"Arginase in *Bacillus circulans*"

Major Project Report submitted in fulfillment of project of

BACHELORS OF TECHNOLOGY

IN

BIOTECHNOLOGY

by

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UNDER THE SUPERVISION OF

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BIOTECHNOLOGY AND BIOINFORMATICS DEPARTMENT

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SOLAN

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DECLARATION

I hereby declare that the major project work entitled "Arginase in *Bacillus circulans*" has been solely submitted to the Biotechnology and Bioinformatics Department, Jaypee University of Information Technology, Wakhnaghat, under the guidance of my supervisor **Dr SAURABH BANSAL.**

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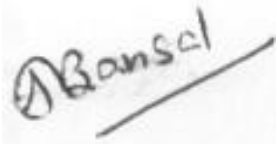
Wakhnaghat.

Date: 21th May, 2021

CERTIFICATE

This is to certify that the project work titled "**Arginase in *Bacillus circulans***" in partial fulfilment of the requirements for the award of the degree of Bachelor of Technology in Biotechnology submitted to the Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat in May 2021 is carried out by **Mr. Vinay Kumar Korram** during his Final year under my supervision. This work on "Arginase in *Bacillus circulans*" has not been submitted partially or wholly to any other Institute or University for the award of any degree or appreciation.



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I would like to take this opportunity to express our first and foremost gratitude to our "DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS" for the confidence bestowed upon me and entrusting my project title **"Arginase in *Bacillus circulans*"**

At this juncture, with proud privilege and profound sense of gratitude I feel honored in expressing our deepest appreciation to **Dr. Saurabh Bansal,** for being a lot more than just a supervisor and going beyond the call of duty in my guidance, support, advice, and motivation throughout. He has been the source of inspiration of come what may these issues cannot bring you down. Sincere thanks for his insightful advice, motivating suggestions, invaluable guidance, help and support in successful completion of this Project and also for his constant encouragement and advice throughout my project work.

Special thanks to my parents for their infinite patience and understanding and for the constant support and most importantly God, who in his mysterious ways, always made things work out in the end.

In gratitude,

Vinay Kumar Korram (171805)

Abstract

The arginine-corrupting and ornithine-creating proteins arginase has been utilized in different applications. It is a protein of interest both in the industry as a biocatalyst for the creation of ornithine from arginine and in medication as antitumoral specialists deciding blood exhaustion of arginine, which is fundamental for developing certain tumors. Arginase, a semi-basic catalyst, assumes significant parts in an assortment of natural capacities, for example, cell multiplication, endurance and protein combination. It is additionally related to the creation of nitric oxide, polyamines, proline, creatinine and glutamate. Lately, specialists found that specific tumors were not proficient at orchestrating arginine autonomously, which take advantage of a decent occasion to win the fight with malignancy. The function of arginase in malignant growth treatment has additionally been considered. L-arginase is an incredible anticancer, L-arginine-draining catalyst. It is dynamic against argininosuccinate synthase communicating tumors by conversion L-arginine to urea and L-ornithine. In this particular study, we have focused on collecting data regarding various characteristics of arginase explicitly acquired from the organism Bacillus *circulans* found in sewage, soil, infant bile, and different food products. We have concentrated mainly on computational analysis of arginase from *Bacillus circulans* using its protein sequence extracted from the Uniprot protein database application. Evaluation of data comprising theoretical physicochemical properties, structural examination, analysis of the genetic and evolutionary relationship among arginase from different sources, and stability and quality estimation was done. The goal was to contemplate the other attributes of arginase in *Bacillus circulans* to become familiar with its applications in various fields.

Chapter 1
Introduction

Arginase is an enzyme that contains Manganese (Mn^{2+}). This enzyme catalyzes the reaction of:

$$\text{Arginine} + H_2O \rightarrow \text{Ornithine} + \text{Urea}$$

It has major role in the final step of the urea cycle. It is also indifferent to every domain of life. Arginase has a place with the ureohydrolase group of proteins.

Arginase enzyme catalyzes the 5^{th} and last advance in the urea cycle, a progression of biochemical responses in well-evolved creatures during which our body discards hurtful alkali. In particular, arginase changes over L-Arginine into urea and L-Ornithine. Arginase enzyme in mammals is dynamic like a trimer. However, some Arginase in bacteria is hexameric in nature. The compound requires a two-atom metal bunch of Manganese (Mn^{2+}) to keep up legitimate capacity. These Mn^{2+} particles facilitate water, arranging and balancing out the atom and permit water to go about like a nucleophile and assault L-arginine and hydrolyzes it into urea and ornithine.

In many vertebrates, two isozymes of the catalyst exist; primary, Arginase I, capacities in the urea cycle, and is being found fundamentally in the cytoplasmic area of hepatocytes. The 2^{nd} isozyme, Arginase II, is being involved in the guideline of intracellular Arginine or Ornithine levels. It is situated in the mitochondria of a few body tissues, with much of the wealth in the kidney and the prostate. It's very well found at the lower levels in the macrophages, lactating mammary organs, and mind. The 2^{nd} isozyme can be found without other urea cycle catalysts.

Arginase inadequacy typically refers to the diminished capacity of arginase I, which is the liver arginase isoform. This lack is usually known as Arginemia or Hyperarginemia. The issue is inherited and is autosomal latent. It is portrayed by brought down the action of arginase in the hepatic cells of the tissue. It is viewed as the most extraordinary of heritable deformities in ureagenesis. Arginase insufficiency, not at all like other urea cycle problems, doesn't altogether forestall ureagenesis. A given justification is that the continuation of arginase work is

recommended by the expanded action of the arginase II inside the kidneys of the subjects with arginase I lack. Scientists accept that the development of arginine is responsible for triggering expanded articulation of the arginase II. The catalysts in the kidney will catalyze the ureagenesis, repaying to some degree for a diminishing in arginase I movement inside the liver. Because of this substitute strategy for eliminating the overabundance of arginine and alkali from the circulation system, subjects with arginase inadequacy will generally have longer life expectancies than the individuals who have other urea cycles absconds.

Mechanism of Arginase:

The dynamic site holds the L-arginine set up through hydrogen holding in between the guanidine bunch with Glu-227. This holding arranges L- arginine for the metal's nucleophilic assault by the metal associated with the hydroxide particle at guanidine gathering. These outcomes in a tetrahedral transitional. The Manganese (Mn) particles act to balance out both hydroxyl bunches in tetrahedral halfway, just like the creating sp-3 solitary pair of electron on the NH-2 bunch as the tetrahedral moderate is framed.

Arginase enzyme's dynamic site's exceptionally explicit. Adjusting substrate structure and additionally, stereo-chemistry seriously brings down the dynamic movement of chemical. This particularity happens because hydrogen is high in number connections among the substrate and catalyst; water-encouraged or direct hydrogen securities exist, soaking every one of the three situations on the alpha-amino gathering and four acceptor positions alpha carboxylate gathering. N-hydroxy L-arginine (NOHA) is a middle road of NO biosynthesis and is the moderate arginase inhibitor. Gem str. of its complex with compound uncovers that it uproots metal spanning hydroxide particle and extensions the binuclear Mn bunch.

Moreover, 2(S) amino-6-boronohexonic acid (ABH) is an L-arginine analogue that makes a tetrahedral intermediate, similar to that intermediate which forms with its natural substrate and is a compelling human arginase I inhibitor.

Chapter 2

LITERATURE REVIEWS

1. Cancer therapy based on Arginase

Little molecules inhibitors of arginase are depicted as promising restorative specialists for treating an assortment of infections, including malignant growth. Arginase enzyme immunization could prompt Th1 irritation in the tumor locales where the administrative myeloid cell, in any case, forestall lymphocyte penetration. Innovative bio labs have broad involvement with malignancy immunizations and can give arginase antibody advancement to disease treatment. Arginase, 1st found by Kossel and Dakin in the mammalian liver in 1904, is an enzyme that converts L-arginine to urea and L-ornithine. It's a binuclear Manganese (Mn) metalloenzyme that helps in catalyzing the conversion of l-arginine to ornithine and urea. It does not withstand its fundamental function in the hepatic ornithine cycle and likewise influences the invulnerable frameworks in mice and people. There are two kinds of Arginase, which results from various qualities that are freely controlled and situated on multiple chromosomes. Arginase1 is prevailing inside the liver, while Arginase-II is communicated in the kidney, mind, small digestive tract, monocytes and macrophage (Ivanenkov YA. 2014).

2. Purification, Expression and Biochemical characteristics of Arginase in *Bacillus subtilis*

The arginine debasing and the ornithine-creating catalysts arginase is being utilized to treat the arginine-subordinate tumors. This examination was completed to acquire microbial arginase from the *Bacillus subtilis,* a significant microorganism found in the matured nourishments, for example. Cheonggukjang (Song et al. 2014). The quality that encodes arginase was segregated from *B. subtilis*168 and is being cloned into the *E. coli* articulation pET32a plasmid. The chemical action was distinguished in the changed supernatant and IPTG instigated cell-remove (Yu et al. 2013). Arginase was refined for homogeneity by partiality chromatography from the supernatant. The particular action of the purged arginase was 150U per mg of protein. SDS-PAGE examination uncovered the sub-atomic size to be 49 kDa. The ideal temperature and pH of the filtered chemical with arginine as a substrate were 45°C and pH of 8.4, separately. The

Km and the Vmax estimations of arginine for the catalyst were 4.6mM and 133.0mM per min per mg of protein individually (Song et al. 2014; Yu et al. 2013). These discoveries can improve the beneficial matured nourishments, for example, Cheonggukjang with the upgraded ornithine level and drug items by giving the critical compound in arginine debasement and ornithine-creation.

3. *Klebsiella* phytase, computational structural study, functional study and phylogenetic analysis

Phytic acid or phytate is the vital stockpiling inedible type of phosphorus in various crops. It is identified as the anti-nutrient component of the human and animal diet as it chelates certain essential divalent minerals make it unavailable to absorb (Wyss et al. 1999; Mullaney and Ullah 2003). The undigested and unabsorbed structure of phosphorus additionally causes phosphate contamination in the dirt by creature squanders. Phytate corrupting catalysts like phytases can be beneficial and monetarily achievable to lessen the danger of phosphate contamination and increment the supplement an incentive in creature takes care of simultaneously. The *Klebsiella* phytases are appropriately used in various food enterprises of the plant starting point for the amazing warm dependability and more pH resilience. From the present computational examination, it was discovered that *Klebsiella* phytases were about 46-47 kDa sub-atomic protein weight of the Histidine phosphatase superfamily having the alkalinity nature and also thermostability (Mullaney and Ullah 2003; Kumar et al. 2012). The thermostability might be accomplished due to the presence of higher alpha-helices and beta-sheets simultaneously and the presence of the higher aliphatic index in the range of 88 and 91 and so forth. Curiously, a solid connection was discovered to be appropriate from phylogenetic investigations of the proteins with their complementary DNA among the two species and their strain level (Huang et al. 2009). Subsequently, the current investigation would be valuable for future analysts to fulfil the need for rural and modern bacterial phytases, especially for rural cultivating.

4. The Immobilized and Stabilized *Bacillus subtilis* and Arginase as for the Bio based Production of the Chemicals Containing Nitrogen

L-Ornithine could fill in as a moderate in the bio-based creation of 1,4 diamino butane from the L arginine. Utilizing the bio-refinery idea, L-arginine can generally be accessible from the biomass squander streams through the nitrogen stockpiling the Polypeptide cyanophycin (Yessie

W Sari et al. 2021). Specific conversion of the L-arginine to L-ornithine is hard to catalyze synthetically; along these lines, the immobilization and adjustment of the arginase in *Bacillus subtilis* (EC 3.5.3.1) were concentrated in a ceaselessly mixed layer reactor framework. Introductory pH of substrate arrangement, expansion of L aspartic corrosive and diminishing specialists all seemed to affect the strength of *B. subtilis* arginase. An astoundingly decent operational strength (complete turnover no. i.e, TTN=1.13·108), arginine free base (pH 11.0) was noticed, which was additionally enhanced with the option of Sodium Dithionite into the substrate arrangement (TTN>1·109). *B. subtilis* arginase was effectively immobilized on the three industrially accessible epoxy activated upholds. immobilization on the Sepa-beads EC EP was generally encouraging, bringing about a recuperated action of 75 percent and the upgraded thermostability (Yunjian Ma et al. 2020). All in all the adjustment and immobilization of the *B. subtilis* arginase has opened up the opportunities for its use in the bio-based creation of nitrogen-containing synthetic compounds as an option in contrast to the petrochemical creation

5. Arginase role in the Asthma treatment: Potential clinical applications

Hypersensitive asthma is a constant infection with huge mortality and dismalness and influences 300 million individuals in the world and ingests many medical care financial plans. The inclination to asthma is directed by hereditary guideline, which is very complex, and asthmatic aggravation itself is portrayed by the transaction of different nearby cells of the bronchial tree and attacking the fiery invulnerable cells (Sabine et al. 2017). These clinical issues of asthma are inferable from irregular aviation route hyper-responsiveness that can persist throughout the illness. Histopathologically, invasion with an assortment of incendiary cells, hypertrophy and smooth muscle cell hyperplasia, flagon cell hyperplasia, and the subepithelial fibrosis is found in the provocative asthmatic tissue (Hagen Schroeter et al. 2013). This is an uncommon report that embarks to survey information on the part of the protein arginase and L-arginine digestion as a binding together component of asthma pathophysiology and an expected objective for future clinical treatment of asthma.

6. The determination of arginine by the Arginase method and its application in the analysis of proteins.

The utilization of the compound arginase for the quantitative determination of arginine was first proposed in 1917 by Jansen. As applied to the investigation of proteins, his strategy included the

synchronous expansion to a somewhat soluble hydrolysate of urease and arginase. At first of these catalysts, any arginine present was parted into urea and ornithine; constantly, the urea was changed over into ammonium carbonate. An appropriate span (24 hours) of the alkali created was dictated by air circulation and standard titration interaction. A controlled with urease alone outfitted the vital revision for prior smelling salts and urea. Jansen depicted his technique somewhat momentarily. The analysis of this lab, from 1922, has shown that its effective business expects consideration regarding numerous subtleties that its creator left unmentioned (Hunter A., Morrell J.A.Tr. Boy. Soc. Canad. (1922). These subtleties dominated, the strategy has been discovered to be not just, as was not out of the ordinary, stringently explicit, yet in addition exceptionally precise. A portion of the prior outcomes we acquired was conveyed to the Royal Society of Canada in 1925, the American Society of Biological Chemists in 1924. They had it inconsistent use from that point forward and feel that we can now unquestionably suggest it as a scientific strategy without a moment's delay dependable and advantageous (Hunter A, Dauphinee J.A. J. Biol. Chem. 1925). The focal rule of the strategy the utilization of arginase as a quantitative reagent-has meanwhile been utilized, in most of the test information of this study are taken from a proposition introduced by James A. Dauphinee in incomplete satisfaction of the necessities for the level of Doctor of Philosophy from the University of Toronto.

7. Novel Arginase Inhibitors for Treatment for Respiratory Inflammatory Disorders and treatment for cancer

Arginase, a manganese activated enzyme, catalyzes the transformation of l-arginine to l-ornithine and urea. Two forms of this protein exist: Arginase 1, present in cytosol, is chemical overwhelmingly mainly in hepatocytes, where it assumes an essential part in eliminating smelling salts through urea combination, and arginase 2, present in mitochondria exceptionally communicated in kidney engaged with the creation of ornithine, an antecedent for prolines and polyamines significant for cell expansion and collagen creation, separately (Gajda, T.; Golebioski, A.; Blaszczyk, R. *Bioorg. Med. Chem.* 2020).

Tumors utilize different invulnerable suppressive instruments to dodge the resistant framework (Kok, V. C. *Front. Oncol.* 2020). One of these is the decrease of L-arginine with expanded

degrees of circling arginase, expanded articulation, discharge of arginase from tumor cells, and enrollment of arginase communicating and emitting myeloid inferred silencer cells.

Pharmacologic hindrance of arginase movement appeared to turn around the low l-arginine actuated safe concealment in creature models. Hence, there is a requirement for potent and particular Arginase inhibitors to invert safe concealment and reactivate anticancer resistance in the patient (Mahdi, A.; Kovamees, O.; Pernow, J. *Int. J. Cardiol.* 2020). The current application depicts a progression of novel arginase inhibitors for the therapy of malignancy and provocative respiratory illnesses)

8. Bacterial Arginase purification.**

This method explains the process of purification of *B. anthracis* arginases and staphylococcus. It comprises extraction of Arginase enzyme by gel filtration on Sephadex G-25 column electrophoresis, autolyzing of the acetone dehydrated bacteria, and horizontal zone electrophoresis on Sephadex G-200. Crystallized proteins were extracted by acetone treatment in the cold. The crystallized forms of the two bacterial arginases are not similar. The arginase, at first, obtained is homogeneous in nature in the immunoelectrophoresis in agar gel test and in double diffusion, and also on electrophoresis on cellulose acetate strips and thin layer gel filtration on Sephadex G-200 superfine. The specificity in the immunological aspect of the two bacterial arginases was represented by immunoelectrophoresis in agar gel and by double diffusion (International Journal of Food Microbiology, Volume 247, 2017).

9. Arginase-1 in macrophage directs bacterial growth and controls pathology of tuberculosis granulomas in hypoxic

Lung granulomas are produced in tuberculosis (Mtb) mycobacteria in infection as a sign of human tuberculosis (TB). Its structure consists mainly of Mtb –uninfected and infected macrophages and Mtb T cells precisely. NO production using granuloma macrophage that expresses nitric oxide synthase-2 by l-arginine and oxygen acts as major protecting step from mycobacteria. Despite this defence, Tuberculosis granulomas are frequently hypoxic and bacteria eliminating in nature by nitric oxide synthase-2 is likely suboptimal in this situation. Arginase-1 (Arg1) also catalyzes l-arginine but without requiring oxygen as a substrate and this

has been shown to contribute to the regulation of nitric oxide synthase-2 by competing for the substrate.

Moreover, Arg1 plays additive roles such as tissue repair and T-cell regulation independent of NOS2 suppression in other infectious diseases in which granulomas occur, such as leishmaniasis and schistosomiasis (Front Cell Infect Microbiol. 2021). For analyzing whether Arg1 could show the same performance in hypoxic regions of TB granulomas, they used a TB murine granuloma model with no NOS2. Expression of arginase-1 in macrophages in this setup gave results of exacerbated lung granuloma pathology and burden of bacteria. Arginase-1 expression correlated with reduced T-cell proliferation in hypoxic granuloma regions, showing that Arginase-1 regulation and expression of T-cell immunity control disease. The observation shows the role of Arginase-1 in the TB control when NOS2 is not affected by hypoxia.

10. **Arginase production from Halophilic Bacteria, mainly with the impact of Manganese Salt**

The impact of manganese salt on the creation of arginase is portrayed. Among 162 different aquatic strains and halophilic microorganisms, one of the disengaged microbes produces L-arginase from arginine-ocean water medium transcendently. Therefore, a suitable environment for the creation of arginase using halophilic bacteria was inspected. Manganese salt didn't provide any acceleratory impact on the chemical composition in the typical medium (H. Hirsch-Kolb and D. M. Greenberg: J. Biol. Chem., 243). Nonetheless, it was tracked down that the protein creation was surprisingly upgraded when manganese salt is expanded after treating the medium with dithizone to eliminate metallic salts. Additionally, the compound arrangement consequently acquired was ultimately enacted explicitly by the option of manganese particles.

Respiratory incendiary illnesses incorporate asthma or constant obstructive aspiratory infection. Further, the application unveils compounds, their planning, use, drug structure, and treatment.

Chapter3

Objectives

- In silico analysis of Arginase in *Bacillus circulans* in various aspects
- Characterization of theoretical physicochemical properties of the enzyme using its protein sequence
- To establish a structural and functional analysis of Arginase specifically in *Bacillus circulans*
- To evaluate the various applications of Arginase based on its biochemical properties in different fields
- To do a hypothetical representation of the evolutionary and genetic relationship of Arginase in *Bacillus circulans* with Arginase in other bacterial species.

Chapter 3
Materials and Methodology

Phylogenetic analysis and extraction of data

For extraction of amino acid sequences of *Bacillus* species showing specific arginase activity, we used the knowledgebase of UniProt. The sequences for amino acid, specifically for the arginase enzyme of *Bacillus circulans* was initially taken from a database of UniProtKB. Then it was used as a target/query sequence to get template sequences with the help of BLAST tool featured in the database of UniProtKB. The selection of amino acid sequences was done to arrange the lowest E-value, maximum query coverage, maximum sequence identity, and maximum bit score. Similarly, ten protein sequences from different *Bacillus* species were extracted in FASTA format for computational analyses. The protein, along with their respective coding sequences from other bacterial species and *Bacillus circulans*, were analyzed in an evolutionary aspect. MEGAX (Molecular Evolutionary Genetics Analysis software) was used to build a phylogenetic tree for the same.

Study of the enzyme sequence

Step1: To study the sequence of the Arginase enzyme in *Bacillus circulans*, we used the Uniprot protein database application. We downloaded the sequence of Arginase in *Bacillus circulans* in fasta form from it

Step2: We downloaded other sequences of Arginase in five different strains, i.e., *Bacillus licheniformis, Bacillus subtilis, Bacillus brevis, Bacillus caldovelox* and *Bacillus anthracis.*

Step3: Multiple sequence alignment is done using these sequences. We put the sequence of arginase from *Bacillus circulans* in the query sequence box and the sequence of *Bacillus licheniformis* in the subject sequence box and blasted both the sequences. And then the result was collected. Similarly, the alignment for four other strains was carried out and their results were noted too.

Structural and functional analysis

Analysis of physio-chemical characteristics of extracted protein sequences

Determination of physio-chemical properties of extracted protein sequence was done using an online tool for characterization, "ProtParam", featured on the Expasy website. The physio-chemical properties can be computed directly from the amino acid sequence and content of a given protein sequence with the help of this tool.

Computation of the secondary structural features comprising secondary elements like coil, sheet, helix, turn, etc., was done by the server—PSIPRED.

Evaluation of comparative protein model of Arginase in *Bacillus circulans* using swish model Workspace

Functional characterization of a protein sequence is quite possibly the most successive issues. This assignment is typically encouraged by the exact three-dimensional (3-D) design of the Arginase protein. Without a tentatively decided design, similar or homology displaying can sometimes give a valuable 3-D model for a protein that is identified with at any rate one realized protein structure.

Similar displaying predicts the 3-D construction of a given protein sequence (target) in light of its arrangement to at least one protein of known structure (layouts). The forecast cycle comprises overlap task, target-layout arrangement, model structure, and model assessment. This unit depicts how to compute similar models utilizing the program and talks about each of the four stages of near displaying, as often as possible noticed mistakes, and a few applications.

UniProt knowledgebase was used for extraction of an amino acid sequence of *Bacillus circulans* having specific arginase activity. Comparative protein structure of Arginase in *Bacillus circulans* was evaluated with Swish model workspace using the amino acid sequence retrieved from Uniprot knowledgebase and observed results.

Chapter 5

Results

Phylogenetic analysis

Phylogram comprises evolutionary distance investigation using arginase amino acid sequences of 10 species of *Bacillus*

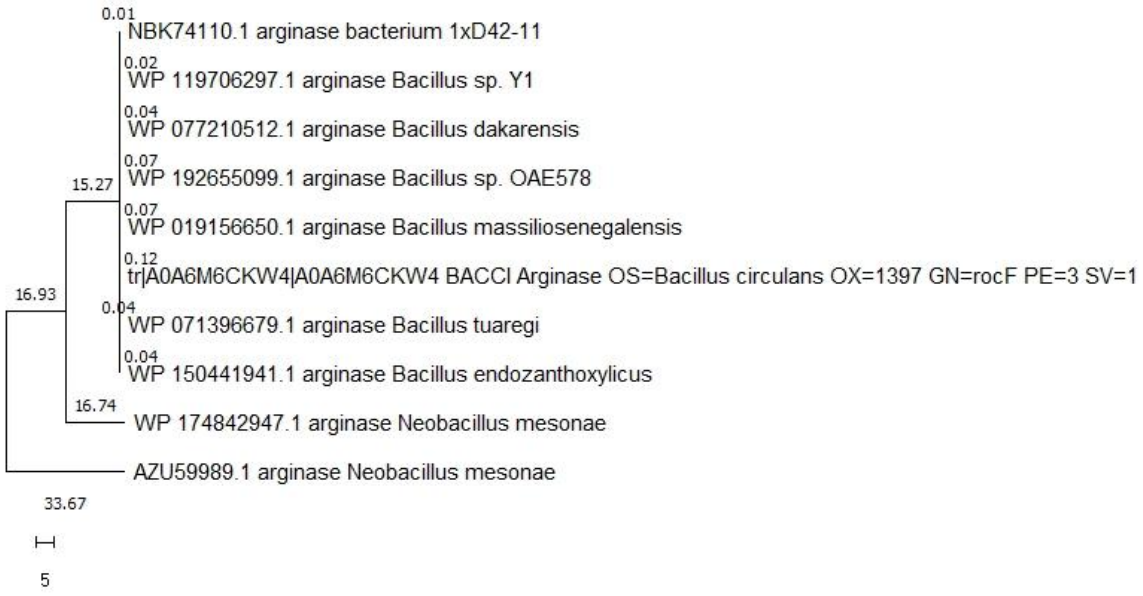


Figure 1. Phylogenetic tree of arginases of different *Bacillus* species

Observations

The phylogenetic tree is a hypothesis that constitutes evolutionary relationships among organisms. The pattern of branching reflects how arginase from different bacterial species evolved from a series of common ancestors. It is based on evolutionary relationships and genetic similarities among the different species. The diagram shows that arginase from *Bacillus circulans* is closely related to arginase from *Bacillus tauregi*, *Bacillus endozanthoxylicus*, *Bacillus massiliosenegalensis*, *Bacillus dakarensis*, etc., and distinctly associated with arginase from *Neobacillusmesonae* (Figure 1).

Results of individual FASTA sequence of *Bacillus* strains

Bacillus circulans

Tr|A0A660BYB0|A0A660BYB0_BACCI Arginase OS=*Bacillus circulans* GN=FHW50_109263 OX=1397 SV=1 PE=3

MRKLTIIGMPMDLGQMRRGVDMGPSAIRYAGIFERLSKLFSSIEDWGDISVGRPETIIDV
QSNLRNLHLIAEKNQMLAEMVDKIIQSKSFPLVLGGDHSIAIGTLAGVSKHYTNLGVIWY
DAHGDLNTAETSPSGNIHGMPLAVSLGYGHELLTNILGASPKIKPEHVVIIGARSLDEGER
RLIKELGIKVFTMHEIDRLGMTKVMESTIDYLKEKTDGVHLSLDLDGLDPNDAPGVGTP
VPGGISYRESHLAMEMLAESGLITSAEFVEVNPILDERNKTASLAVALMGSLFGEKLL

Bacillus licheniformis

>lcl|Query_11171 tr|Q65DS7|Q65DS7_BACLD OS=*Bacillus licheniformis* Arginase (strain / / JCM 2505 / DSM 13 / NBRC 12200 / NCIMB 9375 / NRRL NRS-1264/ ATCC 14580 / Gibson 46) GN=speB PE=3 SV=1 OX=279010

MRFDEAYSGKVFIASRPDWEEADAILYGMPMDWTVSYRPGSRFGPARIREVSIGLEEYS
PYLDRELEEVHFFDAGDIPLPFGNPQKSLDMIEEYVDSILDKGKFPLGMGGEHLVSWPVI
KAMYKKYPDLAIIHMDAHTDLRVDYEGEPLSHSTPIRKAAELIGPGNVYSFGIRSGMKE
EFEWAKENGMHISKFEVLEPLKAVLPKLAGRPVYVTIDIDVLDPAHAPGTGTVDAGGIT
SKELLASIHEIARSDVNVVGGDLVEVAPVYDHSEQTANTASKLIREMLLGWVK

Bacillus subtilis

>lcl|Query_42201 sp|P39138|ARGI_BACSU Arginase OS=*Bacillus subtilis* (strain 168) GN=rocF PE=1 OX=224308 SV=1

MDKTISVIGMPMDLGQARRGVDMGPSAIRYAHLIERLSDMGYTVEDLGDIPINREKIKN
DEELKNLNSVLAGNEKLAQKVNKVIEEKKFPLVLGGDHSIAIGTLAGTAKHYDNLGVIW
YDAHGDLNTLETSPSGNIHGMPLAVSLGIGHESLVNLEGYAPKIKPENVVIIGARSLDEG
ERKYIOSGGYTPKVKAENVVIIGARDLDQGERELIKRIGMKVFTMHEIDKLGMARVMDE
AIAHVSKNTDGVHLSLDLDGLDPHDAPGVGTPVIGGISYREGHVSLEMLADADILCSAEF
VEVNPILDRENM

Bacillus caldovelox

>lcl| P53608 Query_22239 sp| Arginase |ARGI_BACCD OS=*Bacillus caldovelox* OX=33931 PE=1 GN=rocF SV=1

MKPISIIGVPMDLGQTRRGVDMGPSAMRYAGVIERLERLHYDIEDLGDIPIGKAERLHEQ
GDSRLRNLKAVAEANEKLAAAVDQVVQRGRFPLVLGGDHSIAIGTLAGVAKHYERLGV
IWYDAHGDVNTAETSPSGNIHGMPLAASLGFGHPALTQIGGYSPKIKPEHVVLIGVRSLD
EGEKKFIREKGIKIYTMH

Bacillus anthracis

>lcl|Query_53691 tr|A0A1J9VZQ6|A0A1J9VZQ6_BACAN Arginase OS=*Bacillus anthracis* GN=rocF OX=1392 PE=3 SV=1

MKKEISVIGVPMDLGQMRRGVDMGPSAIRYAGVIERIEEIGYDVKDMGDICIEREKEVD
VNTSLRNLTQVATVCNELASKVDHIIEEGRFPLVLGGDHSIAIGTLAGVAKHYKNLGVI
WYDAHGDLNTEETSPSGNIHGMSLAASLGYGHPTLVDLYGAYPKVKKENVVIIGARAL
DEGEKDFIRNEGIKVFTMHEIDRMGMTAVMEETIEYLSHTDGVHLSLDLDGLDPHDAPG
VGTPVIGGLSYRESHLAMEMLAEADIVTSAEFVEVNTILDERNRTATTAVALMGSLFGE
KLK

Bacillus brevis

MNKNMSIVGVPMDLGADRRGVDMGPSAIRYAGVVARLEKMGFNIEDRGDIFVTLPHHF
TETENHKYLDEVVEANEKLANVVSDIMTAGRFPLVLGGDHSIALGTIAGVAKHVKNLG
VICLDAHGDLNTGATSPSGNIHGMPLAASLGYGHERLTNIGGYTPKVKAENVVIIGARD
LDQGERELIKRIGMKVFTMHEIDKLGMARVMDEAIAHVSKNTDGVHLSLDLDGLDPHD
APGVGTPVIGGISYREGHVSLEMLADADILCSAEFVEVNPILDRENMTARVAVALMSSV
FGDKLL

Table 1. Sequence similarity analysis using BLAST

NAME OF THE BACTERIA	SIMILARITY / POSITIVITY	IDENTITY
Bacillus licheniformis	136/295 (46%)	89/295 (30%)
Bacillus subtilis	247/297 (83%)	214/297 (72%)
Bacillus brevis	237/297 (79%)	195/297 (66%)
Bacillus caldovelox	259/299 (86%)	224/299 (75%)
Bacillus anthracis	213/296 (72%)	248/298 (83%)

Observations

After getting the results of multiple sequence alignment of arginase in *Bacillus circulans* with arginase in the other five strains, we observed that the similarity/positivity in sequences is highest with *Bacillus caldovelox* (86%) and *Bacillus subtilis* (83%). The result shows that primary structure of arginase in *Bacillus circulans* resembles the primary structure of *Bacillus caldovelox* and *Bacillus subtilis* Arginases. The primary structure of *Bacillus circulans* arginase shows the highest identity with the *Bacillus anthracis* (83%) and *Bacillus caldovelox* (75%)

arginases, suggesting that all these are all these arginases may have very similar secondary and tertiary structure.

Analysis of physio-chemical characteristics of extracted protein sequences

Determination of physio-chemical properties of extracted protein sequence was done using an online tool for characterization, "ProtParam", available on the Expasy website. The physio-chemical properties can be computed directly from the amino acid sequence and content of a given protein sequence with the help of this tool.

Bacteria: *Bacillus circulans*

Theoretical pI: 5.57

Extinction Coefficient: 28795

Instability Index: 36.50

Aliphatic Index: 98.93

Grand average of hydropathicity: -0.006

Observations

The enzyme attains net charge zero at pH 5.57.

The absorbance of the protein per concentration and path length is 28795 $M^{-1}cm^{-1}$.

The instability index is 36.50, less than 40, indicating that it is probably stable in a test tube.

The aliphatic index of Arginase is 98.93, which indicates that this enzyme is thermally stable and contains high amounts of hydrophobic amino acids.

The grand average of hydropathicity is used to indicate the hydrophobicity value of protein, which calculate the sum of the hydropathy measures of all the amino acids divided by sequence length. Thus, a negative value of GRAVY shows the enzyme is hydrophilic in nature.

Predicted secondary arrangements of the target sequence (arginase in *Bacillus circulans*)

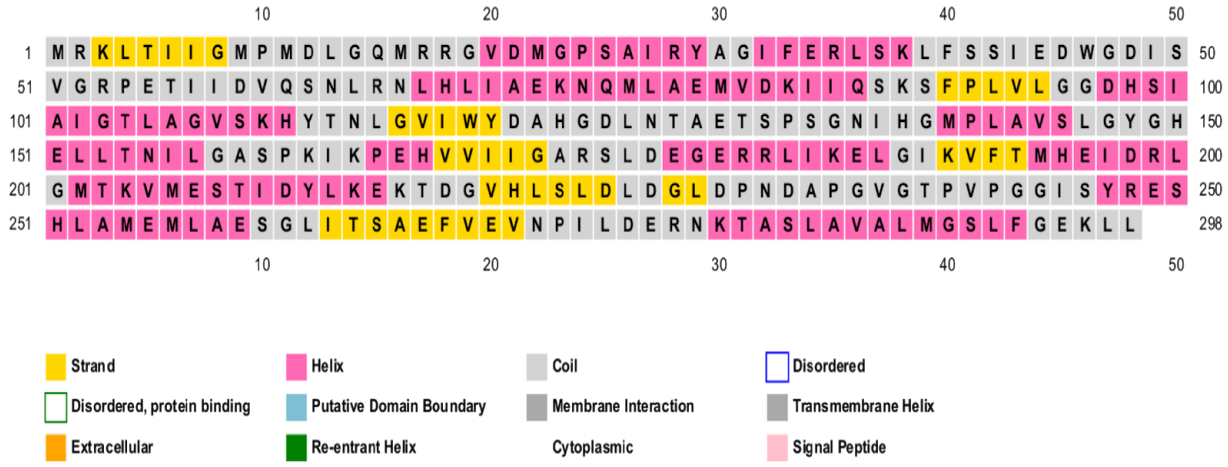


Figure 2. Predicted secondary structure of *B. circulens* using PSIPRED

Observations

The predicted secondary structure of arginase in *Bacillus circulans* aims to investigate its secondary structure using its sequence, highlighting its secondary elements like coil, sheet, turns helix, etc, using server—PSIPRED. The yellow highlighted residues signify the strand region of the protein, the pink residues means helix regions and the grey highlighted residues represents coil regions of the protein (Figure 2).

Evaluation of comparative protein model of Arginase in *Bacillus circulans* using swish model workspace

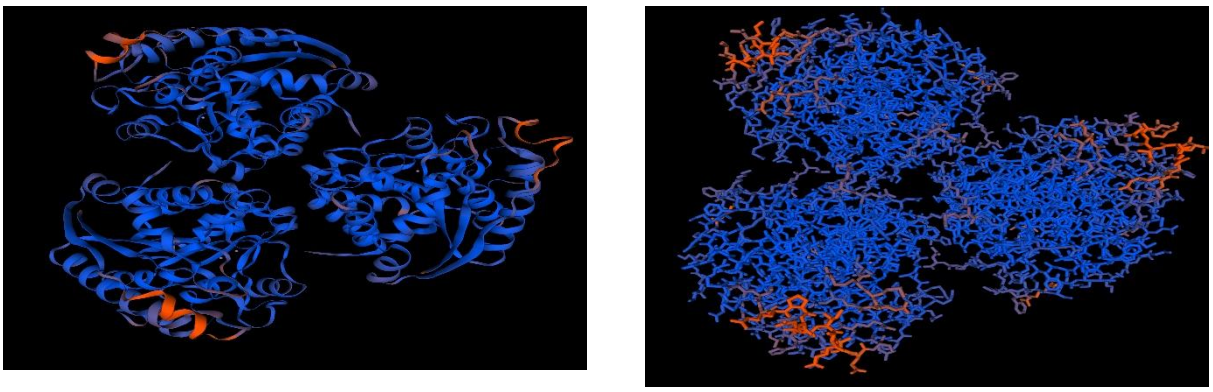
Functional characterization of a protein sequence is quite possibly the most successive issues. This assignment is typically encouraged by the exact three-dimensional (3-D) design of the arginase protein. Without a tentatively decided design, similar or homology displaying can some of the time give a valuable 3-D model for a protein that is identified with at any rate one realized protein structure. Similar displaying predicts the 3-D construction of a given protein sequence (target) in light of its arrangement to at least one protein of known structure (layouts). The

forecast cycle comprises overlap task, target-layout arrangement, model structure, and model assessment.

This unit depicts how to compute similar models utilizing the program and talks about each of the four stages of near displaying, as often as possible noticed mistakes, and a few applications.

Tertiary 3D modelled structure of arginase in *Bacillus circulans* viewed by SWISS-MODEL workspace (Figure 3).

a) **b)**



c) **d)**

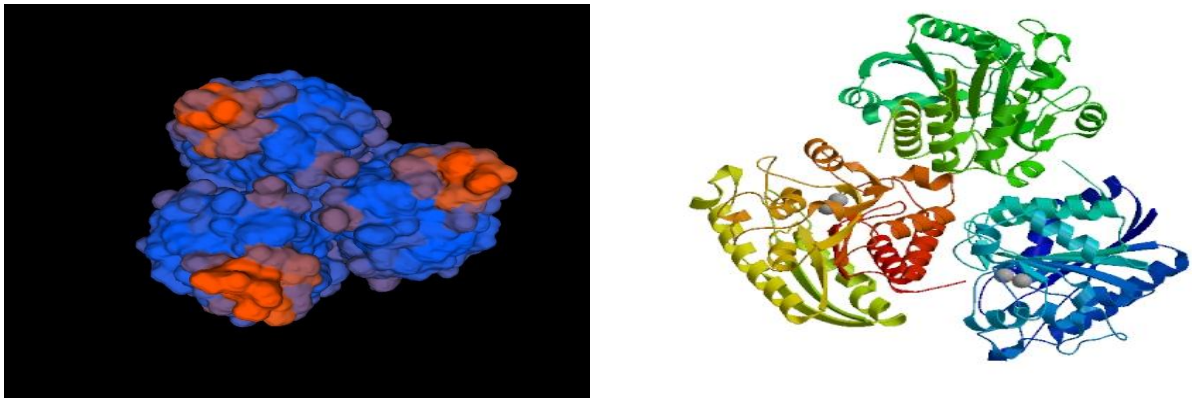


Figure 3. Modelled structure of *B. circulens* arginase using SWISS-MODEL. (a) shows the twists and turns inside the protein, (b) shows the atomic arrangements in protein, (c) is the surface view of the arginase protein showing three chains in different colours, (d) tertiary structure representing prominent secondary adaptations and disulfides (red; helix, yellow; sheet, green; loop, green balls; disulfides)

Quality check of 3D model of arginase:

i) Local Quality Estimate

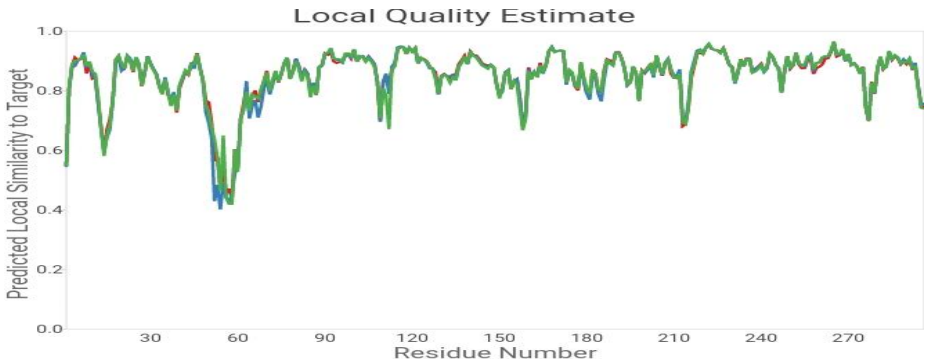


Figure 4. A local quality estimate from the QMEAN server. Local quality estimation of the protein structure is based on predicted local similarity to the target given as per residue number

Comparison with PDB structures (non-redundant set)

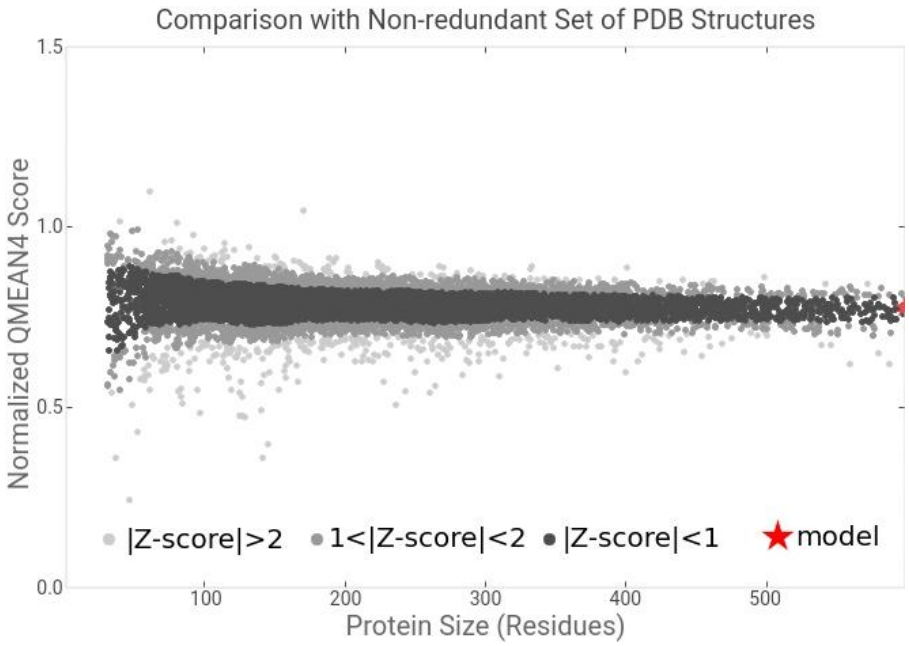


Figure 5. Comparison of predicted model with a non-redundant group of structures from QMEAN server. The plot gives the quality of the protein structure on the basis of normalized QMEAN score compared with Z-score of non reductant group of PDB structures.

Global quality estimate

Figure 6. QMEAN4 score of built protein structure

Observation

In silico identification and modelling homology of arginase protein of *Bacillus circulans* were done on a detailed account. In silico study of biological proteins has resulted in very effective and immense contribution in recent time. In silico biology explains the functional and structural characteristics of the target protein hypothetically.

The functional and structural analysis of the arginase enzyme was discussed in this particular study. The selected target sequence revealed that it was trimeric in structure, i.e. it consisted of three monomeric chains labelled as Chain A-C with the help of the 3D model prepared.

Trimeric arginase have been reported previously both in fungi—*Candida albicans* and bacteria—*Bacillus circulans*. Sheets, loops and helices are described in the colour yellow, green and red, respectively**.** Local quality estimation of the protein structure is based on predicted local similarity to the target given as per residue number. The residue number giving a lower value than 0.6 is considered as of lower quality. In this particular case, the score is higher or equal to 0.6 at every residue number except at residue number 60 (Figure 4). So the overall quality of the protein model is good on a local level.

Compared with the non-reductant set of PDB structutes, we observed that the value of normalized QMEAN score compared with Z-score is between 0.5-1 which signifies that the protein model created is comparable in quality to experimental structure of equal size(figure-5). A Z-score with a negative value indicates that the quality of below average and Z-score with positive value indicates quality above average.

Global Model Quality Estimation is the analysis based on properties of target template and template structure created. Keeping properties on account, including QMEAN value, carbon atom arrangement, all-atom arrangements, salvation and torsion, shows the structure's quality. A disulfide bond was often present in the protein structure that indicates the great stability of the given protein. The Oxidation of thiol groups of cystine residues results in the formation of disulfide bonds in proteins, which provide an advantage in maintaining the protein's stability.

CHAPTER 6

Conclusion

Arginase has been accounted for to assume a critical part in the therapy of neurological issues, unfavourably susceptible asthma, rheumatoid joint pain; it is likewise found to have tumor inhibitory properties and has been licensed significant thought because of its broad scope of action against malignant growth cells which can't biosynthesize arginine because of the absence of articulation of argininosuccinate synthetase-1. Arginase has also been applicable as a biocatalyst in industries and agricultural field as well.

The present study has tried to evaluate information regarding major properties and characteristics of arginase from *Bacillus circulans* hypothetically, using its protein sequence and some bioinformatics tools to execute it in silico analysis. In this study, we extracted the complete protein sequences and used them as target templates on a number of different bioinformatic tools to carry out its biochemical and physicochemical properties. We have also evaluated similarities and identities of the sequences of the arginase from different bacterial sources

The thermostability of the enzyme was deducted using the same target sequences. We concluded that arginase in *Bacillus circulans* is highly thermostable because of the presence of a high amount of hydrophobic amino acids. We have also highlighted the secondary elements in the structure of arginase, including coils, sheets, turns and helix, with the help of predicted secondary structure. The detailed structural investigation was done by creating a comparative protein 3D model and its local and global quality estimation. Evolutionary distance analysis was done to understand the ancestral relationship of arginase in *Bacillus circulans* with arginase from other bacterial species.

In silico investigation of arginase in *Bacillus circulans* was very helpful in characterizing it more accurately. This particular study has covered the inclusive functional, phylogenetic and structural analysis of arginase in *Bacillus circulans*. Computational investigation of the physicochemical features of protein is very important to understand the overview of protein theoretically. Thus, this particular study will be helpful in future research, mainly in silico studies of arginase.

Chapter 7
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