume(SA)**
3	80.911	113.66
4	38.03	8.874
5	27.438	8.094
6	23.763	5.144
7	22.428	3.883
8	17.051	3.685
9	14.566	3.105
10	12.568	2.17
11	11.032	1.517
12	10.846	1.506
13	6.898	1.007
14	3.151	0.573
15	1.7	0.18
16	0.819	0.036
17	0.18	0.006
18	0.116	0.001
19	0.112	0.000
20	0.001	0.000

CASTp data predicts one hundred and thirty-two binding pockets for C5a peptidase. Table 4.7 representing only twenty binding pockets with area and volume of C5a peptidase. The largest binding pocket has surface area 7011.270 whereas its volume is 23365.9 while the smallest binding pocket has surface area 0.001 and volume 0.000.

Figure 4.6 representing the C5a peptidase along with volume and surface area. Red color showing the available binding pocket for protein. Binding pocket is a region where ligands can bind. The number of pockets along with size and volume is already shown in table 4.7.

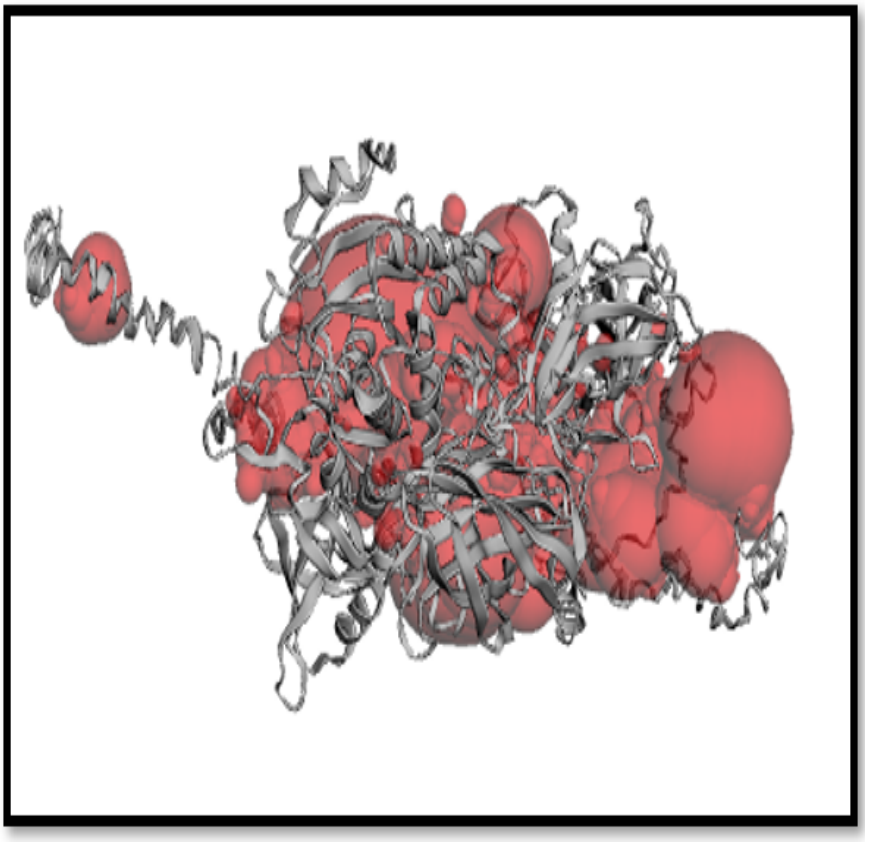


FIGURE 4.6: Structure of C5a peptidase shows the available pockets for ligands.

4.5 Interaction of Ligands and Target Proteins

The interaction of active pockets of the ligands and the protein were calculated for the interpretation of docking results. Two types of interactions were studied, one is hydrogen bonding while other one is hydrophobic bonding interaction. Using Ligplot plus (version v.1.4.5) the protein ligand interaction were studied [107]. By using Ligplot plus the interaction of active conformation of ligands and the target proteins has been identified. The saved conformation for ligand-receptor complex of each molecule were analyzed in detailed. This software automatically generates schematic diagrams of protein ligand interactions of the given ligands in PDB file. The docked files were uploaded in PDB format to get hydrogen and hydrophobic bonding. A significant number of hydrogen and hydrophobic bond interactions were observed between nine ligands and two target proteins. Ligand-receptor complex shows strong hydrogen bonding, hydrophobic interaction and

van der Waal forces [108]. Following diagrams 4.7 to 4.15 representing the ligand-receptor interactions.

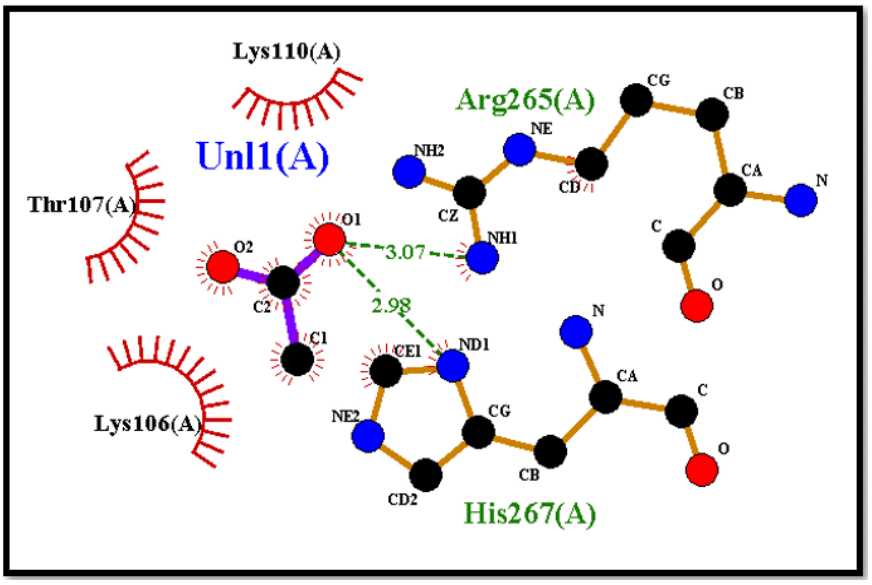


FIGURE 4.7: 2D Representation of docked complex acetate by ligplot plus.

The figure 4.7 predicting the interaction of acetate with receptor protein. It shows acetate made two hydrogen bond and three hydrophobic interactions. The residues involved in hydrogen bonding are His and Arg.

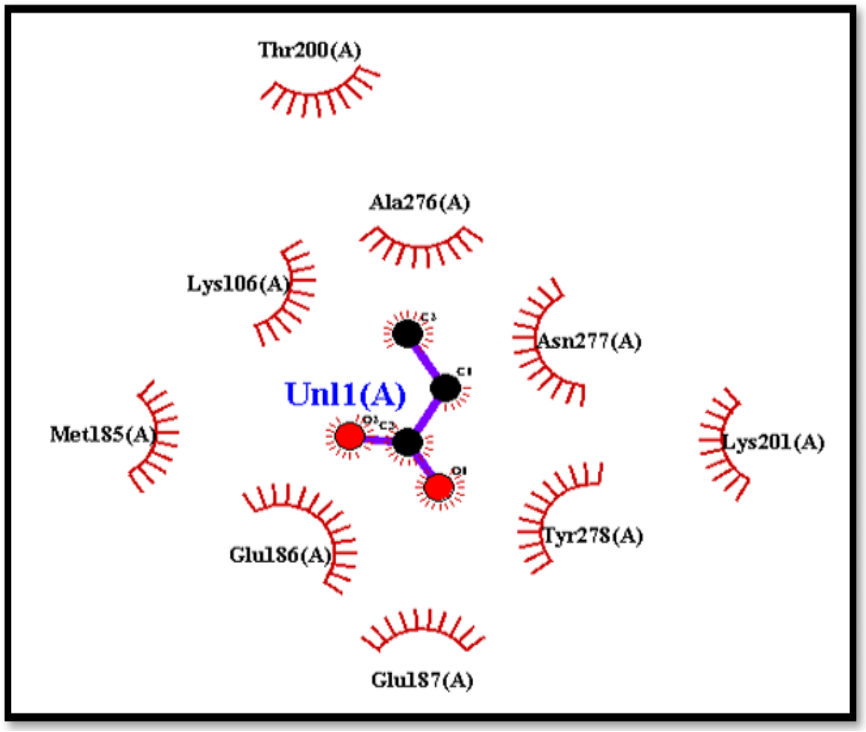


FIGURE 4.8: 2D Representation of docking complex propionate by ligplot plus.

Figure 4.8 showing the interaction of propionate with receptor protein which represents nine hydrophobic interaction and zero hydrogen bond in propionate docked complex.

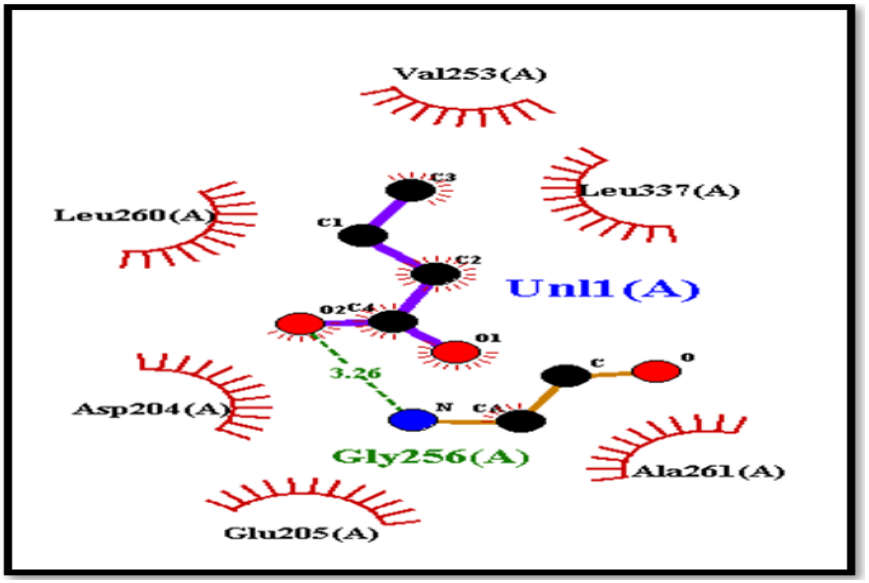


FIGURE 4.9: 2D Representation of docked complex butyrate by ligplot plus.

The docked complex of ligand butyrate made six hydrophobic interaction and one hydrogen bond and residue made hydrogen bond is Gly. Figure 4.9 representing docked complex of butyrate.

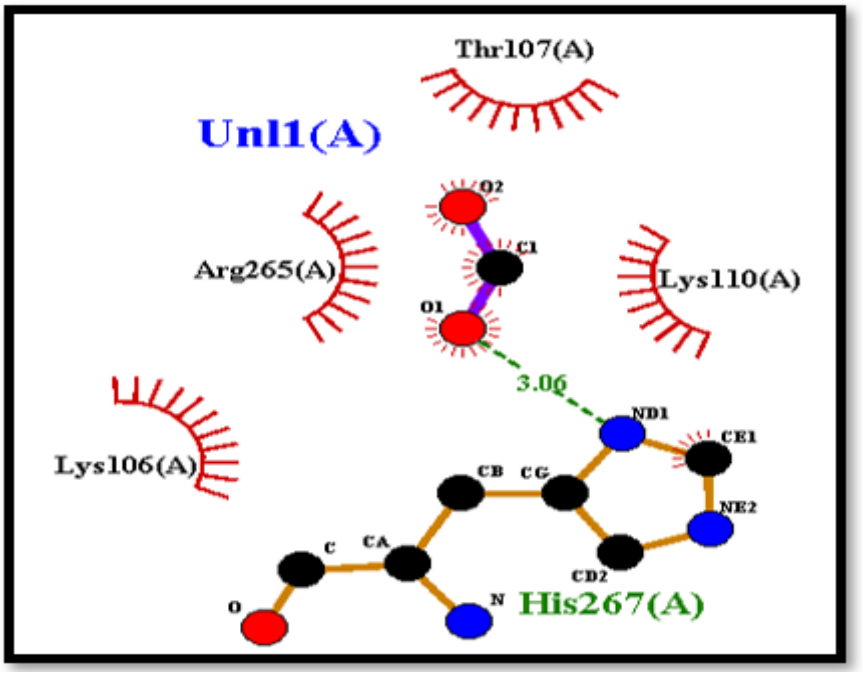


FIGURE 4.10: 2D Representation of docked complex formate by ligplot plus.

Figure 4.10 showing the interaction of formate with receptor protein. It shows formate has formed four hydrophobic interaction and one hydrogen bonds. The residue involve in hydrogen bond formation is His.

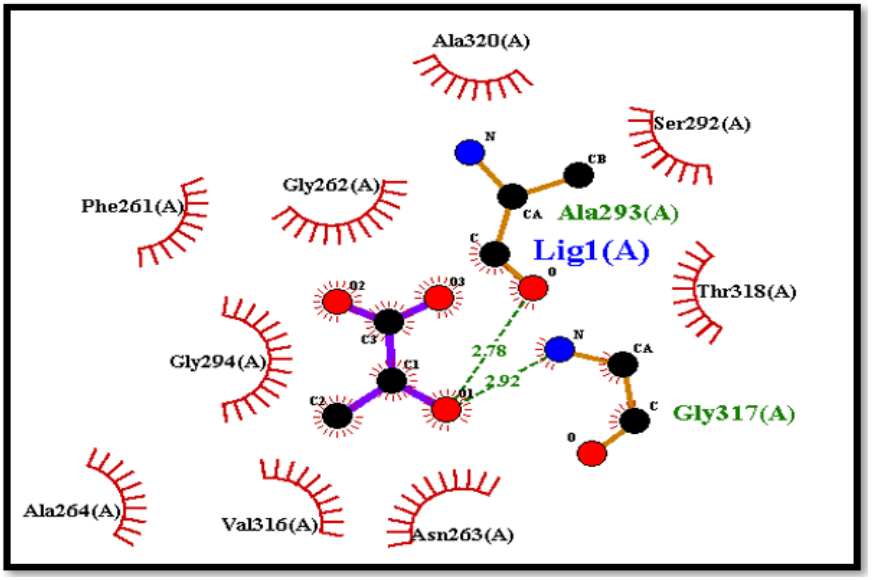


FIGURE 4.11: Representation of docked complex lactate by ligplot plus.

Figure 4.11 representing the lactate ligands has formed 2 hydrogen bond and nine hydrophobic interaction.The residues involved in hydrogen bonding are Ala and Gly and their hydrogen bond distance is 2.78 and 2.92 respectively.

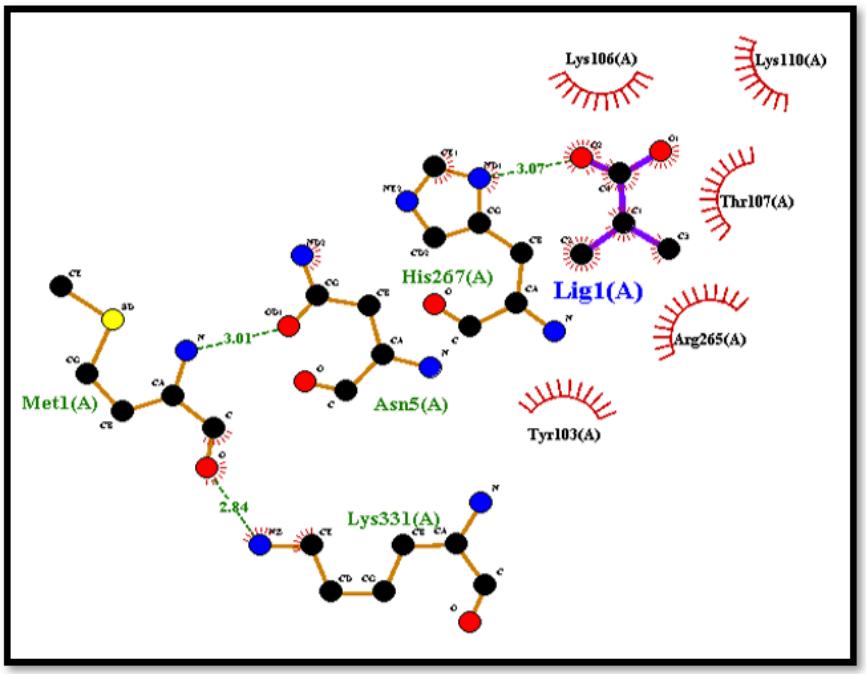


FIGURE 4.12: 2D Representation of docked complex isobutyrate by ligplot plus.

Figure 4.12 shows ligand isobutyrate interactions with receptor protein.The isobutyrate made three hydrogen bond and five hydrophobic interactions.The residue Lys, His and Asn made hydrogen bond along with 2.84, 3.07 and 2.84 distance respectively.

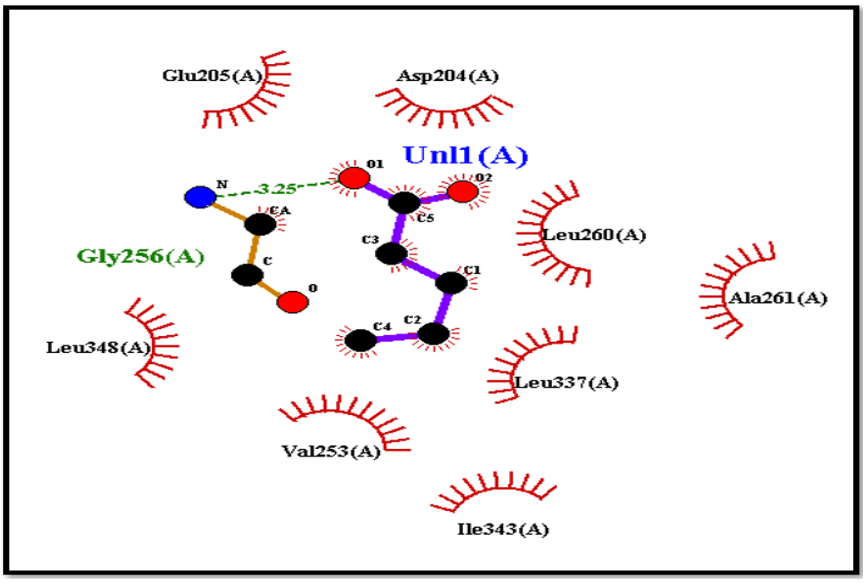


FIGURE 4.13: 2D Representation of docked complex valerate by ligplot plus.

Figure 4.13 showing valerate has made one hydrogen bond and eight hydrophbic interation.

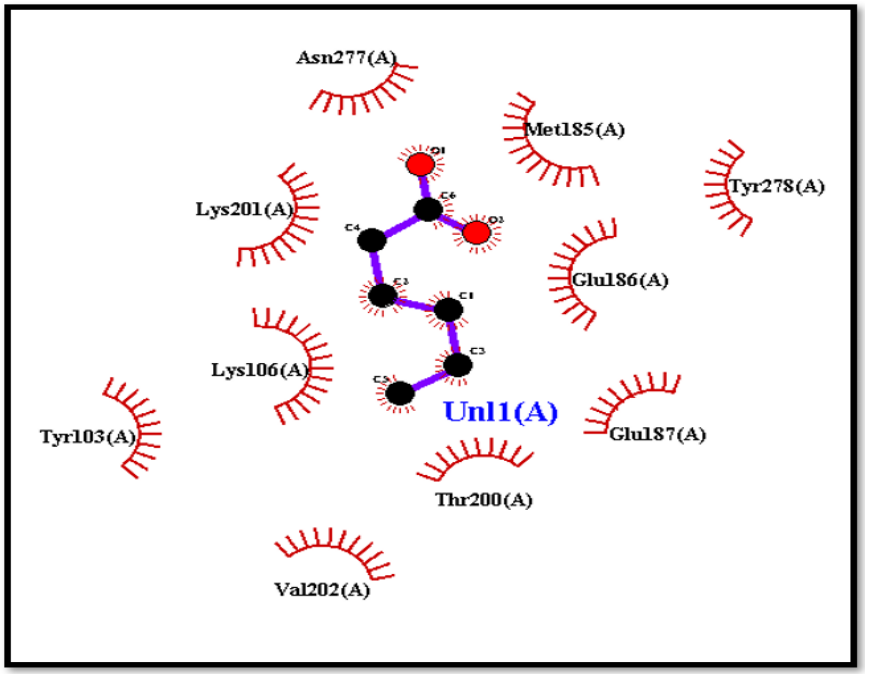


FIGURE 4.14: 2D Representation of docked complex of caproate by ligplot plus.

Figure 4.14 showing caproate has interaction with receptor protein and made zero hydrogen and ten hydrophobic intraction.

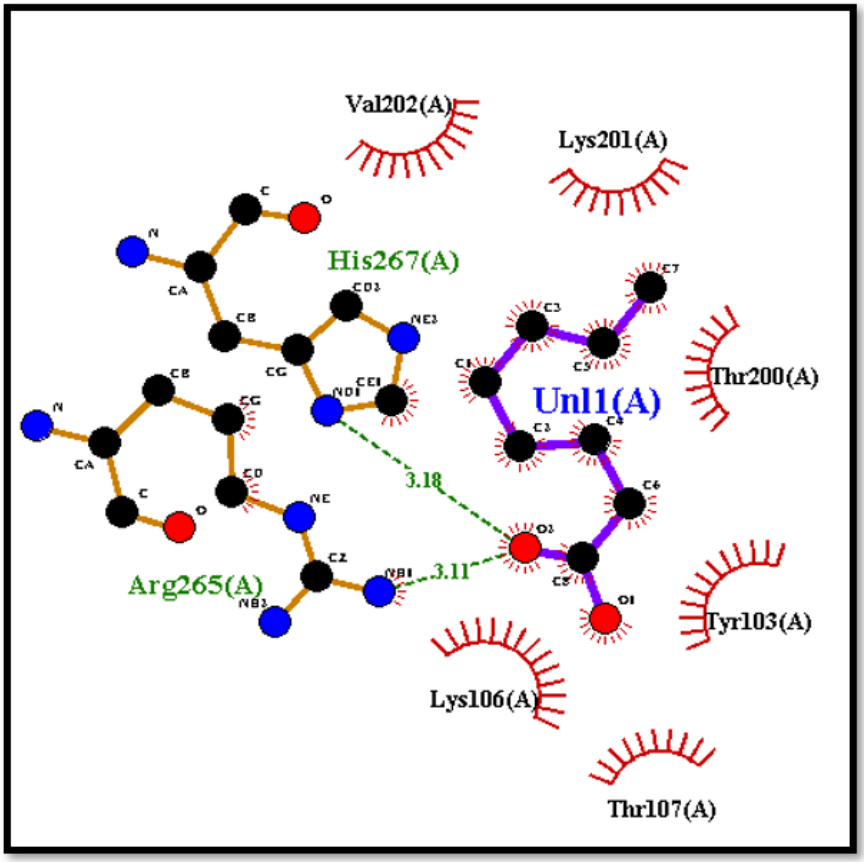


FIGURE 4.15: 2D Representation of docked complex of octanoic acid by ligplot plus.

Figure 4.15 shows octanoic acid interaction with the receptor protein.Otanoic acid formed two hydrogen bond and six hydrophobic interaction.The residues which form hydrogen bond are His267(A) and Arg265(A) with a distance of 3.18 and 3.11 respectively.

TABLE 4.8: Active ligands showing hydrogen and hydrophobic intraction

Sr. #	**Docking Score**	**No. of H-Bond**	**Residues in Molecular Interaction**	**Hydrophobic Residue**
1	-3.2	2	Arg25, His267	Lys110, Thr107, Lys106

TABLE 4.8: Active ligands showing hydrogen and hydrophobic intraction

Sr. #	**Docking Score**	**No. of H-Bond**	**Residues in Molecular Interaction**	**Hydrophobic Residue**
2	-3.7		-	Thr200, Ala267 Lys106, Asn227 Met185, Glu186 Glu187, Tyr278 Lys201
3	-4.3	1	Gly256	Val253, Leu260 Leu337, Asp204 Glu205, Ala261
4	-2.6	1	His267	Arg256, Thr107 Lys110 Gly262, Gly294, Phe261,
5	-4.2	2	Ala293, Gly317	Ser292, Thr318, Gly317 Ala264, Val316 Asn263

TABLE 4.8: Active ligands showing hydrogen and hydrophobic intraction

Sr. #	**Docking Score**	**No. of H-Bond**	**Residues in Molecular Interaction**	**Hydrophobic Residue**
6	-4.3	3	His267, Asn5, Lya331	Lys106, Lys110 Thr107, Arg265,Tyr103
7	-4.9	1	Gly256	Glu205, Asp204, Leu260, Ala261 Leu337, Leu348 Val253, Ile343
8	-5.4		-	Asn277, Met185 Lys201, Tyr278 Glu186, Lys106, Tyr103, Glu187 Thr200 Val202,
9	-5.6	2	His267, Arg265	Lys201, Thr200, Tyr103 Lys106, Thr107

Intractions between receptor proteins and ligands shows the presence of hydrogen bond and hydrophobic intractions.Acetate was bind to Arg25 and His267 residues of CylE and form hydrophobic intraction with Lys110,Thr107 and Lys106 residues respectively.Compound propionate form hydrophobic intraction with Thr200, Ala 267, Lys106, Asn227, Met185, Glu186, Glu187,Tyr278 and Lya201 respectively. Butyate formed one hydrogen bond through Gly256 and formed hydrophobic intraction with Val253,Leu260, Leu337, Asp204, Glu205 and Ala261 residues.

Formte compound was bind to His267 residues of receptor protein residues and form hydrohobic intraction with Leu337, Asp204, Lys106 and Lys 110 respectively.The compound lactate bind to Ala293 residues of CylE protein and fomed hydrophobic intraction with Gly262, Gly294, Phe261,Ser292,Thr318,Gly317,Ala264, Val316 and Asn263 respectively. The compound isobutyrate bind to His267, Asn5 and Lya331 residues of CylE protein residues and formed hydrophobic intraction with Lys106, Lys110, Thr107, Arg265 and Try103.

The compound valerate bind with Gly256 and formed hydrophobic intraction Glu205, Asp204, Leu260, Ala261, Leu337, Leu348, Val253 and Ile343 respectively. Caproate formed hydrophobic intractions with Asn277, Met185, Lys201, Tyr278, Glu186, Lys106, Tyr103, Glu187, Thr200 and Val202. The compound octanoic acid bind with His267, Arg265 and formed hydrophobic intraction with Val202, Lys201, Thr200,Tyr103, Lys106 and Thr107 respectively.

4.6 ADMET Properties of Ligands

The Lipinski's Rule of Five (ROF) is a general guideline for evaluating the likeness of a chemical molecule to a medicine or determining if it has characteristics that would make it likely to be an orally active drug in human [109]. High potency, affinity and selectivity against the molecular target, along with adequate absorption, distribution, metabolism, excretion and tolerable toxicity (ADMET) are all characteristics for effective and safe medications. Safe and effective drugs contain well defined combination of pharmacodynamics (PD) and pharmacokinetics [110].

4.6.1 Pharmacodynamics

Pharmacodynamics is the branch of pharmacology in which we study the effect of drugs on the body.

4.6.2 Pharmacokinetics

In pharmacokinetics we study how drug passes through body and it has five propeties absorption,distribution, metabolic, excretion and toxicity of drug.

4.6.3 Absorption Properties of Ligands

In pharmacology specifically pharmacokinetics, the transfer of a drug from the bloodstream into the tissues is called absorption. So the chemical composition of a drug as well as the environment into a drug is placed work together to determine the rate and extent of drug absorption. A medicine must passed through celluler barriers such as epithelial or endothelial cells in order to be absorbed. Most medications on the other hand pass through celluler barrier by passive diffusion in which they travel from a high concentration area to a low concentration by diffusing through cell membranes.This sort of drug movement does not involve any energy expenditure but it is controlled by the drug size and solubility [111].

The ROF considers drug like properties and ADMET properties include absorption, distribution, metabolism, excretion and toxicity of the molecules. The specified level 0 for absorption property confirms that molecules have good intestinal absorption. Similarly the solubility level 3 confirms good solubility of the compounds. The Blood brain barrier (BBB) level was specified 3 which ensure the low level penetration of the compounds in brain cells [112]. The water solubility and skin permeability of all ligands is low while Caco2 permeability ia normal and intestinal absorption of all ligands is more than 90% and acetate and propiotate is 100%. Acetate, propionate, butyrate and formate showed the positive value for p-glycoprotein and remaining ligands showed negative value. If a compound

is positive for Pgp substrate then its means that it can be easily pumped out of the cells to reduce its absorption.Absorption properties of ligands were shown in tables 4.9, 4.10 & 4.11.

TABLE 4.9: Absorption properties of ligands.

S. No	**Ligands Name**	**Water Solubility**	**$CaCO_2$ Permeability**
1	Acetate	0.728	1.439
2	Propionate	0.376	1.443
3	Butyrate	-0.193	1.444
4	Formate	0.929	1.436
5	Lactate	0.39	1.016
6	Isobutyrate	-0.216	1.576
7	Valerate	-0.79	1.446
8	Caproate	-1.387	1.448
9	Octanoic acid	-2.198	1.565

TABLE 4.10: Absorption properties of ligands.

S. No	**Ligands Name**	**Intestinal Absorption**	**Skin Permeability**
1	Acetate	100	-2.851
2	Propionate	100	-2.329
3	Butyrate	99.361	-2.797
4	Formate	100	-3.273
5	Lactate	90.707	-2.735
6	Isobutyrate	90.797	-2.588
7	Valerate	97.622	-2.773
8	Caproate	99.123	-2.753
9	Octanoic acid	91.705	-1.757

TABLE 4.11: Absorption properties of ligands.

Ligands	**P Glycoprotein Substrate**	**P Glycoprotein I Inhibitor**	**P Glycoprotein II Inhibitor**
Acetate	Yes	No	No
Propionate	Yes	No	No
Butyrate	Yes	N0	N0
Formate	Yes	No	N0
Lactate	No	No	No
Isobutyrate	No	No	No
Valerate	Yes	No	No
Caproate	No	No	No
Ooctanoic Acid	No	No	No

4.6.4 Distribution Properties of Ligands

In pharmacology distribution is the branch of pharacokinetics which deals with the movement of drug within the body from one location to another location.When drug enters the sysytem circulation by absorption , it must be distributed into interstitail and intracelluler fluids [113]. There were four distribution properties of selected ligands, volume of distribution in human, Fraction unbound in humans (Fu),permeabilty of blood brain barrier (BBB) expressed as log BB and log PS exprresed the permeabilty of central nervous system.

VDss is considered low if it is less than 0.71 L/kg and if it is higher than 2.81 L/kg it is considered high.If VDss is high it means that most of the drug is still distributed in tissue than to plasma.If a compound shows more values than it is more effective. BBB pretect the brain from exogenous compounds so BBB is an important property. If the predicted value of log BB greater than 0.3 then

it means given substance can cross the BBB and if its value is less then -1 then it has no harm to brain.Log PS is the product of blood barain permeability and surface area and if its value is greater then 2 it is considered to penetrate into the central nervous system and less the -3 considered to be safe.The VDss value of all ligands is low and Fu value of all ligands is in positive number.The BBB value of all ligands is starting from -0 and log PS value of all ligands in a range of -2 so all the ligands does not penetrate into the central nervous system. Table 4.12 & 4.13 shows the distribution properties of selected ligands.

TABLE 4.12: Distribution properties of ligands.

S. No	**Ligands Name**	**VDss Humaan (L/kg)**	**Fraction Unbound Human(Fu)**
1	Acetate	-0.642	0.788
2	Propionate	-0.78	0.748
3	Butyrate	-0.853	0.71
4	Formate	-0.656	0.833
5	Lactate	-0.703	0.805
6	Isobutyrate	9-1.033	0.655
7	Valerate	-0.855	0.665
8	Caproate	-0.844	0.614
9	Octanoic acid	-0.844	0.462

TABLE 4.13: Distribution properties of ligands.

S. No	**Ligands Name**	**BBB Permeability Humaan (LogBB)**	**CNS Permeability (logPS)**
1	Acetate	-0.329	-2.514

2	Propionate	-0.301	-2.44
3	Butyrate	-0.266	-2.362
4	Formate	-0.345	-2.465
5	Lactate	-0.383	-3.031
6	Isobutyrate	-0.275	-2.311
7	Valerate	-0.226	-2.281
8	Caproate	-0.179	-2.206
9	Octanoic acid	-0.217	-2.252

4.6.5 Metabolic Properties of Ligands

Metabolism is a process of converting one compound into another with the help of enzyme. Mostly metabolic reactions occour in the plasma of blood, liver, intestine and lungs. Metabolic process will convert the drug into more water soluble compound by increaing its polarity. The cytochrome enzymes play a major role in the metabolism of drugs for biotransformation and elimination. Drug-drug interactions have been linked to activate or inhibit CYP enzymes and administration of two or more medications runs the risk of the drug accumulation at dangerous levels owing to CYP enzyme inhibition or being promptly eliminated due to CYP microsomal enzyme activation.

P-glycoprotein is the primary cause of drug resistance and decreased cell susceptibility to medicines in the medical sector. The cytochrome enzymes play a major role in the metabolism of drugs for biotransformation and elimination. P-glycoproteins are the primary cause of drug resistance and decreased cells susceptibility to medicines in the medical flied. P-glycoprotein is primarily associated with activating P-glycoprotein which enhance drug efflux and lowers drug concentrations than minimal which might result in therapeutic failure [114]. All these ligands were the isomers of CYP 450 which is an cleaninsing enzyme found in liver. Lactate, isobutyrate and octanoic acid were shows postive value for the CYP2D6 but the remaining ligands showed negative value. CYP3A4, CYP1AC2,

CYP2C19,CYP2C9,CYP2D6 and CYP34A inhibitors all showed negative value for all the ligands. Table 4.14 & 4.15 illustrates metabolic properties of ligands.

TABLE 4.14: Metabolic properies of ligands.

Ligand Name	**CYP-2D6 Substrate**	**CYP-3A4 Substrate**	**CYP-1A2 Inhibitor**	**CYP-2C19 Inhibitor**
Acetate	No	No	No	No
Propionante	No	No	No	No
Butyrate	No	No	No	No
Formate	No	No	No	No
Lactate	Yes	No	No	No
Isobutyrate	Yes	No	No	No
Valerate	No	No	No	No
Caproate	No	No	No	No
Octanoic Acid	Yes	No	No	No

TABLE 4.15: Absorption properties of ligands.

Ligands	**CYP-2C9 Inhibitor**	**CYP-2D6 Inhibitor**	**CYP-3A4 Inhibitor**
Acetate	No	No	No
Propionate	No	No	No
Butyrate	No	N0	N0
Formate	No	No	N0
Lactate	No	No	No
Isobutyrate	No	No	No
Valerate	No	No	No
Caproate	No	No	No
Ooctanoic Acid	No	No	No

4.6.6 Excretion Properties of Ligands

The organ involved in drug excretion are kidney which play important role in excretion (renal excretion) and liver involve in biliary excretion. Other organs may also involved in excretion such as lungs for volitile or gaseous agents. Drugs also excreated in sweat, sliva and tears. Models of Excretion properties are Total Clearance expressed in log (CL tot)in ml/min/kg and second one is Renal OCT2 substrate which predicts results as Yes or No. The total Clearance of Octanoic acid is 1.48 and other ligands value started from 0. The Rnal OCT2 substrate value of all ligands is negative. Excretion properties are represented in table 4.16.

Table 4.16: Excration properties of ligands.

Ligand Name	**Total Clearance (ml/day)**	**Renal OCT2 Substrate**
Acetate	0.685	No
Propionante	0.433	No
Butyrate	0.453	No
Formate	0.619	No
Lactate	0.74	No
Isobutyrate	0.322	No
Valerate	0.475	No
Caproate	0.499	No
Octanoic Acid	1.48	No

4.6.7 Toxicity Properties of Ligands

PkCSM is an onilne tool used to find the ADMET (Absorption, Distribution, Metabolic, Excretion and Toxicilty) properties of bioactive compounds and drugs. The maximum tolerated dose (MRTD) provides a measures of toxic chemical on individuals. This will help in directing the first recommended dose of the treatment

regimen in phase 1 in clinical trails. MTRD is expressed in the form of logarithms (log mg/kg/day). In given compound MRTD is less than or equal to 0.47 log (mg/kg/day) is considered low and if its value is higher than 0.47(mg/kg/day) then it is considered high.Table 4.17, 4.18 & 4.19 shows the toxicity properties of ligands.

TABLE 4.17: Toxicity properties of ligands.

Ligands Name	**AMES Toxicity**	**Max Tolerated Dose**	**hERG Inhibitor**	**hERGII Inhibitor**
Acetate	No	1.071	No	No
Propionante	No	0.839	No	No
Butyrate	No	0.735	No	No
Formate	No	1.139	No	No
Lactate	No	1.927	No	No
Isobutyrate	No	0.414	No	No
Valerate	No	0.605	No	No
Caproate	No	1.594	No	No
Octanoic Acid	No	0.418	No	No

The hERG I and II inhibitor model is reported to generate chronic QT syndrome and fetal ventriculer arrhythmia by inhibiting pottasuim channels induced by the hERG channels. The amount of a substance that kills 50% of experimental animals such as mice is known as the LD50.The LD50 (mol/kg) predicts toxicity of a probable compound with a significance adverse effect.Exposure to low to moderate chemical doses for a long time is very important in medicine and expressed in a log (kg/mg-bw/day). Hepatotoxicity reveals drug induced liver damage and is a major safety concern for the drug development.Skin sensitivity is a potentail adverse effect of skin care and applied products. *T.pyriformis* is a protozoan bacterium, whose toxin is often used as atoxin and point (IGC50) and inhibit 50% growth. If IGC50 value in log ug/L is greater then 0.5 log ug/ L is considered toxic. The lethal

concentrations (LC50) represent the concentration of molecules needed to cause the death of 50% of Flathead Minnows. In minnow toxicity LC50 value below 0.5-0.3 Mm are regarded as acute toxicity [115]. The maximum tolerated dose of all the ligands is high expect octanoic acid.All ligands hERG I and II inhibitors values is negative.Hepatotoxicity value of all the ligands is No.Isobutyrate,Valerate and caproate shows the yes for skin sensitisation. *T pynformis* toxicity value of all ligands is less than -0.5 log ug /L except lactate. Minnow toxicity value of all ligands is more then 0.5 mM which is considered safe.

TABLE 4.18: Toxicity properties of ligands.

Ligands Name	**Oral Rat Acute Toxicity (LD50)**	**Oral Rat Chronic Toxicity (LOAEL)**	**Hepatotoxicity**
Acetate	1.863	0.864	No
Propionante	1.841	0.957	No
Butyrate	1.758	1.403	No
Formate	1.985	0.787	No
Lactate	1.409	2.69	No
Isobutyrate	1.702	2.654	No
Valerate	1.669	1.12	No
Caproate	1.594	1.188	No
Octanoic Acid	1.526	2.603	No

TABLE 4.19: Toxicity properties of ligands.

Ligands Name	**Skin Sensitisation**	***T.pynformis* Toxicity**	**Minnow Toxicity**
Acetate	No	-0.869	3.052
Propionante	No	-1.056	2.722
Butyrate	No	-1.013	2.431
Formate	No	-0.98	3.169
Lactate	No	0.284	3.23

TABLE 4.19: Toxicity properties of ligands.

Ligands Name	**Skin Sensitisation**	***T.pynformis* Toxicity**	**Minnow Toxicity**
Isobutyrate	Yes	-0.255	2.069
Valerate	Yes	-0.849	2.178
Caproate	Yes	-0.605	1.903
Octanoic Acid	No	-0.02	1.039

4.6.8 Lipinski Rule of Five

For Lipinski score calculations, the ligands in SMILES format was uploaded to pkCSM.The physiochemical properties and Lipinski Rule of Five were also analyzed by pkCSM. Further analysis was not performed on compound that violate more than two of Lipinski's Rule of Five. So Lipinski's Rule of Five were applied to natural compounds and hence analysis of different ligands of *bifidobacteria* was checked and results were shown in table 4.20 respectively. Lipinski rule of five are as follow:

1. Molecular weight (MW) of drug like compound should be less then 500g/mol.
2. The log P value should be less than 5.
3. Hydrogen bond donors (HBDs) should less then 5.
4. Maximum number of hydrogen bond acceptor (HBA) should be limited to 10.
5. The no of rotatory bond should be limited to 5 .

TABLE 4.20: Applicability of lipinski rule of five.

Ligand Name	**M.wt**	**Log P**	**H-bond Doner**	**H-bond Acceptor**	**Rotateable Bond**
Acetate	59.04	-1.24	0	2	0

TABLE 4.20: Applicability of lipinski rule of five.

Ligand Name	**M.wt**	**Log P**	**H-bond Doner**	**H-bond Acceptor**	**Rotateable Bond**
Propionante	73.07	-0.85	0	2	1
Butyrate	87.09	-0.46	0	2	2
Formate	45.01	-1.63	0	2	0
Lactate	89.07	-1.88	1	3	1
Isobutyrate	88.10	0.72	1	1	1
Valerate	101.12	-0.07	0	2	2
Caproate	115.15	0.31	0	2	4
Octanoic Acid	144.21	2.43	1	1	6

Table 4.20 shows the moleculer weight, hydrogen bond doner, hydrogen bond acceptor, log P and rotateable bonds values of ligands of *bifidobacteria*. These rules are to be followed by orally active compounds.The drug like compound is dependent on the mode of administration. A compound is considered a drug when it follows 3 or more rules and if a compound violates two or more rules it is considered poorly absorbed.

4.7 Binding Interaction of Potential Lead Compound

Physiochemical and pharmacokinetics properties determined the final destiny of compound as drug or non-drug compounds.Physiochemical properties or Lipinski's Rule of Five works as a primary filter and pharmacokinetics studies as secondary filter in screening of potential compound.

Acetate, propionate, butyrate, formate, valerate and caproate do not follow the Lipinski's Rule of Five so all of these compound were knock out during primary

screening. On the basis of binding score, ADMET properties, physiochemical properties and Lipinski's Rule of Five, Octanoic acid was selected as lead compound among others which could inhibit target virulence factors of *S.agalactiae*. Octanoic acid showed highest binding score among other ligands such as -5.6 with target protein and selected as lead compound. It also followed Lipinski's Rule of Five as its log P value was 2.43 and its moleculer weight was 144.21g/mol.It has one hydrogen bond doner, one hydrogen bond acceptor and one rotatory bond and its ADME properties analysis was also good among others. So, all these properties determined the final destiny of Octanoic acid as drug. Currently,there are hundreds of thousands of natural compounds that can be used for screening to find new therapeutic targets.Two compounds namely Isobutyrate and Octanoic acid were identified as hit compound in the current study using virtual screening of nine natural compounds from *Bifidobacteria*. The binding affinity of these compounds with S.agalactiea virulence factors (proteins) was -4.3 and -5.9 and their log P value was 0.72 and 2.43 respectively. Intestinal absorption of octanoic acid was 91.705 while it was low for isobutyrate. Our analysis predicted that octanoic acid showed highest binding affinity with CylE protein among other metabolites.However, these findings revealed that Octanoic acid had demonstrated itself as a promising potential anti-virulence agent against *S.agalactiae* proteins.Consequently, it might be an excellent candidate for drugs to treat bacterial infections in neonaes.So, it is concluded that Postbiotics of octanoic acid have ability to suppress the virulence factor of *S.agalactiae* in neonates.

Chapter 5

Conclusions and Recommendations

The aim of this research was to identified effective postbiotics compound using computational method for the treatment of neonatal *Streptococcus agalactiae* diseases that could be used in near future as an efficient drug. After performing detail literature studies in databases nine ligands were selected for the current research work.

The virulence factors that are protein in nature used for virtual screening were CylE (β hemolycin/cytolycin) and C5a peptidase. CB dock automated version of Auto Dock vina was used for the docking studies.

Protein-ligands interactions were analyzed using Ligplot plus version v.1.4.5. After detailed analysis of their binding score, physiochemical properties, ADMET properties two hit compound were selected namely; octanoic acid and isobutyrate. Physiochemical and pharmacokinetics properties determined the final destiny of compounds as a drug or non drug. Octanoic acid was identified as lead compound by virtual screening results. From the above mentioned physiochemical and pharmcokinetics ADMET properties it is concluded that octanoic acid showed best binding with CylE virulence factor. All the software and tools used in this research are authentic and reliable. So its means that octanoic acid have ability

to bind with the virulence factor of *Streptococcus agalactia* and suppress their virulence.

These finding suggest that octanoic acid, a bioactive compound of short chain fatty acid of *bifidobacteria* which were used as a postbotics could be a pomising choice for the treatment of neonatal *Streptoccous agalactiea* diseases. Further research is needed to explore the exact mechanism of action of postbiotics as well as impact on the neonates body and safety concerns. Furthermore, *bifidobacteria* is commonly used in combinations with other strains of bacteria as a probiotics for the treatment of diseases but this experiment shows that these postbiotics have significant pharmacological properties, making it more interesting and important to investigate the medical effects to treat neonatal diseases. Most previous studies on *Streptococcus agalactiea* diseases have concentrated on in vitro studies but the effect of postbiotics on microbiota must be evaluated in neonates and pregnant mothers. *Streptococcus agalactiae* is also known as group B streptococus (GBS) cause diseases in neonates, we should pay attention to the use of postbiotics as an inhibitor and agonists in the future.Future study will aim on the applications of postbiotics to clinical therapies, production and living.The new finding have the potentail to revolutionize our understanding of group B *Streptococcus* pathogenicity and provide novel anti-streptococcus therapeutic targets.

Octanoic acid follows the lipinski rule of fives.Fom the current research study it is suggested that octanoic acid can be used as an alternate drug treatment for GBS neonatel infections which less side effect in comparsion with synthetic drug.

Future Prospective:

In-silico analysis must be evaluated on animal model on their cell lining for further clinical trial and efficacy must also be evaluated as a potential therapeutics.Octanoic acid follows the lipinski rule of fives is considered as a best drug.Fom the current research study it is suggested that in future octanoic acid can be used as an alternative treatment for GBS neonatel infections with less side effect as comparsion with synthetic drug.

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