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Role of Yeast Postbiotics in Antiaging

by

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I dedicate this thesis to my loving and supportive family and friends who have fully helped me in achieving my life goals.

CERTIFICATE OF APPROVAL

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Abstract

Aging is an inevitable biological process characterized by the gradual decline of various body functions, leading to a wide range of health-related issues and visible signs particularly on the skin. The onset of early aging is increasingly prevalent due to lifestyle diseases and stress, prompting the need for effective, natural interventions. Yeast postbiotics have emerged as promising agents in the fight against aging, primarily due to their robust antioxidant, anti-inflammatory, and immune-modulating properties. This study explores the potential anti-aging properties of yeast postbiotics using an in-silico approach. Nicotinamide and beta-glucans were selected as potential postbiotics and examined for their interactions with key aging-related protein mTORC1. The 3D structure of the target protein and the ligands served as the input for docking. The best ligand was selected based on physicochemical properties, ADME/T analysis, docking score, and lipinski rule. By considering all these parameters beta-glucan was seen to obey all drug-like properties with a docking score of -7.5 against mTORC1. The research also underscores the promising ADME/T profile of beta-glucan, indicating its suitability as a therapeutic agent due to its moderate water solubility, good intestinal absorption, and minimal toxicity. To check further effectiveness of beta-glucan, it was compared with commercially available antiaging drug sirolimus. A comparison of all drug-like characteristics showed that beta-glucan is much better in many aspects than sirolimus. Sirolimus showed a docking score of -4.5 while beta-glucan has -7.5, other pharmacokinetic properties of beta-glucan are also good than sirolimus. So, it is concluded here that beta-glucan can prove itself as a potential anti-aging drug candidate in future therapeutics. The study underscores the need for further research and clinical trials to fully understand the mechanisms of yeast postbiotics and optimize their applications in promoting healthy aging.

Contents

List of Figures

List of Tables

Abbreviations

AChE	acetylcholinesterase
ADMET	absorption, distribution, metabolism, excretion, toxicity
BBB	blood brain barrier
CADD	computer aided drug designing
CB-dock	cavity detection guided blind docking
CYP2D6	cytochrome P450 2D6
GRAVY	grand average of hydropathicity
HBA	hydrogen bond acceptor
HBD	hydrogen bond donor
KEGG	kyoto encyclopedia of genes and genomes
MW	molecular weight
NR	negative residues
PR	positive residues
PDB	protein data bank
CNS	central nervous system
VDss	volume of distribution at steady state

Chapter 1

Introduction

Live bacteria known as probiotics have the potential to improve health when taken in sufficient quantities. They are frequently found in some foods, such as yoghurt and vitamins, and are referred to as beneficial or favorable bacteria. It is thought that probiotics help maintain the equilibrium of gut flora and digestive health. Fermented foods such as kefir, sauerkraut, and yoghurt are common sources [1].

Postbiotics are essential for preserving an equal number of good bacteria in the gut which is essential for digestive and immune system function. They can help regulate bowel movements, improve nutrient absorption, and support the body's defense against harmful pathogens. Probiotics are available in supplement form, but incorporating naturally fermented foods into your diet is a more common and diverse way to introduce these beneficial microorganisms. It's crucial to keep in mind that various probiotic strains may provide unique health benefits, so it can be advantageous to use a range of sources [2].

Yeast probiotics, specifically *Saccharomyces boulardii*, are a type of beneficial yeast used as a probiotic supplement. Unlike bacteria-based probiotics, which are mostly lactic acid bacteria, *S. Boulardii* is a non-pathogenic yeast. It's known for its ability to help restore and maintain the balance of gut microbiota, particularly during and after antibiotic treatment [3].

S. boulardii is thought to function by forming chemicals that prevent the growth of dangerous microbes by fighting with them for binding points in the intestines. Its

ability to prevent or treat a variety of gastrointestinal problems, such as antibiotic induced diarrhoea and Clostridium difficile infections, has been investigated. Although it is a form of yeast, it is not to be mistaken with Candida or other forms of yeast that cause illnesses [4].

The probiotic yeast *Saccharomyces boulardii* is commonly employed to address gastrointestinal problems, such as diarrhea symptoms. Given its genetic similarity to the model yeast *Saccharomyces cerevisiae*, there has been continuous discussion as to whether it should be considered a distinct species or a variant of *S. cerevisiae*. In this context, we examine the salient genetic differences between *S. boulardii* and *S. cerevisiae* in order to comprehend the probiotic's capacity for physiologic adaptation to the host [5]. The ability of *S. boulardii* to withstand harsh gastrointestinal conditions, develop at the ideal temperature, and be viable at low pH levels are critical phenotypic characteristics that determine the species' potential as a probiotic. Its probiotic efficacy is mediated through a number of mechanisms, including immunological modulation, trophic effects, competitive elimination of pathogens, production of antimicrobial peptides, and improved gut barrier function. This summary highlights the multifaceted role that *S. boulardii* plays in modifying the host microbiota and intestinal function, as well as how the yeast contributes to these processes [6].

Antiaging refers to efforts and practices aimed at slowing down or minimizing the effects of aging on the body and skin. While aging is a natural process, certain lifestyle choices and skincare practices can contribute to maintaining a more youthful appearance and overall health. Key aspects of antiaging include healthy diet, hydration, sun protection, regular exercise and stress management. It's worth noting that while these practices can contribute to a healthier lifestyle and may have positive effects on aging, there is no definitive way to stop the natural aging process. Genetics, environmental factors, and individual variations play significant roles in how individuals age [7].

There is no way to stop the natural process of aging, which is characterized by the steady decline of various body functions over time. This may often lead to a wide variety of health-related issues as well as the obvious signs of age, which are

most prominent in the skin. Notably, the use of postbiotics generated from yeast as an anti-aging remedy has attracted an abundance of attention recently because due to the fact that it may help slow down the aging process [8]. Active chemicals produced by microorganisms called postbiotics have been demonstrated to have a wide range of positive health benefits on humans. The immune system being strengthened, the gut flora being in better health, and the manifestation of anti-inflammatory and antioxidant properties are some of these impacts. The purpose of this study proposal is to examine the possible function of yeast postbiotics in slowing down the ageing process, with a focus on their potential applications in the nutritional and cosmetics industries [9].

This discovery has a significant amount of potential in terms of tackling the myriad of issues that are linked with becoming older. As the average lifespan of individuals throughout the globe continues to increase, there is an increasing need for the investigation of novel methods that may improve the quality of life in later years. The effects of aging go beyond simple appearances and may have a negative influence on both one's well-being and their health as a whole; as a result, the potential advantages of yeast postbiotics provide an intriguing new direction for research [10].

If we can get a grasp of the mechanisms by which yeast postbiotics alter the aging process, we may be able to design treatments that not only slow the outward manifestations of aging but also enhance cognitive performance, physical vigor, and general health. The use of these substances in both nutrition and cosmetics provides a comprehensive strategy to combating the aging process, one that takes into account both the interior and exterior aspects of the problem [11].

This research has the potential to ultimately bring about a revolution in the way that we think about and approach aging. It has the potential to pave the way for novel products and strategies that contribute to a healthier and more vibrant aging population, while simultaneously creating opportunities for advancements in the fields of nutrition and cosmetics [12]. In addition, the relevance of this study extends well beyond the consequences it may have for business. The socioeconomic burden that is connected with age- related health problems is expanding as the

world's population continues to age at an alarming rate. We may be able to lessen the load on healthcare systems, lower the expenses of healthcare, and enhance the overall quality of life for older folks if we can determine effective techniques to ameliorate these concerns, such as via the employment of yeast postbiotics [13].

In the field of nutrition, adding yeast postbiotics to dietary supplements and functional meals may provide an approachable and hassle-free method of supporting good aging. Consequently, this could lead to a decline in the occurrence of age-related illnesses and enhance the standard of living for the elderly population. The creation of novel goods within the cosmetics sector may assist people in preserving a healthy and youthful-looking complexion throughout their lives, therefore boosting their sense of self-worth and study proposal lays the groundwork for a thorough investigation into the potential of yeast postbiotics in delaying the aging process, with a dual emphasis on nutrition and cosmetics as the primary areas of investigation [14].

The results of this research have the capacity to fundamentally alter the way we perceive and react to aging, which would be advantageous for both individuals and society as whole If we try to strengthen people's health and well-being in their later years, we can contribute to a future that is healthier, happier, and more sustainable. In addition, the investigation will make a contribution to the ever-developing area of biotechnology as well as the study of how the body ages. We may get a deeper comprehension of the complex dynamic that exists between diet, the composition of one's microbiome, and the process of aging if we investigate the ways by which yeast postbiotics exert their impact on the aging process. This understanding has the potential to open doors to additional discoveries in the investigation of bioactive chemical research and anti-aging tactics, which will not only assist persons who are aging but will also advance the areas of medicine and biology more generally [15].

In addition, the possibility of incorporating yeast postbiotics into nutritional supplements and anti-aging cosmetics shows a paradigm change in the way we think about the aging process. It recognizes that aging is a complex process that involves more than just the appearance of wrinkles and a general loss in one's physical

health. We are able to give more complete treatments to promote healthy and elegant aging if we address the underlying cellular and molecular elements of the aging process [16].

In a nutshell, the findings of this study are likely to have significant repercussions not just for the health and happiness of individuals but also for the advancement of scientific knowledge. It is a tribute to the inventive potential of harnessing nature's resources to battle age-related issues and presents a hopeful route toward a future in which aging is not only endured but rather embraced as a chance for vitality, health, and self-confidence. Moreover, it is a monument to the innovative potential of using nature's resources to combat age-related challenges.

1.1 Problem Statement

Aging is a natural phenomenon with no specific solution to avoid it but the onset of early ageing has become increasingly dominant these days. The impact of lifestyle, diseases, and stress is causing early aging. People are digging into various unnatural therapies to reverse the physical non-aesthetic as well as physiological implications rather than exploring natural bioactive compounds with the same potential.

1.2 Hypothesis

The yeast postbiotics might have an active role in the reduction of early aging.

1.3 Aim and Objectives

This research aims to explore potential yeast postbiotics showing antioxidant properties to control early aging.

This research entails the following objectives:

- To screen yeast postbiotics with anti-inflammatory and antioxidant properties.
- To analyze the interaction between specific yeast metabolites at the desired target.
- To identify the impact of docked metabolites as inhibitory molecules against ageing.

Chapter 2

Literature Review

The article "Using yeast as a technique to find anti-aging substances," discusses the application of yeast to the search for anti-aging chemicals, this source qualifies as both useful and reliable. Anti-aging compounds can be identified with the help of yeast.

2.1 Yeast Postbiotics: Overview and Mechanisms

Yeast postbiotics are a type of component produced by yeast that may have anti-aging benefits. One of the proteins involved in this process is Sirtuin, which is known for its role in regulating cellular health and longevity. Sirtuin proteins have been studied for their potential to protect cells from age-related damage and promote overall health and longevity. Research on yeast postbiotics and Sirtuin proteins is ongoing and may provide further insight into their potential as anti-aging agents. Sirtuin proteins are NAD+-dependent deacetylases, meaning they require the coenzyme NAD+ as a substrate. The interaction between Sirtuins and NAD+ is essential for their enzymatic activity and their involvement in regulating cellular health and longevity, thus potentially contributing to anti-aging processes [17].

In addition to NAD+, there are other ligands that have been found to interact with Sirtuin proteins, such as small molecule activators like resveratrol. Red wine

contains a substance called resveratrol, which has been investigated for its capacity to activate Sirtuins and replicate the effects of caloric restriction, which is known to extend life in a variety of creatures. Therefore, the interaction between Sirtuin proteins and their ligands, particularly NAD+ and small molecule activators like resveratrol, is of interest in the context of anti-aging research. To completely comprehend the mechanisms and possible advantages of these interactions for anti-aging therapies, more research is necessary [18]. Addresses the examination of flavonoids' antioxidant and anti-aging characteristics using yeast as a model organism. Model yeast is a useful tool to examine the antioxidant and anti-aging potential of flavonoids, derived from medicinal plants. Flavonoids' anti-aging effects on yeast are specifically investigated [19].

2.2 Role of Yeast in Health and Disease

The article related to probiotics in cosmetic and personal care products discusses the use of probiotics in cosmetics and the potential anti-aging effects of these probiotics. According to Ibrahim et al resveratrol production in yeast, hosts is currently the best method for mass producing this compound. This is a relevant and helpful reference because it discusses resveratrol production in yeast hosts and the compound's potential anti-aging effects [20][21].

According to a study of antioxidative and anti-aging effects of nucleotide-rich yeast hydrolysate by modulating metabolites in Caenorhabditis elegans based on metabonomic analysis was the working title of the research team led by Li and his colleagues. It examines yeast hydrolysate's antioxidative and antiaging properties in the worm model Caenorhabditis elegans. The findings of this study are extremely relevant. We share the research's interest in metabolite modulation as a means to comprehend the mechanisms by which yeast postbiotics affect aging [22].

This is because metabolite modulation is crucial to the lifespan. The findings of this study can be used to expand upon existing knowledge and, perhaps, make a novel that may be used for the creation of anti-aging strategies and cosmetics.

Mushroom extracts and compounds in cosmetics, cosmeceuticals, and nutricosmetics which appeared in Industrial crops and products in 2016. The inclusion of mushroom extracts and components in cosmetics and cosmeceuticals makes this reference appropriate and relevant [23][24].

Saccharomyces cerevisiae fermentation products describe the effects of a postbiotic derived from yeast on the gut microbiota of stressed horses. According to a study published in the International Journal of Food Science & Technology in 2023, antioxidative and anti-aging effects of nucleotide-rich yeast hydrolysate by modulating metabolites in *Caenorhabditis elegans* based on metabonomic analysis was the working title of the research team led by Li and his colleagues. It examines yeast hydrolysate's antioxidative and antiaging properties in the worm model *Caenorhabditis elegans*. The findings of this study are extremely relevant. We share the research's interest in metabolite modulation as a means to comprehend the mechanisms by which yeast postbiotics affect aging. This is because the aging process depends critically on metabolite regulation. The investigation's findings can be applied to improve knowledge currently held and possibly reveal fresh angles that can be used in the creation of cosmetics and anti-aging strategies [25].

Li et al.'s research, which used Caenorhabditis elegans as a model, sheds important new light on the potential anti-aging benefits of yeast hydrolysate. Using this model, the effects of yeast-derived chemicals on aging-related metabolites can be evaluated in a controlled and efficient manner, yielding insights into the underlying processes. Understanding the broader implications of yeast postbiotics in the aging process relies on details like the ones provided by their metabonomic research on how individual metabolites are altered [26].

Furthermore, the reference emphasizes the importance of using model organisms to examine the effects of bioactive chemicals on aging processes. Exploratory research using these models could help guide researchers as they move on to study humans. This strategy is in line with our suggested in vitro and in vivo investigations, which aim to close the knowledge gap between basic science and implementation in the food and cosmetics industries. We can use this useful resource to bolster

the scientific foundation of our study. This is an important and relevant reference because it discusses how Middle-aged mice's oxidative stress can be decreased by probiotics and their metabolite [27].

Protective effects of the postbiotic deriving from cow's milk fermentation with L. paracasei CBA L74 against Rotavirus infection in human enterocytes by Bruno et al., Scientific publications, 2022. The benefits of a postbiotic made from fermented cow's milk against Rotavirus infection are discussed, making this reference both timely and practical [28].

In their 2019 article, Sampaio-Marques and coworkers wrote, Yeast at the forefront of Research on Aging and Age-Related Diseases. Because it highlights the importance of using yeast as a model organism in research on aging and age-related diseases, this reference is timely and applicable. Assessment of the Suitability of Methods for Testing the Antioxidant Activity of Anti-Aging Creams, Applied Science. This source is pertinent and useful because it provides an examination of methods for figuring out the antioxidant properties of anti-aging creams [26].

A rapid, high-throughput method for determining chronological lifespan in budding yeast, by Belak et al., in Journal of biological methods. This reference is appropriate because it discusses a method for calculating chronological lifetime in yeast, which could be used to identify anti-aging agents. The International Journal of Cosmetic Science published an article by Silva et al. titled, Evolution of the Use of Antioxidants in Anti-Aging Cosmetics. This source is appropriate and relevant because it discusses the rise of antioxidant use in anti-aging cosmetics [30].

Research by YADAV et al., probiotic in cosmetics, appeared in the international journal of pharmaceutical sciences and life sciences research that same year. This document is useful and timely because it surveys the landscape of cosmetics patents involving probiotics. Unripe pear fruit extract induces the transcriptional activity of sirtuin-related genes to extend the chronological lifespan of *Saccharomyces cerevisiae* by Murata et al., Advancement in medicinal plant research with thanks to Murata and coworkers. This source is relevant and helpful because it investigates

the effects of unripe pear fruit extract, which has anti-aging properties, on the lifespan of yeast [31].

2.3 Antioxidant Properties of Yeast Derived Compounds

Yeast probiotics, particularly *Saccharomyces boulardii*, are a type of beneficial yeast that can positively influence the digestive system. Here are some key aspects:

2.3.1 Diarrhea Prevention

The potential of *Saccharomyces boulardii* to prevent and treat different kinds of diarrhoea has been investigated. It might be useful in lessening the intensity and length of traveler's diarrhoea, antibiotic-associated diarrhoea, and infectious diarrhoea [32].

2.3.2 Balancing Gut Flora

These probiotics made of yeast aid in preserving the equilibrium of the gut microbiota. Proper digestion and nutrition absorption depend on a healthy microbiome. The balance of gut flora is maintained by postbiotics, which are the byproducts of metabolism of probiotics or inactive probiotic cells. By boosting the strength of the intestinal barrier, regulating immunological responses, and encouraging a favourable microbial balance, they can positively impact the gut environment. Postbiotics can help maintain the balance of the microbiota in the gut and improve overall gut health whether consumed in food or supplement form [33].

2.3.3 Intestinal Barrier Function

Saccharomyces boulardii might help to maintain the intestinal barrier's strength. This barrier is essential for keeping dangerous substances out of the bloodstream

where they could cause inflammation or other health problems. Their potential to improve the integrity of the intestinal lining by encouraging the production of mucins and strengthening the tight junctions between intestinal cells has been investigated [34].

2.3.4 Immune Modulation

The yeast probiotics can modulate the immune system in the gut, enhancing the body's defense mechanisms against harmful pathogens. Yeast, particularly beta-glucans found in their cell walls, are known to stimulate immune responses. They can enhance macrophage activity and promote the production of certain cytokines, contributing to immune modulation. This interaction is being explored for potential therapeutic applications in immune-related conditions [35].

2.3.5 Inflammatory Bowel Diseases

Certain inflammatory bowel diseases, like Crohn's disease and ulcerative colitis, may be better managed by reducing inflammation and improving gut health, according to some research. Because of their anti-inflammatory properties, yeast postbiotics may help treat inflammatory bowel issues. These metabolites have the ability to control the immune system and lessen intestinal inflammation. They might also aid in restoring the integrity of the gut barrier, which is frequently weakened by inflammatory bowel disorders. It may be possible to help people with inflammatory bowel issues by include yeast postbiotics in a comprehensive management strategy, however, it's imperative to see a healthcare professional for precise advice [36].

2.3.6 Antimicrobial Properties

Saccharomyces boulardii has antimicrobial properties, which means it can inhibit the development of harmful bacteria in the gut. This can be particularly beneficial during and after antibiotic treatment, helping to prevent imbalances in the

microbiota. Yeast postbiotics can exhibit antimicrobial properties, contributing to their potential role in promoting a healthy microbial balance. These byproducts may help inhibit the growth of harmful microorganisms in the gut, supporting the overall antimicrobial defense mechanisms. This property can be beneficial in maintaining a diverse and balanced microbiota, essential for gut health and immune function [37].

2.3.7 Support During Antibiotic Therapy

Taking yeast probiotics alongside antibiotics may help mitigate the disruption of the gut microbiota caused by antibiotic treatment. Yeast, specifically *Saccharomyces boulardii*, is recognized for its potential in supporting antibiotic therapy. It can help prevent or alleviate antibiotic-associated diarrhea by maintaining a balanced gut microbiota. Additionally, yeast may contribute to immune modulation, potentially aiding the overall effectiveness of antibiotic treatment. Individual reactions could differ, though, so it's best to speak with a healthcare provider [38].

It is important to remember that different people react differently to probiotics, so it is best to speak with a healthcare provider before starting a probiotic regimen, particularly if you have a weakened immune system or other medical issues. "*Lactobacillus casei*-Derived Postbiotics Elevate the Bio accessibility of Proteins via Allosteric Regulation of Pepsin and Trypsin and Introduction of Endopeptidases," explain how *Lactobacillus casei*-derived postbiotics can improve protein digestibility. It addresses the bioacessibility of proteins via postbiotics produced from *Lactobacillus casei* [39].

Tintor´e and colleagues released their, "In Vitro and in Vivo Anti-Inflammatory Effects, Protection of Gut Barrier Integrity and Stimulation of Phagocytosis of ABB C1, a Synergistic Combination of -Glucans and Selenium- and Zinc-Enriched *Saccharomyces cerevisiae*,". This source is relevant because it describes the anti-inflammatory properties observed both in vitro and in vivo, as well as the protective benefits of the combined effects of beta-glucans and *Saccharomyces cerevisiae* on the intestinal barrier [40].

Preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" is the definition of postbiotics. Postbiotic elements includes short-chain fatty acid exopolysaccharides, vitamins, teichoic acids, bacteriocins, enzymes, and peptides in a non-purified inactivated cell preparation.

Postbiotics are still being investigated, but there is mounting evidence that they might have an impact on human health. Specifically, research has shown that several postbiotics improve gut health by strengthening the intestinal barrier, reducing inflammation, and increasing the activity of microorganisms that fight gut infections [41].

This review concentrates on three of these substances and their outcomes the impact of specific short-chain fatty acid chains on gut health, the impact of exopolysaccharides on reducing inflammation in the gut environment and fortifying the gut barrier and the impact of bacteriocins produced by particular bacteria on enhancing their antimicrobial properties against gut organisms. Acetate, propionate, and butyrate are three SCFAs that the gut microbiota produces and are crucial for human health. Cross feeding in the human colon and bacterial fermentation of food fibres are the two sources of SCFAs.

Among other ways, SCFAs guard against intestinal inflammation by stimulating G-protein-coupled receptors (GPCRs), which are SCFA-sensing receptors that help maintain the intestinal epithelial barrier and immune system [42].

Many lactic acid-producing bacteria produce substances that increase the synthesis of ZO-1 and occludin, two essential proteins that reinforce the intestinal barrier. Examples of intestinal inflammation that can be prevented include Crohn's disease and ulcerative colitis [43].

2.4 Postbiotics in Aging: Current Research

Yeast postbiotics can play a role in immune modulation by influencing the activity of immune cells. Some studies suggest that these byproducts may enhance the

production of certain cytokines, supporting a balanced immune response. However, the specific mechanisms and optimal applications in immune modulation are areas that require more research for a comprehensive understanding. Potential immunomodulatory effects of yeast postbiotics, including compounds like mannans and beta-glucans, have been investigated. A schematic illustration of yeast-derived membrane vesicles (EcN@YM) and their involvement in influencing mucosal immunity and fostering gut microbiota balance is shown in figure 2.1 (A) depicts the procedure of removing yeast membranes (YMs) and encapsulating Escherichia coli Nissle 1917 (EcN) to create EcN@YM. (B) depicts the interaction between EcN@YM and the gut immune system, highlighting EcN@YM survival, M cell absorption, and subsequent activation of immunological responses in Peyer's patches, increasing mucosal immunity and balancing gut microbiota [44].

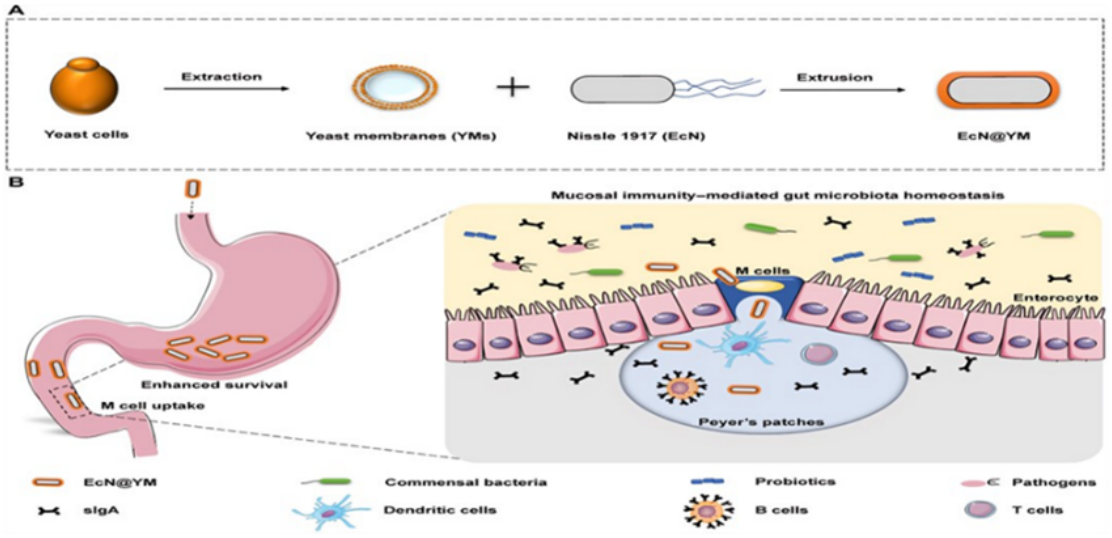


FIGURE 2.1: Development and Function of EcN@YM for Enhanced Gut Immunity and Microbiota Homeostasis, EcN@YM, a yeast-derived membrane vesicle, plays a crucial role in gut microbiota homeostasis and mucosal immunity. Its increased survival, absorption by M cells, and subsequent immune response activation in Peyer's patches support mucosal immunity.

Although encouraging, it's important to remember that this field of study is still in its infancy and that the precise processes and best dosages for immune regulation are still being investigated. As the understanding of yeast postbiotics advances, their potential applications in supporting immune health may become clearer. If immunity of your body is good then it leads to better health & long life. Probiotic organisms are said to offer a number of useful properties, immune system

stimulation being one of them. The purpose of this research is to present comprehensive data on the mechanisms by which probiotics strengthen the immune system. The probiotic cultures Lactobacillus rhamnosus GG, *Lactobacillus casei* Shirota, Bifidobacterium animalis Bb-12, Lactobacillus johnsonii La1, Bifidobacterium lactis DR10, and *Saccharomyces cerevisiae* boulardii have been the most extensively researched for their capacity to influence immunity [45].

Probiotics can improve the nonspecific cellular immune response, which is typified by the strain-specific and dose-dependent production of different cytokines, natural killer (NK) cells, antigen-specific cytotoxic T-lymphocytes, and macrophage activation. Different cytokine responses may be induced by different probiotic bacteria types (gram-positive and gram-negative). Probiotic supplementation throughout infancy may help avoid immune-mediated childhood illnesses [46].

2.5 Yeast Postbiotics and Anti-bacterial Roles

Probiotic bacteria's secreted chemicals have frequently been explored as possible medications to treat infections brought on by bacterial or yeast infections. The goal of the current investigation was to determine if secreted probiotic filtrates, also known as postbiotics, generated from Lactobacillus plantarum cells might inhibit the growth of harmful microbes such *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. It was discovered that the postbiotics had no discernible impact on the planktonic growth of the studied pathogens, but rather reduced their biofilms.

The diagram 2.2 depicts a complete picture of probiotics' functional route, focusing on both antibacterial qualities and immunological regulation capabilities. Probiotics are obtained from a variety of sources, including fermented foods (such as yogurt) and the gastrointestinal system, where they are extracted for further research. Once separated, these probiotics can produce bacteriocins, which are antimicrobial peptides that hinder the development of harmful bacteria. The lower route in the picture depicts how probiotics directly attack harmful bacteria by

producing bacteriocin, which results in antimicrobial action. These natural antibiotics damage the structure of dangerous bacteria, limiting their capacity to colonize and cause diseases. This shows how probiotics affect the host's immune system, causing immune cells to develop a stronger defense [47].

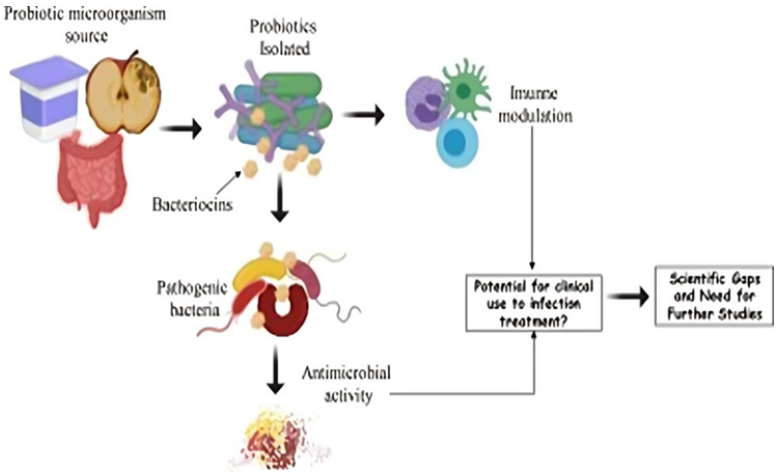


FIGURE 2.2: Exploring Probiotics for Immune Modulation and Antimicrobial Activity, The picture illustrates probiotics' antibacterial action and immunological responses, highlighting their genesis, separation, bacteriocin synthesis, and potential clinical uses and research gaps in infection therapy.

An important part of preserving skin health is the diverse group of bacteria known as the skin microbiome. The gram-positive *Micrococcus luteus* bacteria s one of these microorganisms that may help to improve skin health. The postbiotics generated from M. luteus YM-4, a strain isolated from human skin, are the subject of this investigation.

Using real-time PCR for gene expression analysis and fibroblast migration tests the effects of the YM-4 culture filtrate were investigated on human keratinocytes and fibroblasts under various conditions. To create an RNA-Seq sample from HaCaT cells treated with the YM-4 culture filtrate, a model simulating dehydration was employed. Protein-protein interaction mapping, gene ontology term enrichment analysis, and k-means clustering were used to identify and functionally classify differentially expressed genes [48].

The diagram 2.3 provides a comprehensive overview of solar radiation and its interaction with human skin. It categorizes solar energy into four wavelengths: UVC, UVB, UVA, visible light, and infrared. UVC is the most damaging and

absorbs most of the ozone layer. The diagram also shows how UV light penetrates the skin's three major layers, highlighting the potential for skin damage [49].

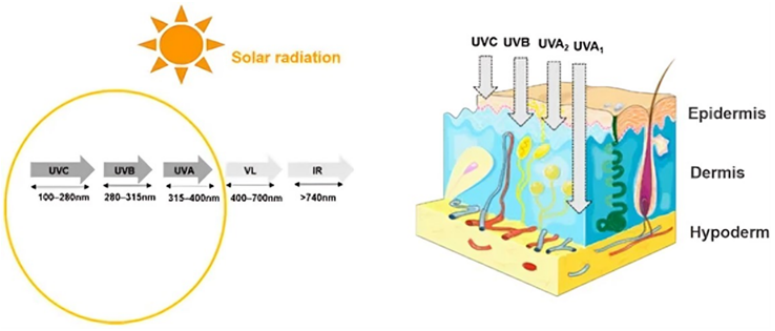


FIGURE 2.3: Solar Radiation and Its Effects on Skin Layers, this diagram depicts the spectrum of solar radiation, which includes UVC, UVB, UVA, visible light (VL), and infrared (IR), as well as their interaction with the three layers of skin: epidermis, dermis, and hypodermis.

2.6 Immunomodulatory Effects of Yeast Postbiotics

2.6.1 Role of Postbiotics in Food Packaging & Bio-preservatives

Metabolic by-products are included in the referred to as postbiotics of probiotics along with additional beneficial microbes, particularly lactic acid bacteria. These are becoming more and more popular as a practical replacement for improving food safety and quality. Postbiotics, comprising organic acids, bacteriocins, exopolysaccharides, and bioactive peptides, have a wide spectrum of antimicrobial qualities that make them easy to incorporate into food formulations and packaging materials. You can find them in liquid and dry versions. They also possess antibacterial and antioxidant properties. Postbiotics can prolong the shelf life of food goods by inhibiting the growth of bacteria that cause rotting and illnesses [50].

The use of yeast postbiotics in this natural preservation method is in line with the growing need for environmentally friendly and sustainable food packaging options.

Research in this area explores how these postbiotics can contribute to food safety and preservation without the need for synthetic additives. Compared to synthetic preservatives, which can have detrimental effects on the environment, postbiotics are seen to be a safer and more sustainable option because to their ease of manufacturing and lack of need for intensive processing. Furthermore, postbiotics can be easily incorporated into food formulations by food makers without requiring major changes [51].

2.6.2 Role of Postbiotics in Oral Health

Studies point to a possible connection between the equilibrium of oral microbiota and dental illnesses. Beneficial bacteria's metabolites called postbiotics may help to preserve dental health. Nonetheless, additional research is required to completely comprehend their influence and create focused solutions. Oral disease is one of most common disorders that people encounter both internationally and in their lifetimes. The composition of the oral microbiota is essential to the growth of tooth decay, a complicated illness. Moreover, it is believed that Streptococcus mutans is the main species responsible for tooth caries [52].

Historically, scientists have frequently employed terms such as "cell free the supernatant biogenic," "abiotics," "metabiotics," "pseudoprobiotics," "ghost probiotics," "paraprobiotics," and "postbiotics" to incorporate non-viable elements or probiotic metabolites. Furthermore, postbiotic has been the most widely used. By definition, metabolites, cytoplasm extracts, or fragments of cell walls produced by food that is fermented and gut-dwelling probiotics are classified as postbiotics.

The diagram 2.4 depicts how probiotics affect gut health by producing postbiotics and then influencing inflammatory responses. At the top, probiotics are portrayed as helpful microbes that, when consumed, lead to the development of postbiotics—probiotic-produced metabolites. These postbiotics play an important role in gut health by modulating the immune system. The figure depicts the pathways that contribute to both pro-inflammatory and anti-inflammatory responses, emphasizing interactions with different immune cells and signaling molecules. It stresses

the dynamic balance between inflammation and the body's regulatory mechanisms, demonstrating how probiotics might improve health by shifting the inflammatory response to an anti-inflammatory state [53].

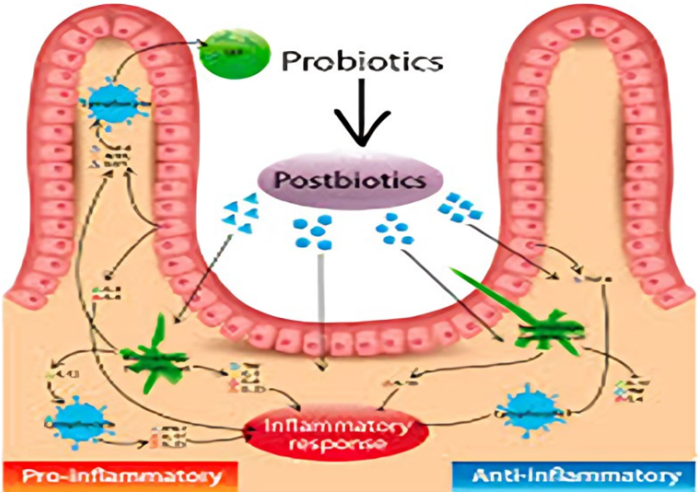


FIGURE 2.4: The Role of Probiotics and Postbiotics in Modulating Inflammatory Responses, this picture depicts the interplay of probiotics, postbiotics, and inflammatory reactions in the gut, emphasizing the balance of pro-inflammatory and anti-inflammatory pathways.

2.7 Comparative Analysis of Probiotics and Postbiotics

This section looks at the differences and similarities between probiotics and postbiotics, including their classifications, modes of action, and health effects. Probiotics are living bacteria that, when ingested in suitable proportions, provide health advantages. They are frequently found in fermented foods such as yogurt and kefir. In contrast, postbiotics are non-viable microbial products or metabolic byproducts formed during fermentation that provide health advantages. Probiotics work by colonizing the gut, regulating immunological responses, and creating helpful metabolites including short-chain fatty acids (SCFAs). Postbiotics, on the other hand, work by reducing inflammation, improving gut barrier integrity, and altering the microbiome without requiring living microorganisms [54].

Probiotics have been linked to improved gut health, digestive issues, and immunological function, as well as possible benefits to mental health via the gut-brain axis. Emerging research suggests that postbiotics can reduce inflammation, boost immunity, and improve skin health, making them interesting candidates for anti-aging treatments. While probiotics can occasionally offer a concern to immunocompromised patients, postbiotics have a decreased risk of infection due to their non-viability. This section also includes clinical trials that show the usefulness of both in a variety of health settings [55].

2.8 Molecular Mechanisms of Anti-Aging in Yeast

Interest in probiotics has significantly expanded due to their potential as beneficial bacteria strains for health. in research employing them to alter the gut microbiome. Comprising accessible nutrients, microorganisms, and host cells, the animal stomach is a complex ecosystem. The microbiota shields both people and animals from a variety of degenerative illnesses by means of immunomodulation. Numerous probiotic species and strains are investigated along with the gut microbiota's effects on the nutrition of humans, metabolism, anatomy, and immunity this will allow you to comprehend the regulation of the gut microbiota better. This paper provides a thorough review of different Lactobacillus species, Bifidobacterium species, and other coliform bacteria and their role in preventing chronic illnesses such as obesity, diabetes, cancer, cardiac malignancy, disease of the liver, and inflammatory bowel disease. Lactobacillus is one of the leading probiotic species. This review also discusses a recent study using Saccharomyces spp. that found that producing short-chain fatty acids enhanced proinflammatory immune function, meaning that inflammation was prevented. A summary of the altered gut flora is also provided, along with potential future directions [56].

The term probiotics refers to live, non-pathogenic microbes that improve host health when given in the right dosages. The yeast *Saccharomyces boulardii* and bacteria from the genera Lactobacillus and Bifidobacterium, which are members

of LAB referred to as lactic acid bacteria groups, are currently included in the category of probiotic microorganisms. Probiotics are protective against oral infections caused by Candida spp., particularly colonization. By inhibiting Th17 cells' ability to secrete, which is how this illness is pathophysiological caused, an overwhelming cytokine response is caused, which damages periodontal tissues, Lactobacillus slows the course of chronic periodontitis. a comprehensive review of research findings regarding the clinical assessment of pro- biotics' efficacy in gingivitis treatment [57].

Probiotic use on a regular basis during orthodontic treatment considerably lowers salivary levels of Streptococcus mutans bacteria and significantly minimizes the release of mediators of inflammation and an overreaction by the immune system. Probiotics can be used effectively in the treatment of oral diseases due to their main mechanisms of action, which include stimulating both specific and non-specific immune responses by activating T lymphocytes and promoting the production of cytokines, as well as the removal and suppression of pathogenic microbes through rivalry for receptor regions and the production of metabolites with antibiotic effects [58].

2.9 Molecular Docking

Using a specific scoring function, a technique known as molecular docking evaluates the strength of the contact between a ligand and a target protein. It seeks to ascertain the precise location of the ligand within the target's binding site. making it a common strategy in structure-based drug design, as it necessitates knowledge of the target protein's three-dimensional structure. Molecular Docking serves to identify the accurate positioning of a ligand and assess binding strength with target proteins using a designated scoring function. Additionally, it aids in identifying new small molecular compounds by revealing crucial properties like strong interaction with the that protein and favorable absorption, distribution, metabolism, and excretion. This information is valuable in selecting lead compounds for further development [59].

2.9.1 Obtaining Protein Structure

The first step is acquiring the three-dimensional structure of the target protein, typically downloaded from databases like the Protein Data Bank.

1. Molecule Size Requirements:

The molecules or compounds involved in the process, including virtual com- pounds within a database, must meet a minimum size for effective docking.

2. Computational Framework:

A computational framework is essential to execute the docking process and perform scoring. This involves sophisticated algorithms and calculations to predict the interaction between the ligand and the target protein accurately.

Protein and ligand docking plays a pivotal role in molecular docking, gaining significant popularity for its contribution to structure-based drug design. Widely used algorithms in molecular docking include molecular dynamics, distance geometry method, and genetic algorithms. Commonly employed software for molecular docking includes AutoDock Vina, AutoDock, CBDock, and ICM, among others. These tools facilitate the exploration of interactions between proteins and ligands, aiding in the design and optimization of drug compounds [60].

2.10 Future Directions in Yeast Postbiotics Research

Brewer's or baker's yeast are terms used to refer to the yeast species *Saccharomyces cerevisiae*. Important details regarding *Saccharomyces cerevisiae* are as follows.

1. **Classification**

   It belongs to the kingdom Fungi, phylum Ascomycota, and class Saccharomycetes.

2. Usage in Baking and Brewing

   Saccharomyces cerevisiae is extensively used in the food industry for baking and brewing. In baking, it ferments sugars to produce carbon dioxide, causing dough to rise. In brewing, it is crucial for the fermentation process that converts sugars into alcohol.

3. **Genome**

   The yeast has a well-studied and sequenced genome, making it a model organism for genetic and molecular biology research.

4. **Single-Celled Organism**

   Saccharomyces cerevisiae is a unicellular organism, which means it consists of a single cell. This makes it convenient for laboratory studies and industrial applications.

5. **Research Significance**

   Due to its simple and well-understood biology, it has been widely used in research to understand fundamental cellular processes. Many discoveries in cell cycle regulation, DNA repair, and other molecular biology fields were initially made using *Saccharomyces cerevisiae*.

6. **Fermentation**

   The yeast is involved in alcoholic fermentation, converting sugars into ethanol and carbon dioxide. This property is harnessed in various industries, including the production of alcoholic beverages.

7. **Mitochondrial DNA**

   Saccharomyces cerevisiae has both nuclear and mitochondrial DNA. The study of its mitochondria has contributed to our understanding of eukaryotic cell biology.

   It's important to note that *Saccharomyces cerevisiae*'s versatility and ease of manipulation make it an invaluable organism for scientific research and various industrial applications. *Saccharomyces cerevisiae*, in its role as yeast postbiotics, refers to the beneficial metabolites and compounds produced by

this yeast after fermentation. These postbiotics can have positive effects on the host organism, contributing to health and well-being. Here are some aspects of *Saccharomyces cerevisiae* as yeast post- biotics [61].

8. **Beta-Glucans**

Saccharomyces cerevisiae is known to produce beta-glucans during fermentation. Beta-glucans are polysaccharides that have been associated with various health benefits, including immune system modulation and potential anti-inflammatory effects. These beta-glucans have been studied for their immunomodulatory effects, potentially enhancing the immune system's response. They are often used in various forms, including yeast extracts or supplements, with claims of supporting immune health and providing other potential benefits. Research suggests that beta-glucans from yeast can stimulate certain immune cells and contribute to overall immune system modulation.

(a) **Mannans**

Another class of compounds produced by *Saccharomyces cerevisiae* includes mannans. Mannans may contribute to the overall health of the digestive system and could have prebiotic-like effects, supporting the growth of beneficial gut bacteria.

(b) **Metabolites**

Saccharomyces cerevisiae ferments food to produce a variety of metabolites, including peptides, vitamins, and organic acids. These substances may improve gut health by influencing the surroundings in the gut.

(c) **Immunomodulation**

Immune modulation refers to the alteration or regulation of the immune system's activity to achieve a desired therapeutic outcome. This can involve enhancing or suppressing the immune response, depending on the specific medical goal, such as treating autoimmune diseases, preventing organ rejection after transplantation, or boosting the immune response against infections or cancer. Some studies suggest that

the postbiotics derived from *Saccharomyces cerevisiae* may have immunomodulatory effects, helping to regulate the immune system and enhance its responsiveness [62].

9. **Gut Microbiota Balance**

   The diversity and peaceful cohabitation of bacteria in the gastrointestinal tract are referred to as gut microbiota equilibrium. An equilibrium of beneficial bacteria in the gut is necessary for healthy digestion, absorption of nutrients, and general health. Dysbiosis, or imbalances in this balance, can affect other body functions and cause gastrointestinal disorders as well as other health concerns. Diet, antibiotic use, and lifestyle choices can all affect the gut microbiota's makeup. For the immune system and digestive tract to function at their best, a healthy and diversified gut microbiome must be maintained.

   Postbiotics containing *Saccharomyces cerevisiae* may aid in preserving a healthy gut microbiome. An optimal gut microbiota is linked to general health and can influence multiple facets of well-being, such as immunological response and digestion

10. **Antioxidant Properties**

    Certain compounds produced during fermentation, such as peptides and antioxidants, may contribute to the overall antioxidant capacity of the postbiotics, potentially providing protection against oxidative stress. It's important to remember that postbiotic research is still in progress. Although there is encouraging data on the health advantages of postbiotics produced from *Saccharomyces cerevisiae*, More research is necessary to fully understand their workings and potential applications [63].

2.10.1 NAD+

The underlying cause of many inherited and acquired human disorders is the reduction of nicotinamide adenine dinucleotide (NAD+), an important redox cofactor

and required substrate for major metabolic enzymes. Impaired biosynthesis is the primary cause of primary deficits in NAD+ homeostasis. On the other hand, increasing use of NAD+ or insufficient intake of vitamin B3 precursors can lead to secondary deficiencies. NAD+ depletion can lead to diverse pathological conditions, ranging from rare inherited disorders with congenital malformations, retinal degeneration, and/or encephalopathy to more common age-related diseases with multiple contributing factors. In this discussion, we delve into the biochemistry and metabolism of NAD+ and offer insights into the causes and pathological effects of disruptions in NAD+ metabolism in humans. Additionally, we explore the current status of potential therapeutic applications of NAD+ replenishment for enhancing overall health and addressing both rare and common diseases. The exploration ex- tends to various NAD+-enhancing agents as potential avenues for achieving these therapeutic goals [64].

2.10.1.1 NAD+ and It's Antiaging Role

According to the geroscience theory, treating the biology of ageing may be able to directly stop the emergence of several chronic diseases or lessen their severity. Realising the potential of the geroscience hypothesis requires an understanding of how important components of the biological markers of ageing interact with one another. Notably, cellular senescence is one of the molecular characteristics of ageing that the nucleotide nicotinamide adenine dinucleotide (NAD) interfaces with. It has also been demonstrated that alterations in NAD metabolism contribute to ageing [64].

NAD metabolism and cellular ageing appear to interact intricately. One could contend that the accumulation of damaged DNA and mitochondrial dysfunction caused by low NAD+ speed up the ageing process. But because both this secretory phenotype and the onset of cellular senescence are highly metabolically requiring, the low NAD+ state associated with ageing may inhibit the synthesis of SASP. However, little is known about how NAD+ metabolism contributes to the development of the cell-senescence phenotype. Therefore, to properly comprehend the implications of these processes, it is imperative to consider how NAD metabolism

and NAD supplementation therapies collaborate with other aspects of ageing, such as cellular senescence. We propose that a comprehensive understanding of the interplay between senolytic medications and NAD boosting strategies is necessary to advance the research [65].

Nicotinamide adenine dinucleotide is an essential metabolite in the processes of protein deacetylation, DNA repair, and redox equilibrium. Pharmacological or genetic inhibition of NAD+-degrading enzymes, exogenous replenishment of NAD+ precursors, and transgene amplification of NAD+-generating enzymes can all significantly improve the prevention of age-related illnesses and metabolic health. The three main risk factors for cardiovascular disease are normal ageing, diabetes, and hypertension, these illnesses all tend to cause a decrease in NAD+ pools. NAD+ replenishment reduces blood pressure, avoids metabolic syndrome, and extends life in preclinical models. Moreover, an experimental elevation of NAD+ improves a number of heart failure types, such ischemic, diabetic, arrhythmogenic, hypertrophied, and atherosclerotic cardiomyopathies. Here, we compare and evaluate the preclinical efficacy of different NAD+ precursors, critically examine the circuitries of NAD+ metabolism unique to cardiomyocytes, and raise significant concerns about how best to plan clinical trials assessing supraphysiological NAD+ elevations or NAD+ restoration for preventing or treatment of major cardiac conditions. We hypothesise that patients with heart failure who nonetheless have an intact ejection fraction and other heart conditions that were once thought to be incurable would benefit by taking NAD+ precursors. Such NAD+-centered treatments will require conceptual and technological advances in the fine administration of NAD+ metabolism [66].

2.10.2 Beta Glucans

Although yeast β-glucans are known to have immune-modulating qualities, the study used a systematic review and meta-analysis approach to investigate their effect on infections of the upper respiratory tract in healthy adults. This study examined the effects of adding selenium enriched yeast, mannan- oligosaccharide- and β glucan rich yeast postbiotics, and live yeast to the meals of sheep. The

outcomes demonstrated higher postpartum milk production, greater activity of antioxidants in blood plasma, decreased levels of β hydroxybutyric acid in the blood of treated ewes, and improved milk quality. Additionally, throughout the peripartum phase, the supplementation decreased PR inflammatory gene ex- pression in monocytes and neutrophils, indicating favourable impacts on energy, milk production, oxidative status, and immune system-related gene expression [67].

2.10.3 Nicotinamide riboside

Nicotinamide riboside is a form of vitamin B3 that has gained attention for its potential health benefits. NR is a precursor to nicotinamide adenine dinucleotide (NAD+), an important coenzyme involved in various cellular processes. Studies suggest that NR supplementation may help increase NAD+ levels in the body, which could have positive effects on metabolism, aging, and mitochondrial function. Some research indicates NR may improve metabolic health, support cognitive function, and provide neuroprotective effects, though Further investigation is still required to completely comprehend its therapeutic uses. NR is considered a safe and well-tolerated supplement, making it an area of ongoing interest for nutritional and biomedical research [68].

2.10.3.1 mTORC1

Cells use resources for biosynthesis and bioenergetic demands in order to promote development and division. During the highly controlled process of the cellular absorption of nutrients from the environment and their intracellular metabolism, growth signals and metabolic processes interact with one another. When faced with constant changes in the food source and environmental signals, normal cells restore metabolic homeostasis to sustain cellular activity and prevent illness. The rapamycin mechanistic target is an essential signaling protein that unites growth and metabolism. mTORC1 forms two protein complexes: mTORC1 (rapamycin sensitive) and mTORC2 (not directly affected by this medicine). Rapamycin has facilitated the identification of the several metabolic roles of mTORC1. Genetic

models that disrupt mTORC1 and mTORC2 in cells, organs, and entire systems have contributed to a better understanding of these enzymes' actions in metabolism. But we haven't kept up with the most recent discoveries about the functions and control of mTORC2, particularly in metabolism. Since mTOR is a major target for getting older, cancer, and other metabolism-related disorders, our ability to understand the distinct and overlapping regulation and actions of the two mTOR complexes is critical to the development of more effective therapeutic methods. This review includes the key findings and the majority of recent studies on the regulation and metabolic functions of mTOR complexes. We present results from cancer models and address potential applications of mTOR mediated reprogramming of metabolic pathways in stem and immunity cells, type 2 diabetes/obesity, neurological disorders, and ageing [69].

2.10.3.2 Ethanol

Ethanol, a byproduct of yeast fermentation, has been found to have antibacterial effects. While ethanol is well known for its usage in alcoholic drinks, its ability to suppress the growth of dangerous microbes in the stomach suggests that it has potential therapeutic uses in gut health [62].

2.10.3.3 Glycerol

Glycerol is obtained during yeast fermentation and used as a carbon source for yeast development. Its hydrating characteristics make it a possible candidate for skin care treatments. Glycerol improves skin hydration and barrier function, making it an important element in cosmetic formulations [62].

2.10.3.4 Acetic Acid

Yeast produces another metabolite called acetic acid during fermentation. It is known for its antibacterial qualities, which can help keep the gut microbiota healthy by suppressing harmful microorganisms. Additionally, acetic acid has

been related to enhanced metabolic health and may aid with weight management [63].

2.10.3.5 Mannan Oligosaccharides

Mannan oligosaccharides are complex carbohydrates generated from the yeast cell wall, specifically Saccharomyces cerevisiae. These chemicals are renowned for their prebiotic properties, which encourage the establishment of good gut flora and improve gut health. They also aid in immunological regulation and may help avoid gastrointestinal infections.

2.10.3.6 Trehalose

Trehalose is a disaccharide made up of two glucose molecules and is generated by a variety of yeast species. It has been examined for its capacity to protect cells from stress, improve energy metabolism, and provide neuroprotective properties. Trehalose may potentially aid in anti-aging by enhancing cellular resilience and lowering oxidative stress [64].

Chapter 3

Methodology

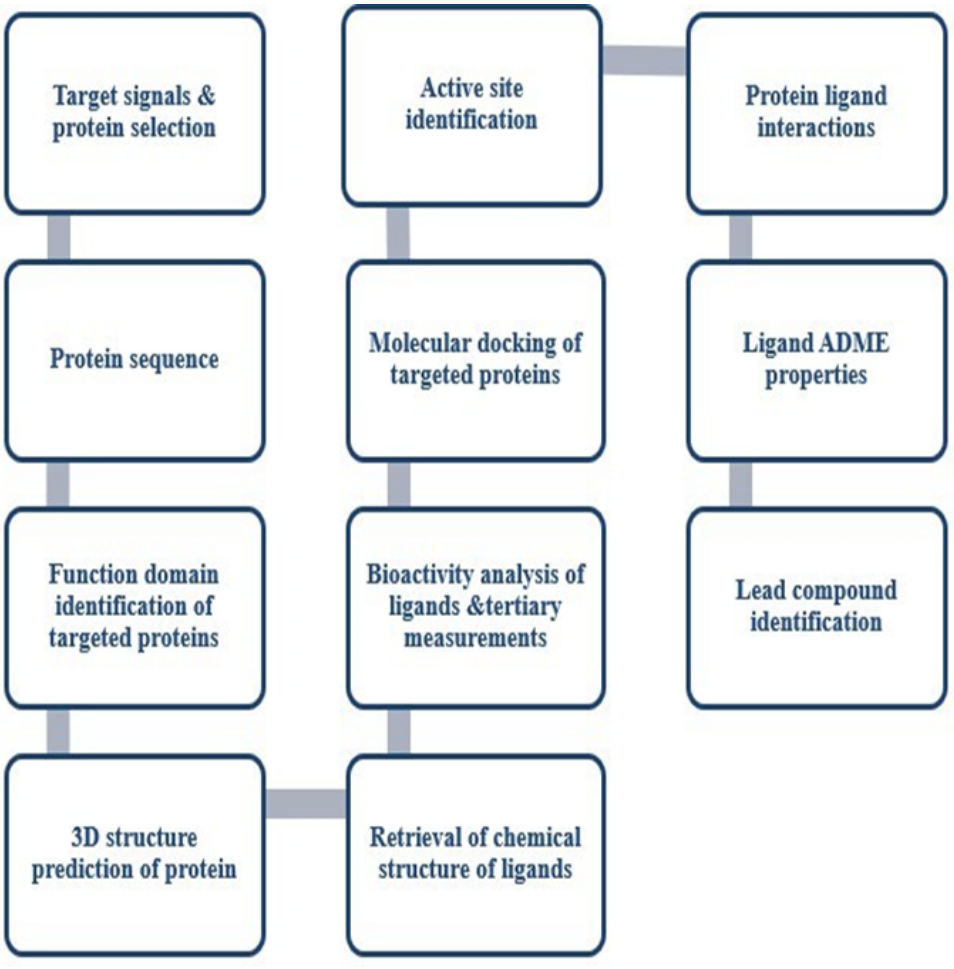


FIGURE 3.1: Methodology Flowchart

3.1 Selection of Problem

Aging is an unavoidable biological process however; people do not wish to grow older because of the associated risk factors of several chronic diseases. Lifestyle, diseases, and stress are major causes of early aging.

People are using different unnatural therapies to slow the process of aging but they may cause physiological implications. Alternative sources are required to lessen the extent of this problem. mTORC1 is a potential protein responsible for the aging mechanism. Nicotinamide riboside and beta-glucans can be potential metabolites to control early aging.

3.2 Selection of Target Proteins

The main purpose of selecting the respective protein is to regulate the differentiation of cells, signaling, and metabolism. Balancing mTORC1 signaling is a key target for interventions aimed at promoting healthy ageing and longevity [70].

3.3 3D Structure Prediction of Protein

The 3D structures can be predicted through PDB www.rcsb.org. Alternatively, I-TASSER can be used if some structures are not available on PDB. I-TASSER zhanglab.ccmb.med.umich.edu/I-TASSER stands for Interactive Threading Assembly Refinement.

This software is available online and predicts proteins' three-dimensional structure and function. First of all, it identifies the structural model of the PDB through various strategies which include the atomic models of full length and they are built by using simulations of the different threading fragments [71]. The 3D structure of proteins is also predicted by the I-TASSER and this server gives us five 3D structures of proteins so based on the C-score we can select the best 3D structure

of the protein [72]. Alphafold alphafold.com is another reliable source for the prediction of the 3D structure of proteins [73].

3.4 Retrieval of Protein FASTA Sequence

The FASTA sequence of the selected target protein mTORC1 was taken from the protein sequence database UniProt http://www.uniprot.org/. Alternatively, PDB or NCBI www.ncbi.nlm.nih.gov databases can also be used for sequence retrieval of target proteins [74].

3.5 Analysis of Physicochemical Properties of Target Proteins

The function of the proteins is significantly influenced by their physicochemical characteristics. ProtParam expasy tool web.expasy.org/protparam was used for the prediction of these features of mTORC1. ProtParam was used to calculate the number of negatively charged residues (Asp+ Glu) and positively charged residues (Arg+ Lys), theoretical pI, molecular weight, aliphatic index, grand average of hydrophobicity, instability index, Ext coefficient (Cys included), and Ext coefficient (Cys not included) [75].

3.6 Protein Structure Analysis and Refinement by Use of PyMol

PyMOL pymol.org is an open-source molecular graphics program that has been used extensively worldwide for the three-dimensional examination and representation of several proteins and small compounds including nucleic acids, densities of different electrons and varying surfaces, and also the trajectories. It is also used for editing the molecules, tracing the ray, and also to make animations and

movies. This is software that is based on Python and also contains many plugin tools to enhance its use and facilitate drug targeting and designing by the use of PyMol software.

This is software that is based on Python and also contains many plugin tools to enhance its use and facilitate drug targeting and designing by the use of PyMol software.

The excess components linked to the protein must be deleted after downloading the protein structure which was done by the use of an open-source system PyMol [76].

3.7 Functional Domain Identification of Targeted Proteins

Interpro www.interpro.com is a database that was utilized to determine the targeted protein mTORC1 functional domains [77]. Sequence, structure, and relationships are all involved in conserved domains.

Sequence, structure, and relationships are all involved in conserved domains. Sequence, structure, and relationships are all involved in conserved domains.

3.8 Active Site Identification

The area in which the target protein's active site is located is where the ligand exhibits the greatest or maximal interaction with the protein. Amino acids have a major role in the ligand-protein complex building process.

CASTp sts.bioe.uic.edu/castp software was used for the detection of protein binding pockets [78].

3.9 Selection of Active Metabolic Ligands

After an extensive literature review, those ligands were selected that have previously shown some antiaging properties. These include nicotinamide riboside and beta-glucans.

3.10 Retrieval of Chemical Structure of Ligands

PubChem pubchem.ncbi.nlm.nih.gov is the world's largest repository of easily accessible chemical information databases. So the chemical compounds that were selected as potential ligands were taken from the PubChem database in SDF format [79]. If in case the selected ligand structure is not available then our next attempt would be to download the canonical smiles from PubChem and then insert them in the software ChemDraw after obtaining the 3D structure repeat the energy minimization step using Chem3D ultra. At the end, PDB format will be selected to save the energy-minimized structure of the ligand.

3.11 Energy Minimization of Ligands

Ligands energy was minimized by using Chem3D ultra. It is a necessary step to refine the ligands before performing docking otherwise, there will be unreliable docking scores [80].

3.12 Virtual Screening of Ligands by Application of Lipinski Rule of Five

An essential criterion for determining whether ligands are likely to be drugs is the lipinski rule. Certain chemicals are likely to be utilized as active pharmaceuticals

in humans if they adhere to the lipinski rule of five. pkCSM omictools.com/pkcsm-tool is an online tool that helps to check whether ligands obey the lipinski rule or not [81]. The rules are described as under:

1. The log P value should be in the range of five.
2. Maximum number of H-bond acceptor should be limited to ten.
3. Maximum number of H-bond donor should be limited to five.
4. The molecular weight should be below five hundred grams.
5. Rotatable bonds should be limited to five.

3.13 Ligands ADME/T Analysis

After filtering the ligands by applying the lipinski rule, the next step of the study was the prediction of pharmacokinetic and toxicity properties. pkCSM omictools.com/pkcsm-tool was used for the prediction of the pharmacokinetic properties of selected ligands. The weak candidates of the drug would be eliminated during ADME/T analysis. The remaining candidates can be selected as potential drugs against the disease. The target proteins were used to optimize the ADME/T (Absorption, Distribution, Metabolism, excretion, and toxicity) associated with the human body [82].

3.14 Molecular Docking

For performing the molecular docking between the protein and the ligand, CB-dock (Cavity detection guided blind docking) was used. CB Dock clab.labshare.cn/cbdock/blinddock finds the sites of docking automatically. CB-Dock is a method of protein and ligand docking that indicates the sites of bonding, the size, and the center calculated. The box size is adjusted according to the ligand and then docking is performed. The docking is performed through AutoDock Vina. Its

accuracy ratio is greater because the docking process is more focused on cavity binding [83]. We uploaded the protein to do docking, using the 3D structure in PDB format and the ligand's 3D structure in SDF format. After this docking is performed, the result would be 5 different poses of interaction. To select the best pose, we would look at the lowest docking score which is given in KJ/m-1. CB-Dock will provide an interactive 3D visualization of results in 5 different poses. Based on the lowest vina score expressed in (kJ/m1), the optimal position was chosen [84].

3.15 Analysis of Docked Complexes via Ligplot

The interaction between the ligand and the active pockets of the protein was computed in order to interpret docking results. The two kinds of interactions that were examined are hydrophobic and hydrogen bonding. Ligplot Plus (v.1.4.5) was used to analyze the protein-ligand interactions. The protein-ligand interactions for the designated ligands in the PDB file are automatically schematically diagrammed by this tool [85].

3.16 Lead Compound Identification

The most active inhibitor was found after a thorough examination of docking scores, pharmacokinetic studies, toxicity features, and protein and ligand interactions. Our lead compound was the one that followed all these parameters.

3.17 Reference Anti-aging Drug Selection

The purpose of this step is to identify the commercial drugs that are already in use for antiaging disease treatment purposes. KEGG www.genome.jp/kegg and Drug Bank go.drugbank.com databases were used for this purpose because they provide details about drugs and their pathways [86].

3.18 Comparison between Lead Compound and Reference Drug

Docking values, molecular interactions, and pharmacokinetic features were compared between the reference anti-aging drug and the suggested lead molecule.

Chapter 4

Results and Discussion

4.1 3D Structure Prediction and Refinement of Selected Proteins

3D structures of target protein mTORC1 were taken from PDB under PDB ID 6BCX, Using PyMol, the protein structures were created by eliminating any ligands and water molecules. To obtain the stable conformation After the ligands, the absent polar hydrogens were added, and other atoms were removed to prevent overlaps and the modified file was saved in PDB format. The refined structures are shown in figure 4.1 below.

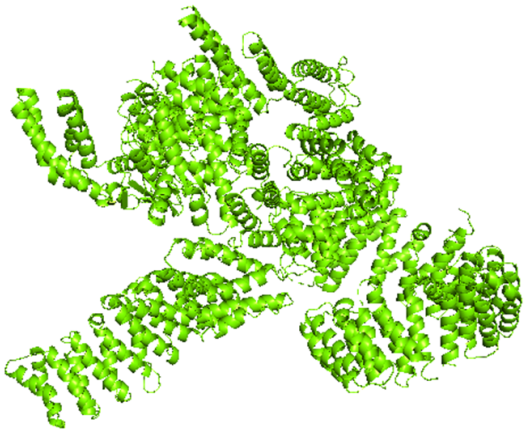


FIGURE 4.1: Refined Structure of mTORC1

The mammalian target of rapamycin complex 1 (mTORC1) is a central regulator of aging. It integrates diverse signals, such as growth factors and nutrients, to control cellular processes like protein synthesis, autophagy, and mitochondrial function. Sustained mTORC1 activation is linked to accelerated aging and age-related diseases, while its inhibition extends lifespan in model organisms. Modulating mTORC1 signaling has emerged as a promising approach for promoting healthy longevity [87].

4.2 Primary Sequence Retrieval

Using UniProt, the chosen protein mtorc1 FASTA sequence was obtained. The FASTA sequence was downloaded from UniProt under accession number P42345 containing Chain A with 1-2549 amino acid residues length as shown in figure 4.2.

>6BCX_1|Chains A, E[auth B]|Serine/threonine-protein kinase mTORC1| Homo sapiens
(9606)MLGTGPAAATTAATTSXXXXXXXXXXXXXXSRNEETXXXXXXXXXXXXXXXXXXEMSQEEXTRFYDQLNHHIFELVSSSDANERKGGILAIASLIGVE
GGNATRIGRFANYLRNLLPSNDPVVMEMASKAIGRLAMAGDTFTAEYVEFEVKRALEWLGADRNEGRRHAAVLVLRELAISVPTFFFQQVQPFFDNIFVAV
WDPKQAIREGAVAALRACLILTTQREPKEMQKPQWYRHTFEEAEKGFDETLAKEKGMNRDDRIHGALLILNELVRISSMEGERLREEMEEITQQQLVHDK
YCKDLMGFGTKPRHITPFTSFQAVQPQQSNALVGLLGYSSHQGLMGFGTSPSPAKSTXXXXXXXXXXXXXXXXXXXXXXXXXXXRNSKNSLIQMTILNLLPR
LAAFRPSAFTDTQYLQDTMNHVLSCVKKEKERTAAFQALGLLSVAVRSEFKVYLPRVLDIIRAALPPKDFAHKRQKAMQVDATVFTCISMLARAMGPGIQQ
DIKELLEPMLAVGLSPALTAVLYDLSRQIPQLKKDIQDGLLKMLSLVLMXKPLRHPGMPKGLAHQLASPGLTTLPEASXVGSITLALRTLGSFEFEGHSLTQF
VRHCADHFLNSEHKEIRMEAARTCSRLLTPSIHLISGHAHVVSQTAVQVVADVLSKLLVVGITDPDPDIRYCVLASLDERFDAHLAQAENLQALFVALNDQV
FEIRELAICTVGRLSSMNPAFVMPFLRKMLIQILTELEHSGIGRIKEQSARMLGHLVSNAPRLIRPYMEPILKALILKLKDPDPDPNPGVINNVLATIGELAQVS
GLEMRKWVDELFIIIMDMLQDSSLLAKRQVALWTLGQLVASTGYVVEPYRKYPTLLEVLLNFLKTEQNQGTRREAIRVLGLLGALDPYKHKVNIGMIDQSRD
ASAVSLSESKSSQDSSDYSTSEMLVNMGNLPLDEFYPAVSMVALMRIFRDQSLSHHHTMVVQAITFIFKSLGLKCVQFLPQVMPTFLNVIRVCDGAIREFLF
QQLGMLVSFVKSHIRPYMDEIVTLMREFWVMNTSIQSTIILLIEQIVVALGGEFKLYLPQLIPHMLRVFMHDNSPGRIVSIKLLAAIQLFGANLDDYLHLLLPPIV
KLFDAPEAPLPSRKAALETVDRLTESLDFTDYASRIIHPIVRTLDQSPELRSTAMDTLSSLVFQLGKKYQIFIPMVNKVLVRHRINHQRYDVLICRIVKGYTLA
DEEEDPLIYQHRMLRSGQGDALASGPVETGPMKKLHVSTINLQKAWGAARRVSKDDWLEWLRRLSLELLKDSSSPSLRSCWALAQAYNPMARDLFNAA
FVSCWSELNEDQQDELIRSIELALTSQDIAEVTQTLLNLAEFMEHSDKGPLPLRDDNGIVLLGERAAKCRAYAKALHYKELEFQKGPTPAILESLISINNKLQ
QPEAAAGVLEYAMKHFGELEIQATWYEKLHEWEDALVAYDKKMDTNKDDPELMLGRMRCLEALGEWGQLHQQCCEKWTLVNDETQAKMARMAAAAA
WGLGQWDSMEEYTCMIPRDTHDGAFYRAVLALHQDLFSLAQQCIDKARDLLDAELTAMAGESYSRAYGAMVSCHMLSELEEVIQYKLVPERREIIRQIW
WERLQGCQRIVEDWQKILMVRSLVVSPHEDMRTWLKYASLCGKSGRLALAHKTLVLLLGVDPSRQLDHPLPTVHPQVTYAYMKNMWKSARKIDAFQHM
QHFVQTMQQQAQHAIATEDQQHKQELHKLMARCFLKLGEWQLNLQGINESTIPKVLQYYSAATEHDRSWYKAWHAWAVMNFEAVLHYKHQNQARDEK
KKLRHASGANITNATTAATTAATATTTASTEGSNSESEAESTENSPTPSPLQKKVTEDLSKTLLMYTVPAVQGFFRSISLSRGNNLQDTLRVLTLWFDYGHW
PDVNEALVEGVKAIQIDTWLQVIPQLIARIDTPRPLVGRLIHQLLTDIGRYHPQALIYPLTVASKSTTTARHNAANKILKNMCEHSNTLVQQAMMVSEELIRVAI
LWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISKQLPQ
LTSLELQYVSPKLLMCRDLELAVPGTYDPNQPIIRIQSIAPSLQVITSKQRPRKLTLMGSNGHEFVFLLKGHEDLRQDERVMQLFGLVNTLLANDPTSLRKN
LSIQRYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKKILLNIEHRIMLRMAPDYDHLTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSSEVWFDRRTNYT
RSLAVMSMVGYILGLGDRHPSNLMLDRLSGKILHIDFGDCFEVAMTREKFPEKIPFRLTRMLTNAMEVTGLDGNYRITCHTVMEVLREHKDSVMAVLEAFV
YDPLLNWRLMDTNTKGNKRSRTRTDSYSAGQSVEILDGVELGEPAHKKTGTTVPESIHSFIGDGLVKPEALNKKAIQIINRVRDKLTGRDFSHDDTLDVPT
QVELLIKQATSHENLCQCYIGWCPFW

FIGURE 4.2: FASTA sequence of mTORC1

4.3 Physicochemical Characterization of Proteins

Scientists utilize an online tool called ProtParam to forecast a variety of parameters, including the molecular and structural properties of specific proteins. Table 4.1 lists the physiochemical characteristics of mTORC1.

TABLE 4.1: The physicochemical properties of mTORC1

Target Protein	**MW**	**PI**	**NR**	**PR**	**Ext Co1.**	**Ext Co2.**	**Instability Index**	**Aliphatic Index**	**GRAVY**
mTORC1	288891.6	6.73	278	274	310370	308370	39.45	93.26	-0.184

The aliphatic index indicates the aliphatic composition of a protein. The protein's thermostability is shown by the high aliphatic index value.

The aliphatic index indicates the aliphatic composition of a protein. The protein's thermostability is shown by the high aliphatic index value. Protein residues with both positive and negative charges are included in molecular weight. Low GRAVY shows better interaction with water molecules. The total number of negatively charged residues and the molecular weight (Asp +ve Glu), total number of positively charged residues (Arg +ve Lys), and theoretical isoelectric point (when protein is neutral and free of charge) are represented by the symbols MW, pl, NR, and PR.

Grand average of hydropathicity is represented by GRAVY, extinction coefficients are represented by Ext.Co1 when all pairings of Cys residues form cystines, and extinction coefficients are represented by Ext. Co2 when all Cys residues are reduced [88].

4.4 Functional Domain Identification of Protein

To ascertain the domains and functional sites of specific proteins, Interpro was utilized. It is a useful tool for functional study of protein sequences. Sequence, structure, and relationships are all involved in conserved domains. Proteins can have many functional domains, each of which carries out a distinct task.

The functional domain of a protein is the active region that engages in interactions between proteins and other substances [89].

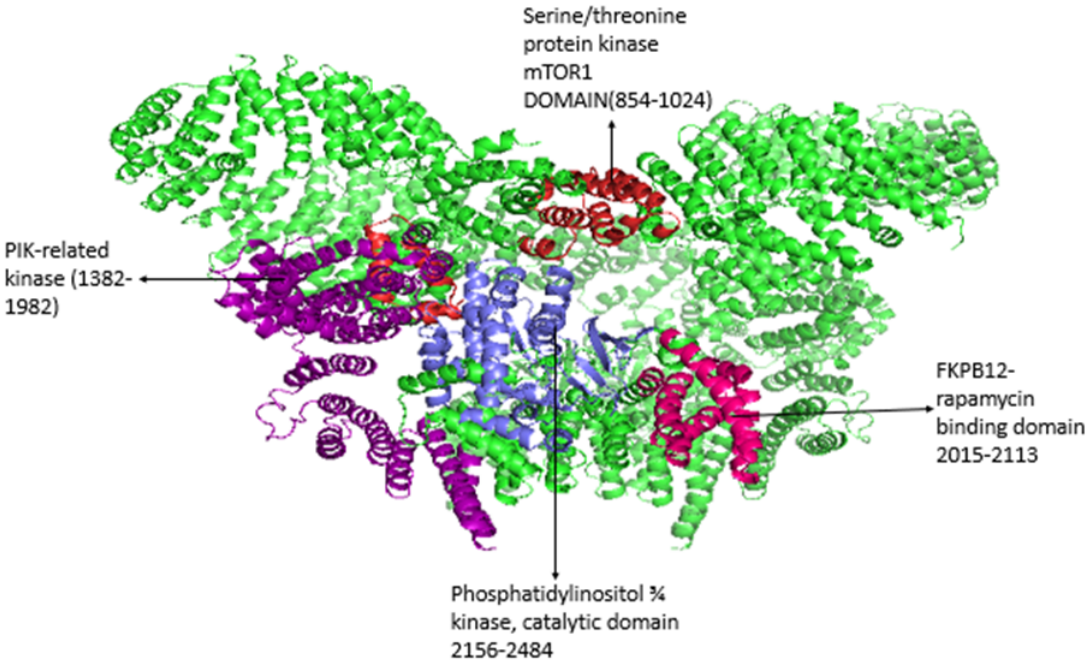


FIGURE 4.3: Domains of mTORC1

mTORC1protein consists of four domains as shown in figure 4.3. The serine/threonine protein kinase mTORC1 domain is shown in red colour, it starts from 854 and ends at 1024 amino acid residue. Phosphatidylinositol ¾ kinase, catalytic domain shown in blue colour, it starts from 2156 and ends at 2484 amino acid residue. FKPB12-rapamycin binding domain is shown in, it starts from 2015 and ends at 2113 amino acid residue.

The PIK-related kinase domain is shown in purple colour, it starts from 1382 and ends at 1982 amino acid residue.

4.5 Active Site Identification

The CASTp software, which determines the number of pockets that can be bound and gives details on their surface area and volume, was utilized to determine the active sites of the protein. Figure 4.4 below illustrates the areas and volumes of target protein mTORC1. The red area depicts the active sites available for a particular protein.

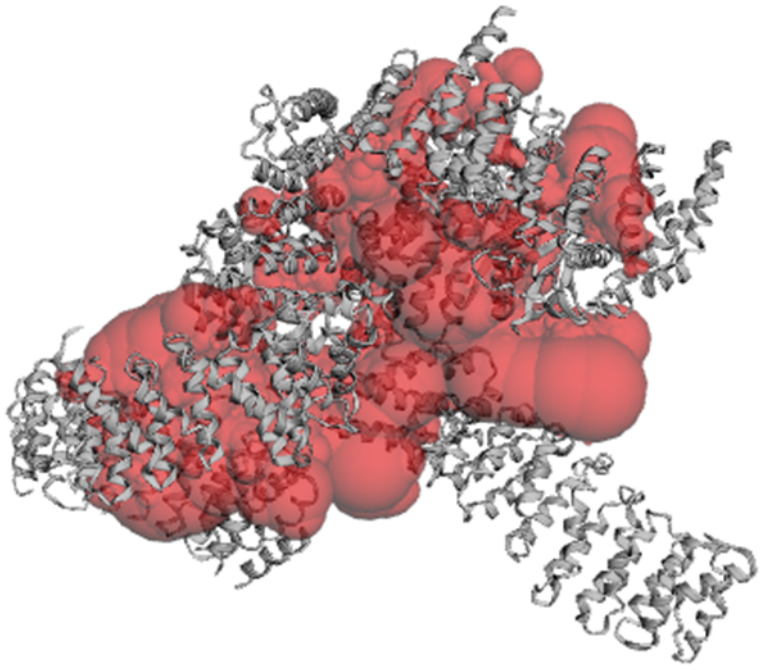


FIGURE 4.4: Active sites of mTORC1

CASTp data depicts different numbers of pockets for each protein. According to CASTp data, the mTORC1 consists of 331 pockets. The area of the largest pocket is 21016.072 while the area of the smallest pocket is 2.865. The area and volume of some pockets of mtorc1 are shown in table 4.2.

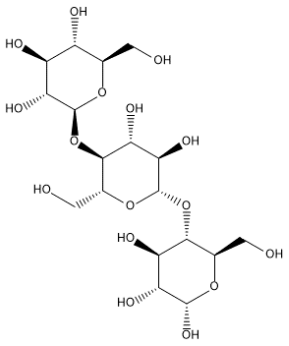
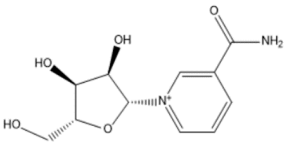
TABLE 4.2: Area and volume of mTORC1 binding pockets

Pocket ID	**Mtorc1**		**Pocket ID**	**Mtorc1**	
	Area	**Volume**		**Area**	**Volume**
1	21016.07	61373.8	11	60.45	44.303
2	524.585	723.989	12	79.711	38.122
3	284.768	215.968	13	60.45	35.404
4	93.003	150.579	14	81.02	34.678
5	47.311	82.494	15	76.262	32.679
6	120.825	68.621	16	60.243	32.308
7	117.633	63.292	17	119.518	32.286
8	87.758	56.774	18	55.293	30.942
9	48.799	50.469	19	65.565	30.115
10	89.986	47.005	20	53.859	27.292

4.6 Retrieval of Chemical Structure of the Ligands

The ligand to be selected should be on the best resolution structure with that based on crystal-chemical class and their binding affinities. With that what matters is the conformational selection of the ligand. A ligand preferentially binds to one of the conformers in this selection process, boosting its numbers in comparison to the overall population and fortifying it of that protein. The largest chemical databank in the world, PubChem, was searched for ligands, or fungal compounds [90]. These ligands' 3D structures were extracted in SDF format from the PubChem database. Nicotinamide riboside and beta-glucan were selected as bioactive metabolites by Fatima et al and followed the same methodology for ligand acquisition [91]. Table 4.3 shows all the selected ligands with the information regarding their structure.

TABLE 4.3: Chemical structure of ligands

S.No	Ligands Name	Molecular Formula	Structure
1	Beta-glucan	$C_{18}H_{32}O_{16}$	
2	Nicotinamide riboside	$C_{11}H_{15}N_2O_5$	

4.7 Energy Minimization of Ligands

After downloading the structures of the ligands that were selected the next step that was performed was minimizing the energy of these ligands. This step is an important one as we can't use simply the downloaded structure as the ligands are

unstable and it can directly affect the docking vina scores. The refined structures of ligands obtained after energy minimization are given in figure 4.5.

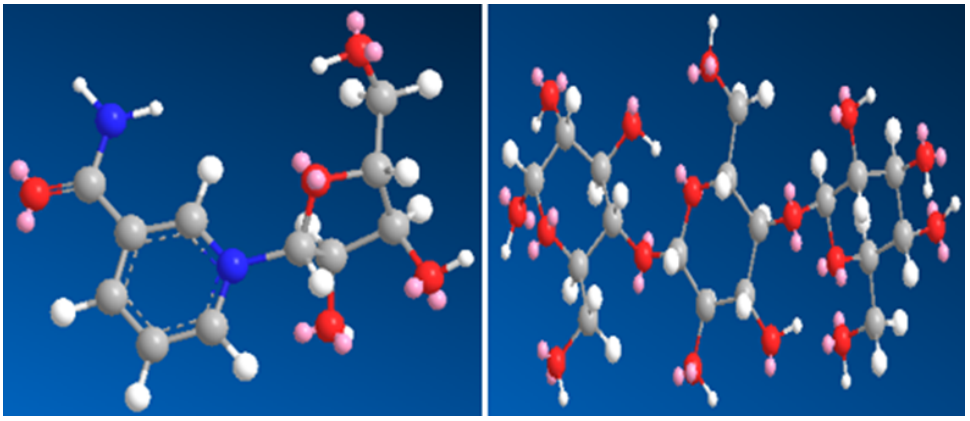


FIGURE 4.5: Energy minimization of (a) nicotinamide riboside (b) beta-glucan

4.8 Virtual Screening of Ligands

For compounds to be separated as both drug-like and nondrug-like virtual screening and pharmacokinetic properties are followed. The Lipinski rule deals with certain parameters like molecular weight which should be ≤ 500, log P ≤ 5, H-bond donors ≤ 5, H-bond acceptors ≤ 10, and rotatable bonds ≤ 5. These rules are to be followed by orally active compounds. The drug-like is dependent on the mode of administration. A compound is considered a drug when it follows 3 or more rules and if a compound breaks more than two rules then its absorption is not good [92]. Virtual screening of ligands is shown is table 4.4.

TABLE 4.4: Virtual screening of ligands

Ligands	**Log P value**	**Molecular weight**	**H-bond donor**	**H-bond acceptor**	**Rotatable bonds**
Nicotinamide riboside	-2.3155	255.25	4	5	3
Beta-glucan	-0.1623	498.438	5	10	5

Table 4.4 shows that both ligands follow the lipinski rule. The log P value of all ligands is below 5 and the molecular weight of all is also below 500. The hydrogen bond donor, acceptor, and rotatable bonds are also in range for all ligands.

4.9 ADME/T Analysis of Ligands

A second investigation was conducted utilizing the online program pkCSM to determine the ADME/T properties of ligands as a measure of pharmacokinetics after the lipinski rule. There are two general words in pharmacology: pharmacodynamics and pharmacokinetics. Within the field of pharmacology, pharmacodynamics examines how medications affect the body. In pharmacokinetics, we investigate how medications are absorbed, distributed, metabolized, and excreted [93].

4.10 Absorption Properties of Ligands

The CaCO2 solubility helps in predicting the absorption of the drugs which are administered orally. Value >0.90 (log Papp in 10-6 cm/s) is considered as high CaCO2 permeability. The water solubility of the ligands is given as log mol/L, this indicates the compound solubility in water at 25 C. Hence the lipid-soluble drugs will be less soluble than the water-soluble drugs. Intestinal absorption indicates the value or proportion of the compound that will be absorbed into the intestines. A value less than 30% is considered poorly absorbed [94].

P-glycoprotein is an ABC transporter that functions to extrude toxins or other xenobiotics from the cells by acting as a biological barrier. P-glycoprotein inhibition can be a therapeutic target or it can act in contradiction. Skin permeability is important for developing transdermal drugs. Any compound with a value > -2.5 has a low skin permeability [95]. The absorption properties of nicotinamide riboside and beta-glucan are given in table 4.5.

TABLE 4.5: Absorption properties of ligands

ADMET Properties		**Nicotinamide riboside**	**Beta-glucan**
Absorption	Water solubility	-2.707	-1.455
	$CaCO_2$ Permeability	-5.6	-6.25
	Intestinal absorption(human)	90.73	93.938
	Skin permeability	-2.41	0.97
	P-glycoprotein substrate	No	No

Table 4.5 continued from previous page

ADMET Properties		Nicotinamide riboside	Beta-glucan
	P-glycoprotein I inhibitor	No	No
	P-glycoprotein II inhibitor	No	No

All ligands have good water solubility and skin absorption, normal $CaCO_2$ permeability, and more than 90% intestinal absorption. For any ligand, skin permeability is modest. A chemical can be readily pumped out of the cells to lessen its absorption if it tests positive for Pgp substrate.

4.11 Distribution Properties of Ligands

The theoretical volume or VDss indicates the entire dosage of the medication that must be dispersed evenly to produce a concentration similar to that of blood plasma. The medication is more widely disseminated in the tissues than in the plasma if the VDss value is more than 2.81 L/kg. The VDss will be low if the value is below 0.71 L/kg. Many drugs in the plasma exist in an equilibrium between a bounded and an unbounded state of the serum proteins. As a drug binds more to the serum proteins it will have less efficiency of diffusion to cellular membranes [96].

The blood-brain barrier reduces the number of exogenous substances that can reach the brain directly while protecting it. If a compound has a value of logBB >0.3 then it will easily cross the BBB barrier hence been effective and if it is logBB<-1 then it is poorly distributed. Compounds with a value of logPS>-2 penetrate the CNS whereas value logPS<-3 does not penetrate the CNS [97]. Table 4.6 shows the distribution properties of nicotinamide riboside and beta-glucan. The table indicates all ligands have safe range which is given below.

TABLE 4.6: Distribution properties of ligands

ADMET Properties		Nicotinamide riboside	Beta-glucan
Distribution	VDss (human)	0.84	0.03
	Fraction unbound (human) Fu	0.48	0.56

Table 4.6 continued from previous page

ADMET Properties	Nicotinamide riboside	Beta-glucan
BBB permeability log BB	0.972	0
CNS permeability log PS	-3.83	-4.31

The parameters through which the distribution properties are determined include VDss which is in the given range to be distributed in the blood and the tissues. The values of the fraction unbound of these ligands show that out of the total dose, this fraction will not be bound to the protein. All these ligands mentioned in above table cannot cross the blood-brain barriers and the CNS.

4.11.1 Metabolism Properties of Ligands

The enzyme cytochrome P450 is in charge of the liver's detoxification process. Many drugs get deactivated by this enzyme but certain drugs are capable of activating. This enzyme's inhibitors can directly affect the metabolism of the drug hence should not be used. Similarly, CYP2D6 and CYP3A4 are responsible for the drugs' metabolism. Inhibition of these affects the pharmacokinetics of the drug in use [98]. The prediction of the metabolism of ligands is given below. Table 4.7 shows the metabolic properties of nicotinamide riboside and beta-glucan.

TABLE 4.7: Metabolism properties of ligands

ADMET Properties		Nicotinamide riboside	Beta-glucan
Metabolism	CYP2D6 substrate	No	No
	CYP3A4 substrate	No	No
	CYP1A2 inhibitor	No	No
	CYP2C19 inhibitor	No	No
	CYP2C9 inhibitor	No	No
	CYP2D6 inhibitor	No	No
	CYP3A4 inhibitor	No	No

All ligands mentioned are neither the CYP2D6 substrates nor they are CYP2C19, CYP2C9, CYP2D6, and CYP3A4 inhibitors whereas the rest parameters are shown in the table. Table 4.7 shows the metabolic properties of selected ligands.

It indicates that both ligands mentioned are not CYP2D6, or CYP3A4 substrates. Whereas these ligands are not CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor and CYP1A2 inhibitor.

4.11.2 Excretion Properties of Ligands

Two organs are involved in drug excretion, the liver, which is engaged in biliary excretion, and the kidneys, which are involved in renal excretion. Excretion may also include other organs, such as the lungs in the case of volatile or gaseous substances. Moreover, drugs can be expelled through tears, saliva, and perspiration. The excretion values of the ligands are given in table 4.8.

TABLE 4.8: Excretion properties of ligands

ADMET Properties		**Nicotinamide riboside**	**Beta-glucan**
Excretion	Total Clearance	3.74	4.4
	Renal OCT2 substrate	No	No

The Renal OCT2 substrate acts as a transporter that helps in clearing the drugs and other compounds. Total clearance indicates hepatic clearance which means the drug is metabolized and renal clearance indicates the drug is excreted [99]. Since none of these ligands are renal OCT2 substrates, the body would not be able to eliminate them, as the above table illustrates and hence the total clearance values are given accordingly.

4.11.3 Toxicity Properties of Ligands

An online program called pkCSM is used to check the ADMET i.e. absorption, distribution, metabolism, excretion, and toxicity values of drugs and bioactive substances. By using this tool we will determine the toxicity of the ligands selected for this different methods are used to test whether a given ligand is toxic or not. AMES toxicity test is used to test the mutagenic potential of the compound by using bacteria. If it shows a positive response, then the ligand is mutagenic which

can also act as a carcinogen. The toxicity of *T. Pyriformis* (protozoa bacterium) is used as a toxic endpoint in the *T. Pyriformis* toxicity method. Any value >-0.5 log ug/L is considered toxic. The values predicted in the Minnow toxicity test are used to represent the concentration at which the compound could cause the death of 50% of the minnows. The value below 0.5 mM is regarded as acute toxic [100].

In the oral rat chronic toxicity test, the concentration of the drug that necessitates a certain amount of treatment time is related to the expected log value of the lowest observed adverse impact, expressed in log mg/kg bw/day. A hepatotoxicity test predicts that if a compound could affect liver functioning or not. A hazardous substance's maximum tolerated dose (MRTD) indicates how dangerous it is to a certain person. In phase 1 clinical studies, ligand toxicity information helps guide the initial recommended dosage of a medication. The logarithmic representation of the MRTD value is log mg/kg/day. If a chemical's value is less than or equal to 0.477 log (mg/kg/day), it is said to have a low MRTD; if it is more than 0.477 log (mg/kg/day), it is said to have a high MRTD [101]. The toxicity values of all ligands are given in table 4.9.

TABLE 4.9: Toxicity values of ligands

ADMET Properties		**Nicotinamide riboside**	**Beta-glucan**
Toxicity	AMES toxicity	No	No
	Max tolerated dose (human)	2.05	1.72
	hERG I inhibitor	No	No
	hERG II inhibitor	No	No
	Oral rat acute toxicity (LD50)	1.74	1.47
	Oral rat chronic toxicity (LOAEL)	2.45	3.77
	Hepatotoxicity	No	No
	Skin sensitization	No	No
	T.Pyriformis toxicity	-0.37	0.285
	Minnow toxicity	0.18	-0.91

No inhibition of hERG I or hERG II was seen in any ligand. None of the ligands demonstrated hepatotoxicity, cutaneous sensitivity, or AMES toxicity and skin sensitivity. Every ligand's MRTD value is within the range. *T. pyriformis* activity

was seen in all ligands at least 0.5 log µg/L. All ligands had minnow toxicity values above the acceptable threshold of 0.5 mM.

4.12 Molecular Docking

To carry out docking, the three-dimensional structures of the protein and ligands are used. An online blind auto docking program called CB dock is utilized for this. CB Dock computes the cavity sizes and predicts the protein binding locations. CB Dock provides us with the top five possess and receptor models upon docking. Based on the cavity size and the vina score, the optimal position was chosen among these five [102]. Figure 4.6 shows the best docking complexes of ligands and proteins.

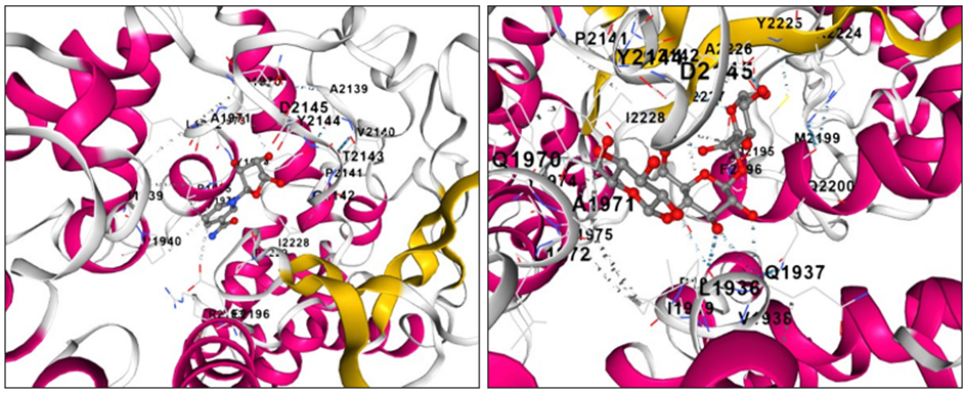


FIGURE 4.6: Docking complexes of mTORC1 with (a) nicotinamide riboside (b) beta-glucan

Molecular docking is performed by using mTORC1as the receptor protein and the two ligands i.e. nicotinamide riboside and beta-glucan are selected. The ligands are in SDF format, while the protein is in PDB format. After verifying the input files, CB Dock uses Open Babel and MGL tools to transform them into files in the pdbqt format. Next, CB dock determines the receptor's cavities as well as the diameters and centers of the top five cavities. The protein-ligand interaction's high-affinity score determines which of the five optimal conformations is the best [103]. The scores obtained after the docking of proteins and ligands are shown in tables 4.10.

TABLE 4.10: Docking score of ligand-protein complexes

Target Protein	**Ligands**	**Docking score**	**Cavity volume**	**Docking size(x,y,z)**
mTORC1	Nicotinamide riboside	-6.8	827	26,19,26
	Beta-glucan	-7.5	827	24,24,24

Table 4.10 shows the docking result of two selected ligands that are nicotinamide riboside and beta-glucan. It shows that beta-glucan has the highest binding score of -7.5, with mTORC1 protein. Nicotinamide riboside has a binding score of -6.8 with mTORC1 protein. A similar methodology was adopted by Dhamodiran et al for docking of cycloleonuripeptide_B-AMPK$\alpha 2\beta 1$, R734_AMPK$\alpha 2\beta 1$ and cycloleonuripeptide_B-AMPK$\gamma 1$. The best one was cycloleonuripeptide|_B-AMPK$\alpha 2\beta 1$complex with a docking score of -9.91 [104].

4.13 Analysis of Docked Complexes via Ligplot

To understand docking data, how the ligand and protein interact in active pockets was estimated. Hydrogen bonding and hydrophobic bonding interactions were the two types of interactions that were investigated. Ligplot Plus (v.1.4.5) was used to analyze the interactions between proteins and ligands. By using Ligplot the interaction of the active conformation of ligands and the target protein has been identified [105].

The saved conformations for the ligand-receptor complex of each molecule were analyzed in detail. This program creates schematic representations of the protein-ligand interactions between the specified ligands in the PDB file automatically. The docked files were uploaded in PDB format to get hydrogen and hydrophobic bonding. A significant number of hydrophobic and hydrogen bond interactions were observed between the ten ligands and the four target proteins [106]. Ligand-receptor complex shows strong hydrogen bonding and hydrophobic interactions. The following diagrams show the ligand-receptor interactions.

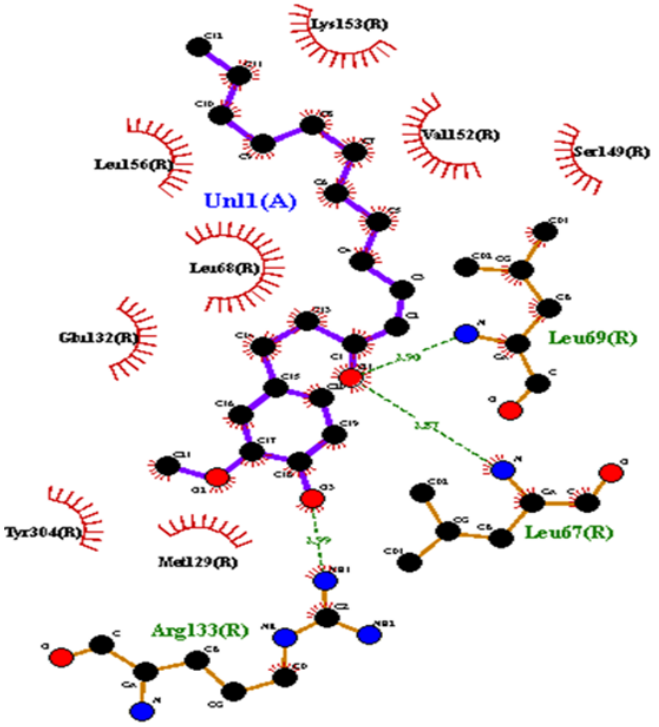


FIGURE 4.7: Interaction analysis of nicotinamide riboside- mTORC1

Above figure shows that there are three hydrogen bonds and eight hydrophobic interactions in nicotinamide riboside- mTORC1 complex

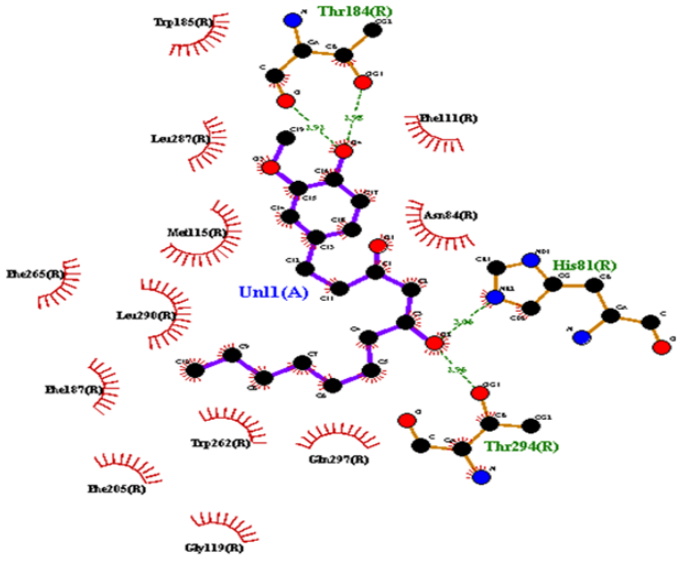


FIGURE 4.8: interaction analysis of betaglucan- mTORC1

Above figure shows that there are four hydrogen bonds and twelve hydrophobic interactions in betaglucan- mTORC1 complex. The complete detail of these interactions along with name of amino acids and hydrogen bond distance is shown in table 4.11.

TABLE 4.11: Docking interaction analysis

S.No	**Docking complex**	**Binding energy**	**No of HBs**	**Amino acids**	**H-bonding distance**	**Hydrophobic interactions**
1	Nicotinamide riboside-mTORC1	-6.8	3	2.9	Leu69	Ser149
				2.57	Leu6	Val152
				2.99	Arg133	Lys153
						Leu156
						Leu168
						Glu132
						Tyr304
						Met129
2	Betaglucan-mTORC1	-7.5	4	3.06	His81	Phe111
				2.96	Thr294	Asn84
				2.93	Thr184	Trp185
				2.93	Thr184	Leu287
						Met115
						Ehe265
						Leu290
						Ehe187
						Trp262
						Gln297
						Ehe205
						Gly119

Table 4.11 below shows the details of hydrogen and hydrophobic interactions between the receptor protein and selected ligands. The values show that beta-glucan forms the highest hydrogen bonds i.e. four while the highest hydrophobic interactions are also shown by beta-glucan. Nicotinamide riboside also form a considerable number of hydrophobic interactions and hydrogen bonding but their

number is less than beta-glucan. Bisht et al predicted the 3D conformation and 2D interaction plots of best-docked peptides and ligands in the protein binding site. Ligands exhibited comparatively interesting interactions with the receptors, further validating the docking methodology [107].

4.14 Lead Compound Identification

The ligands' pharmacokinetic and physiochemical properties determine their fate as for being drug or non-drug compounds. Lipinski's rule is the first filter and pharmacokinetics is the second filter for this identification. All ligands were seen obeying the lipinski rule of five so they all get selected for docking. The next knock-out stage is pharmacokinetic screening. In this screening beta-glucan was selected as it showed the best ADME/T values over nicotinamide riboside concerning moderate water solubility, good intestinal absorption, and minimal toxicity. Number of hydrogen bonds, docking score and hydrophobic interactions of beta-glucan are also good than other ligands, so beta-glucan was selected as lead compound.

4.15 Reference Anti-aging Drug Identification

Sirolimus, also known as rapamycin, exerts its immunosuppressive effects by inhibiting the mammalian target of the rapamycin (mTOR) pathway, a crucial regulator of cell growth, proliferation, and survival. Specifically, sirolimus binds to the intracellular protein FKBP-12 (FK506 binding protein 12), forming a complex that directly interacts with mTOR complex 1 (mTORC1). This interaction leads to the inhibition of mTORC1's kinase activity, resulting in the blockade of key downstream signaling pathways that promote protein synthesis, cell cycle progression, and angiogenesis. Consequently, sirolimus effectively suppresses T-lymphocyte activation and proliferation, which are essential processes in the immune response. By inhibiting these pathways, sirolimus prevents organ rejection in transplant recipients and also exhibits potential therapeutic benefits in various diseases characterized by abnormal cell proliferation and growth [108].

4.16 Sirolimus and Lead Compound Comparison

To identify the better treatment for aging and the best fungal bioactive metabolite for controlling aging mechanisms, a comparison between sirolimus and beta-glucan was done. The comparison was performed through parameters like ADME/T properties, lipinski rule and docking complexes analysis.

4.17 Sirolimus Structure Prediction

First of all sirolimus structure was downloaded in SDF format from PubChem. Then its energy was minimized by using chem3D pro to get the refined structure. The chemical formula of sirulimus is C51H79NO13 and refined structure of sirolimus is given in figure 4.9.

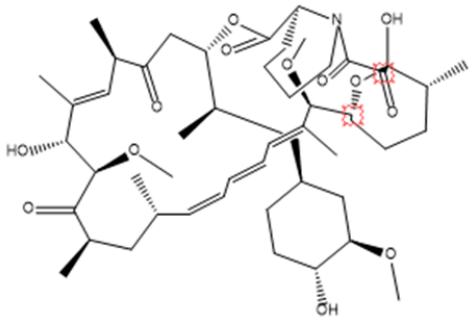


FIGURE 4.9: Refined structure of sirolimus

4.18 Lipinski Rule Comparison

The sirolimus and beta-glucan were compared to observe their response to the lipinski rule. To evaluate their pharmacokinetic properties to assess their bioavailability, safety, efficacy, and drug-likeness. The comparison is given in table 4.12.

TABLE 4.12: Comparison of lipinski rule

Ligands	**Log P value**	**Molecular weight**	**H-bond donor**	**H-bond acceptor**	**Rotatable bonds**
Sirolimus	6.1806	914.187	3	13	6

Table 4.12 continued from previous page

Ligands	Log P value	Molecular weight	H-bond donor	H-bond acceptor	Rotatable bonds
Beta-glucan	-0.1623	498.438	5	10	5

Table 4.12 shows that sirolimus is seen violating more than three lipinski rules whereas beta-glucan is following lipinski rule of five.

4.19 ADMET Properties Comparison

To identify a better drug candidate, the absorption, distribution, metabolism, excretion, and toxicity properties of the medication and the lead chemical were compared using the ADME/T properties.

4.19.1 Absorption Properties Comparison

The comparison between sirolimus and beta-glucan for checking absorbance models is given in table 4.13

TABLE 4.13: Absorption properties of ligands

ADMET Properties		Beta-glucan	Sirolimus
Absorption	Water solubility	-1.455	-3.984
	$CaCO_2$ Permeability	-6.25	-4.87
	Intestinal absorption(human)	93.938	64.393
	Skin permeability	0.97	-1.51
	P-glycoprotein substrate	No	Yes
	P-glycoprotein I inhibitor	No	Yes
	P-glycoprotein II inhibitor	No	Yes

Absorbance in the stomach of beta-glucan is more than sirolimus. The skin permeability value of both is in range. The substrate model for P-glycoprotein is not well absorbed, since P-glycoprotein serves as a biological barrier and an ABC transporter, the P-glycoprotein substrate model is important.

4.19.2 Distribution Properties Comparison

The comparison between the distribution properties of sirolimus and beta-glucan is given in table 4.14.

TABLE 4.14: Distribution properties comparison

ADMET Properties		**Beta-glucan**	**Sirolimus**
Distribution	VDss (human)	0.03	3.05
	Fraction unbound (human) Fu	0.56	0.99
	BBB permeability log BB	0.0	0.0
	CNS permeability log PS	-4.31	-3.09

The above table shows the comparative distribution properties of beta-glucan and sirolimus. Other parameters are in range except for CNS permeability. The CNS, or central nervous system, model is predicated on the idea that drugs with a log PS value more than -2 may readily permeate the CNS, but those with a log PS value less than -3 cannot. Because of its low value, beta-glucan cannot enter the central nervous system.

4.19.3 Metabolism Properties Comparison

The comparison between the metabolism properties of sirolimus and beta-glucan is given in table 4.15.

TABLE 4.15: Metabolic properties comparison

ADMET Properties		**Beta-glucan**	**Sirolimus**
Metabolism	CYP2D6 substrate	No	No
	CYP3A4 substrate	No	Yes
	CYP1A2 inhibitor	No	No
	CYP2C19 inhibitor	No	No
	CYP2C9 inhibitor	No	No
	CYP2D6 inhibitor	No	No
	CYP3A4 inhibitor	No	No

Mostly located in the liver, cytochrome P450 is an enzyme involved in detoxification because it oxidizes foreign substances to make them easier for the body to eliminate. It either deactivates or activates some medicines.

Therefore, determining whether a chemical is a P450 substrate or not, as well as if it is a P450 inhibitor, is crucial. Table 4.15 illustrates how the metabolic characteristics of the reference medication and lead compound are almost identical.

4.19.4 Excretion Properties Comparison

The comparison between the excretion properties of beta-glucan and sirolimus is given in table 4.16.

TABLE 4.16: Excretion properties comparison

ADMET Properties		**Beta-glucan**	**Sirolimus**
Excretion	Total Clearance	4.4	8.17
	Renal OCT2 substrate	No	No

The value of total clearance is a combination of hepatic and renal clearance and is important so that the dose rates of the drugs can be assessed. Compared to beta-glucan, sirolimus has a higher overall clearance.

The renal OCT2 (organic cation transporter 2) model is the second one, and it aids in the renal clearance of medications and other substances. Concerning inhibitors, one may experience negative effects from being an OCT2 substrate. The medication and the main ingredient are not substrates of renal OCT2.

4.19.5 Toxicity Properties Comparison

The comparison between the toxicity properties of beta-glucan and sirolimus is given in table 4.17.

TABLE 4.17: Toxicity properties comparison

ADMET Properties		**Beta-glucan**	**Sirolimus**
Toxicity	AMES toxicity	No	No
	Max tolerated dose (human)	1.72	0.58
	hERG I inhibitor	No	No
	hERG II inhibitor	No	No

Table 4.17 continued from previous page

ADMET Properties	Beta-glucan	Sirolimus
Oral rat acute toxicity (LD50)	1.47	3.64
Oral rat chronic toxicity (LOAEL)	3.77	2.21
Hepatotoxicity	No	No
Skin sensitization	No	Yes
T.Pyriformis toxicity	0.285	-27.34
Minnow toxicity	-0.91	34.66

Nine models are used to assess the toxicity of the lead ingredient and the standard medication. No one is mutagenic, according to AMES toxicity model 1. According to the second maximum tolerated dosage model, a value is deemed low if it is equal to or less than 0.477 log mg/kg/day, whereas a higher value is deemed high. The chart indicates that the tolerable dosage of beta-glucan is minimal.

The third model involves hERG I and II inhibitors, none of which is an inhibitor. The relative toxicity is evaluated using the fourth model of oral rat acute toxicity.

Model 5 of oral rat chronic toxicity provides the lowest dosage values that might have a negative outcome. Hepatotoxicity model 6 indicates that a medicine may not harm the liver. The table demonstrates that sirolimus and beta-glucan are not hepatotoxic. The number seven is used to verify the dermal goods model's sensitivity to the skin.

The skin has exhibited sensitivity to sirolimus. To test for toxicity, model 8 employs *T. Pyriformis*, whereas model 9 utilizes minnows. The relative toxicity values of beta-glucan and sirolimus are displayed in table 4.17.

4.20 Docking Score Comparison

The target protein mTORC1 was docked with both the lead and standard compounds, and the docking result provided us the highest binding score. The dock complex of sirolimus against mTORC1 are shown in figure 4.10.

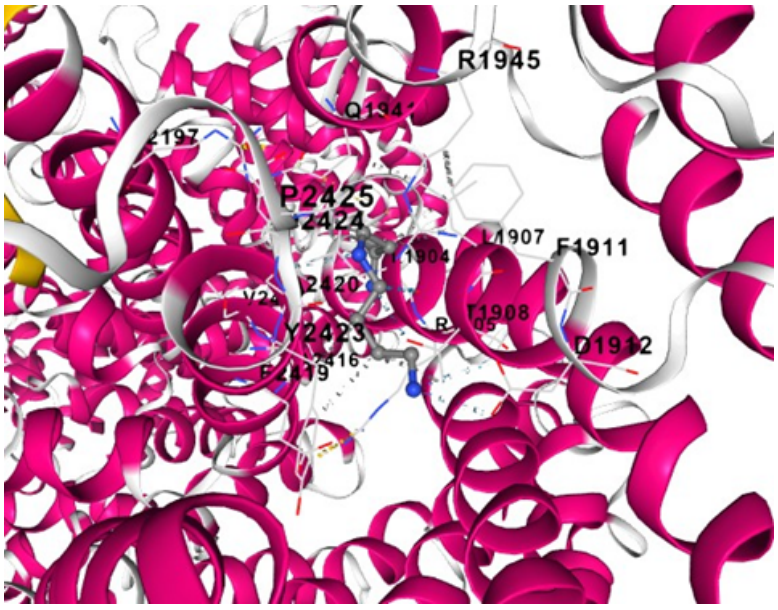


FIGURE 4.10: Docking complex of sirolimus- mTORC1

Table 4.18 shows the docking score comparison of the standard drug sirolimus and lead compound beta-glucan.

TABLE 4.18: Docking comparison of beta-glucan and sirolimus

Target Protein	**Ligands**	
mTORC1	Sirolimus	Beta-glucan
	-4.5	-7.5

As can be shown in table 4.18, the vina score of the lead compound beta-glucan is significantly greater than that of the generic medication sirolimus. The docking score of the sirolimus against target protein mTORC1 is -4.5 while for beta-glucan it is -7.5. These results show that lead compound beta-glucan can bind with target protein mTORC1 more efficiently than the of standard drug sirolimus.

4.21 Docking Analysis Comparison

Based on the quantity of hydrogen bonds, hydrophobic interactions, interacting amino acids, and steric interactions, ligplot evaluates the docking results. The following figure show the docking analysis of standard drug sirolimus with target protein mTORC1.

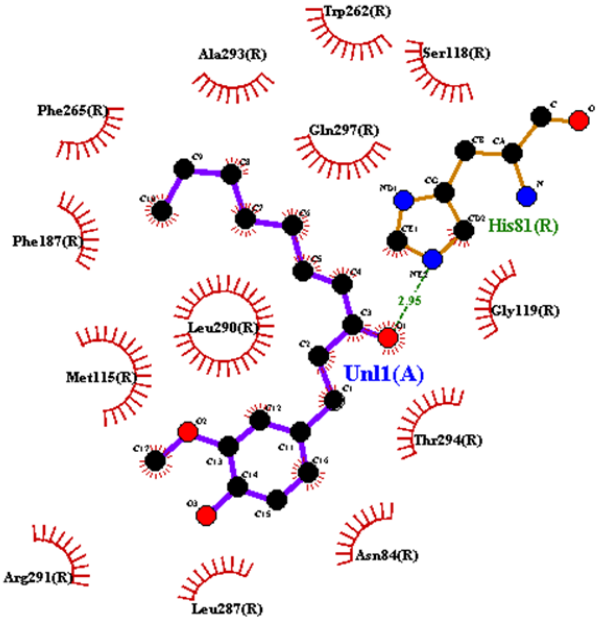


FIGURE 4.11: Interaction analysis of sirolimus- mTORC1

Above figure shows that there is one hydrogen bond and thirteen hydrophobic interactions in dock complex of sirolimus and mTORC1.

The complete details of the docking analysis of standard drug sirolimus and lead compound beta-glucan are shown in table 4.19.

TABLE 4.19: Interaction analysis comparison

S.No	**Docking complex**	**Binding energy**	**No of HBs**	**Amino acids**	**H-bonding distance**	**Hydrophobic interactions**
1	Betaglucan-mTORC1	-7.5	4	3.06	His81	Phe111
				2.96	Thr294	Asn84
				2.93	Thr184	Trp185
				2.93	Thr184	Leu287
						Met115
						Ehe265
						Leu290
						Ehe187
						Trp262
						Gln297
						Ehe205
						Gly119

Table 4.19 continued from previous page

S.No	Docking complex	Binding energy	No of HBs	Amino acids	H-bonding distance	Hydrophobic interactions
2	Sirolimus-mTORC1	-4.5	1	2.95	His81	Trp 262 Serl18 Ala293 Gln297 Phe265 Phe187 Met115 Leu290 Arg291 Leu287 Asn84 Thr294 Gly119

Table 4.19 shows the comparison of docking analysis of standard drug sirolimus with lead compound beta-glucan. A comparison shows that the sirolimus- mTORC1 complex consist of one hydrogen bond and thirteen hydrophobic interactions while betaglucan- mTORC1 complex consist of four hydrogen bond and twelve hydrophobic interactions.

An overall comparison shows that lead compound beta-glucan showed the best physicochemical and pharmacokinetic properties over reference drug sirolimus. The number of hydrogen bonds and docking score of beta-glucan are also good as compared to the reference drug sirolimus. It shows that beta-glucan can act as a promising anti-aging therapeutic candidate in the future.

Chapter 5

Conclusion and Future Prospects

The role of yeast postbiotics in anti-aging has garnered considerable attention due to their potential to modulate various biological processes associated with aging. Yeast postbiotics, which include bioactive compounds produced by yeast, have demonstrated significant antioxidant and anti-inflammatory properties that are crucial in mitigating oxidative stress and inflammation, key contributors to the aging process. By enhancing gut health, modulating immune responses, and improving the integrity of the skin barrier, these postbiotics provide a holistic approach to promoting healthy aging. Furthermore, the incorporation of yeast postbiotics in both nutritional supplements and cosmetic products represents a comprehensive strategy to address both internal and external manifestations of aging [109]. The findings of this study suggest that yeast postbiotics not only improve overall health and well-being but also have the potential to revolutionize the fields of nutrition and cosmetics by introducing innovative anti-aging solutions.

This research is based on the anti-aging characteristics of yeast postbiotics, including beta-glucan and nicotinamide glucoside which indicates a prospective avenue for therapeutic implementation. According to molecular docking studies, beta-glucan has a considerable affinity for important ageing-related proteins like mTORC1. Numerous hydrogen bonds and hydrophobic contacts define these interactions, indicating a strong and stable relationship that is essential for blocking aging-related processes. Given their strong binding, beta-glucan may be able to

control these pathways effectively, thereby delaying or even stopping some elements of the ageing process.

Furthermore, beta-glucan's ADME/T profile provides additional support to its prospective use as a medicinal substance. Oral administration of the chemical is advantageous due to its modest solubility in water and a good range of intestinal absorption. Moreover, it also exhibits minimal toxicity, rendering it a more secure substitute in contrast to other anti-aging therapies now accessible. Beta-glucan exhibits better pharmacokinetic qualities and a higher binding affinity than sirolimus, a common anti-aging prescription. These findings highlight beta-glucan's potential as a safer and more effective anti-aging treatment.

The future prospects of yeast postbiotics in anti-aging research and application are promising. Further studies should focus on elucidating the precise mechanisms by which these postbiotics exert their anti-aging effects at the molecular and cellular levels. Additionally, clinical trials are necessary to validate the efficacy and safety of yeast postbiotics in human populations. Exploring the synergistic effects of combining yeast postbiotics with other bioactive compounds could lead to the development of more potent anti-aging formulations. The potential for yeast postbiotics to be incorporated into a wide range of products, from dietary supplements to advanced skincare solutions, opens new avenues for innovation in the health and wellness industries.

Based on the findings of this study, it is recommended that yeast postbiotics be incorporated into daily health and skincare routines to promote overall health and slow the aging process. Health practitioners should advocate for the inclusion of these postbiotics as they offer significant antioxidant and anti-inflammatory benefits. Companies within the nutrition and cosmetics sectors should invest in the development of products that harness the power of yeast postbiotics, emphasizing their natural origin and health-promoting properties. Additionally, further research is necessary to fully explore the mechanisms through which yeast postbiotics exert their anti-aging effects, and clinical trials are essential to validate their efficacy and safety in humans.

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