Arginase isolation from Bacillus Megaterium and characterization

Major project report submitted in partial fulfilment of the requirement for the degree of Bachelor of Technology

in

Biotechnology Engineering

By

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UNDER THE SUPERVISION OF

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DECLARATION

I do hereby declare that this dissertation is titled “Arginase isolation from B. Megaterium and characterization” submitted towards attainment for the award of degree of bachelors of technology in biotechnology under the guidance of DR. Saurabh Bansal, department of biotechnology and bioinformatics, Jaypee university of information and technology, is wholly based on the study and results carried out. Therefore, the declaration made by the student is true and genuine.

Gaurav Kumar 181829

CERTIFICATE

This is to certify that the work which is being presented in the project report titled " arginase isolation from B. megaterium" in partial fulfilment of the requirements for the award of the degree of B.Tech in biotechnology And Engineering and submitted to the Department of biotechnology, Jaypee University of Information Technology, Waknaghat is an authentic record of work carried out by "Gaurav Kumar (181829)" during the period from August 2021 to December 2021 under the supervision of "Dr. Saurabh Bansal", Department of biotechnology and bioinformatics, Jaypee University of Information Technology, Waknaghat.

Gaurav Kumar (181829) ( )

supervisor sign

Name of supervisor: Dr. Saurabh Bansal

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Waknaghat , solan HP

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Date: _____

Gaurav Kumar (181829)

LIST OF FIGURES

ABSTRACT

Arginase is a widely known enzyme of the urea cycle that catalyzes the hydrolysis of L-arginine to L-ornithine and urea. The action of arginase goes beyond the boundaries of hepatic ureogenic function, being widespread through most tissues. B.megaterium is an rod shaped , a natural PHB producer, gram positive aerobic spore forming bacteria usually found in the widely diverse habitats and its among biggest bacteria.in this experiment we used bacillus megaterium and inoculated it into nb broth and used that growth to see the growth of the bacteria and then afterwards being used for the staining process in which we will come to see the gram positive bacteria after the staining in which the stain usually binds with the bacteria then either the bacteria will stay purple or then turn to pink if then it turn to pink then it's a gram positive which will show some rod shaped like mainly in chains and concluded that we able to see the proper growth of the bacillus megaterium ,it's been also called as a big beast.

INTRODUCTION

- ## Arginase

Is a ubiquitous enzyme found in bacteria, yeasts, plants, invertebrates, and vertebrates. Some bacteria possess a related enzyme, agmatinase , which belong to Archaebacteria, and the arginase optimum ph. of cotyledon arginase was found to be 10.0 and the human type 1 and type 2 arginase is related to 58% sequence identity, it was found back then in the mammalian liver tissue by the Kossel and Dakin 1904.it's the 6^{th} and the only final enzyme for the urea cycle and converts arginine to urea and ornithine. Arginase have a commission number which is 3.5.3.1 it usually shows the chemical reaction which they catalase and the ec 3 is a hydrolase forming 2 products. The deficiency of it involved into the arginase deficiency which is results into argenimia . Arginase is found in many organisms in nature and has been well studied in bacteria, fungi, lichens, and plants. Arginase functions as a lectin that binds to the cell wall of algae, facilitating cell-to-cell communication, It has a wide range of functions in plants, including seed germination, nitrogen mobilization, and defensive functions. Arg is the primary amino acid storage form in seedlings and during germination. Arginase is found in mitochondria and aids in the assembly of nitrogen-containing biomolecules.

- ## Bacillus megaterium

represents an increasingly used alternative for the high yield intra and extracellular synthesis of protein, the cell often occurs in pairs and chains. And the nitrogen fixation has been done in some strains of megatherium can be found in various food items including honey. Also, the bacillus megaterium plays a major role in dissolving the unnecessary phosphorus compound in the soil rendering them available for growing crops, and helps in the Remedition of the lead, cadbium and other contaminated soil. Bacillus megaterium have a very efficient protein secretion system, grows in different carbon system increased the richness of soil bacteria and most of the fungal communities

History:

The species was discovered by the De Bary in 1884 and is generally ubiquitous in in environment around us, generally can be found in the cow feces or the greater wax moth frass. It's basically known to produce the poly glutamic acid and further can be used for the production of pyruvate and b12 and antiviral properties. it's been recognized as an endophyte and usually been a potential agent for the biocontrol of plant disease. B.megaterium have a very efficient protein secretion system, grows in different carbon system. Increased the richness of soil bacteria and most of the fungal communities

Application

1. waste water treatment
2. bioremediation/biodegradation
3. cleaning/deodorizing
4. drain and septic treatment
5. chemical productions

Arginase activity has 2 main homeostatic purposes. Remove ammonia from the body by urea synthesis to produce ornithine, which is a precursor of polyamines and prolines. Arginase plays an important role in health a disease, and it's involved in diseases and injuries affecting the peripheral cardiovascular system and (CNS). It is a manganese metalloenzyme which catalyzes conversion of l arginine to the l ornithine and the urea.

Commercial arginase:

1. The metalloenzyme arginase could be a therapeutically relevant target related to tumour growth. To combat cancer immunosuppression, arginase activity will be modulated by little chemical inhibitors that bind to its chemical change site.

2. While some arginase inhibitors, such as BEC and ABH, have been offered commercially for pre-clinical use and have been used in humans to date, clinical trials of arginase in patients with cardiovascular disease have been limited to small proof-of-concept studies using local Involvement limits transdermal administration.

3. Promising results have been reported for the treatment of patients with coronary artery disease and type 2 diabetes, congestive heart failure, hypertension and post-cardiac arrest resuscitation

ARGINASE:

Arginase is a widely known enzyme of the urea cycle that catalyzes the hydrolysis of L-arginine to L-ornithine and urea. It was back then first appeared in the bacteria. The action of arginase goes beyond the boundaries of hepatic ureogenic function, being widespread through most tissues. Arginase 1the highly cytosolic enzyme. Usually measured by immunoassay. It's been shown predominantly in the RBC. Although traditionally considered the last enzyme in the urea cycle, the enzyme is found in a variety of tissues outside of the liver, Arginase is used to lower blood serum levels of arginine to starve cancer cells that are auxotrophic to arginine. Arg1 ac in the activity of function of urea cycle and the arg 2 helps in the regulation of the level of ornithine, and as the overly expressive use of arginase activity it can lead to the disease, can somewhere cause structural problem or can the vascular one.

Diseases related to arginase:

1. Hypertension
2. Sickle cell disease
3. Atherosclerosis
4. Erectile dysfunction
5. Kidney failure

Types of arginases:

1) Arginase 1:

It is generally expressed in lymphocytes or erythrocytes measured by immunoassay; its size is approximately. About 5-10 kb long and is a liver-specific hydrolase. Also known as arginine amidinase this codes protein of around 322 amino acids. It's been early onset marker for the liver injury. It’s a hepatocellular marker, which is most sensitivity.

Km value: 3.3
Vmax value: 34+_ nmol
Ph: 7.4
Molecular weight: 35 kDa

2)Arginase 2:

It's an arginase protein which is human encoded by the arginase, it's been located in the mitochondria and is being expressed in the extra hepatic tissue, majorly in the kidney.it majorly plays role in the nitric oxide metabolism. Helps in the progression melanoma migration too. The increase in the activity of it related to the coronary heart disease. And the hypertension.

Km value: 1.9mM
Vmax value: 10(4)
Ph:
Molecular weight:110 kda

Morphology:

1. It’s a manganese containing enzyme.
2. Belongs to the euro hydrolase family
3. Primarily found in the cytoplasm of liver
4. Usually play role in detoxification of ammonia in the urea cycle
5. Should be stored at1 4-degree temperature
6. Ph:9.2

•

names	Work not done	Work done	link
Pyro coccus furiosus	no		
Bacillus subtilis		Yes (yes (Expression, characterization work done))	Control of Ornithine Carbamoyltransferase Activity by Arginase in *Bacillus subtilis*
Bacillus megaterium	no		
Bacillus circulans	no		
Bacillus licheniformis		Yes ((No purification and characterization work)	Regulation of arginine and proline catabolism in Bacillus

			licheniformis
Peniophora lycii	no		
Lactobacillus spp.	no		
Streptococcus spp.	no		
Pseudomonas spp.		yes	Cloning, expression, and characterization of a thermostable l-arginase from Geo bacillus.thermodenitrificans NG80-2 for l-ornithine production
Geobacillus spp.	no		Cloning, expression, and characterization of a thermostable
Aspergillus Niger		yes	Contribution of arginase to manganese metabolism of *Aspergillus niger*
A.oryzae	no		https://environmentaljournals.org/article/isolation-screening-and-characterization-of-l-arginase-producing-soil-fungi-in-saudia-arabia

Arginase as a potential target in treatment of cardio vascular disease: reversal of arginine steal?

In this the probity towards the importance for vascular function which is in that regulating endothelial function of (NO) and we know that arginase being plays an important role in the regulation of NO production through L-arginine and a common substrate. Reduction in the NO production will help in making of different oxygen species and increase in activity will reduce availability in L-arginine which in turn in the end will lead towards the endothelial disfunction, its basic property being invisible in hypertension, atherosclerosis and congestive heart failure and many more others due to the increase in the expression of the arginase in different cardiovascular diseases, how it is uses and different development of disease for therapeutic strategies.

It's being playing an important role in homeostasis and the inflammation and have been playing a very different role in many different diseases, the main cause for the disfunction is the reduced production and the increment in the endothelial derived N0. As we know that nitric oxide is being produced through L-arginine by (eNOS) and it converts L-arginine into ornithine and into urea. In this the potential use of arginine has an inhibition for the normal therapeutic strategies being used.

Arginase, NO production and eNOS:

In this l-arginine being utilized as a common substrate for both of them and interaction between these enzymes. It's been seen that reduced bio availability of NO and the up regulation of arginase been a cause, the most effective enzyme for the cardio vascular system being the arginase and NOS. Generally being seen in many models of ageing, diabetes etc. The cytosolic arginase too in hypoxia is being localized with the eNOS so therefore the role of arginase in the cardio vascular disease being identified.

Diseases:

- **STROKE:**

Regarding this there is a very limited & detailed experimental review are there for the ischemic stroke, it's been shown that being a biomarker, but didn't reveal any information about its functional role in stroke, but as per now many studies been lacking for the activity in the stroke but some of the information been reviewed and seen that vascular function been influenced through the arginase.

- **HEART FAILURE:**

The low bioavailability of NO plays an important role in heart failure patients, increased plasma arginase activity also increases risk of heart failure. Various model organisms like rabbits & cat have been used to show the effect of arginase. Arginase 1&2 reduces NO bioavailability thus negatively affecting heart patients. Interestingly, arginase inhibitors increased basal contractility and improved micro-circulation by an NO-dependent mechanism.

- **AGEING:**

It's been known that it helps in age induced impairment of the vascular function in, especially in humans but not sure that it's been related to all the ageing & some observation been examined and came to know that arginase is being up regulated. With the increasing age factor and have been involved in the

development of the dysfunction of the ageing blood vessels of rats & mice's, its increase in activity been majorly seen in form of arginase 1 isoform

ARGINASE AS A PROMISING CANCER FIGHTING STRATEGY

Arg is a semi-essential vital amino acid that is involved in a variety of metabolic processes, signaling pathways, and cancer cell proliferation. As a result, Arg depriving enzymes (ADE) like arginase, arginine decarboxylase (ADC), and arginine deiminase (ADI) may be effective in cancer therapy

Therapeutic enzyme treatments cause cell death by inducing autophagy, apoptosis, and the production of reactive oxygen species. Asparaginase, arginase, methionase, and arginine are enzymes. Many deiminases have already been studied for their anticancer properties, and many are currently in clinical trials. Because Arg is a versatile biomolecule, studies have revealed that ASS is a critical enzyme in its metabolism, and most cancer cells are auxotrophic to this amino acid. Cancerous cells' ASS auxotrophic nature opens the door to novel therapy by altering the microenvironment (restricted Arg) surrounding uncontrolled cell proliferation.

Arg decarboxylase (ADC):

ADC catalyzes the conversion of Arg into agmatine and CO2. Agmatine is thought to have anti-tumor activity. ADC plays a role in preventing solid proliferation by interfering with polyamine biosynthesis

and the cell cycle. Agmatine dosage also inhibited the progression of human hepatoma cells (HepG2) due to the elimination of ODC expression. It interferes with the G1 and G2 phases of the cell cycle without appearing to be apoptic .

Arginase deiminase (ADI)

Arg iminohydrolase (ADI) catalyzes the conversion of the semi-essential amino Arg to l-citrulline and ammonia. ADI is found primarily in prokaryotic systems (mycoplasma) and is absent in mammalian systems. One of the most important discoveries of ADI was its anti-proliferative effect on human leukemia cells, which is 100 times more effective than asparaginase. Bacillus, pseudomonas, enterococcus, lactobacillus, streptococcus, and mycoplasma are the most common ADI sources.

The combination of ADI-PEG-Gemcitabine inhibits pancreatic cancer growth by inhibiting the phosphorylation of NF-B p65. The combination of ADI-PEG and Doxorubicin slows the progression of breast cancer. Combinatorial ADI-PEG therapy with chemotherapeutic agents (cisplatin, pemetrexed, doxorubicin) is also being investigated in the treatment of prostate and small lung cancers. The mechanism by which ADI inhibits cancer progression is based on the fact that it reduces Arg in cancer cells' microenvironment. This physiological change in Arg homeostasis causes a variety of intracellular changes, which are then activated and deactivated sequentially, eventually leading to cancerous cell death

ARGINASE:

Several studies have demonstrated that using human arginase, covalently binding the human arginine to

PEG, and replacing the metal ion with cobalt resulted in killing both ASS and OCT cancer cells. Because melanoma cells lack ASS, they require extra Arg from outside sources to meet their needs.

Arginase induces autophagy (programmed cell death) by increasing membrane potential, ROS, the Akt/mTOR signaling pathway, Erk1/2 activation, tumor-associated macrophage activation, and other pro-apoptotic factors. All of these events culminate in the death of cancer cells.

ARGINASE ASSAY:

Arginase activity can be determined using both quantitative and qualitative assays. While administering arginase as a therapeutic drug, it is critical to monitor the Arg level. Advanced bioanalytical techniques such as fuorometric and biosensing methods are used in addition to spectrophotometric methods. The spectrophotometric method, which relies on recording absorbance at a specific wavelength to form a colored complex, is the most reliable quantitative and qualitative analysis method.

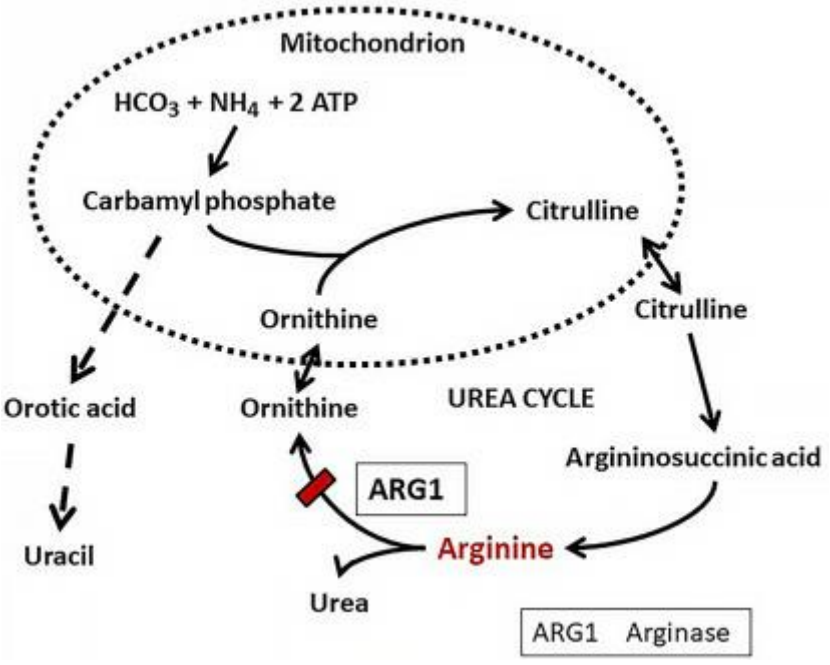
Another conventional method used in thin layer chromatography (TLC). Ornithine reacts with reagent ninhydrin to produce an orange color.

FUTURE PERSPECTIVE AND CONCLUSION:

As we have been considered that arginase plays a major role in the urea cycle for a long time in the ammonia detoxification, and it's been seen that the activity in liver of the arginase is higher than in the vasculature. It holds very great promises in the treatment of all the other disease which is related with the cardiovascular and some other also like cancer, it's still not been identified that which strain or the part of its effective and is beneficial. therapeutic test been also tested with almost all of the positive results, and this paper concludes by stating the relationship between arginase and NO –bioavailability & now limiting arginine levels.

Urea cycle:

It is a sixth end enzyme of the urea cycle that converts the enzyme arginine to urea in ornithine. Urea is normally excreted from the body and ornithine is recycled. It provides protection from excess ammonia, but ornithine is necessary for cell proliferation and other physiological functions.in this reaction, as we see that urea being formed which occurs originally in the mitochondria and in the cytosol of, the main enzyme associated with the cycle is carbamoyl phosphate synthase.



Therapeutic application:

It usually plays a role in the regulation of the tumor growth and the metastasis and plays a role in the therapy of cancer. Arginase plays an important role in health a disease, and it's involved in diseases and injuries affecting the peripheral cardiovascular system and (CNS). the b megaterium have been used as an alternative for the high yield in the protein syntheses. Arginase been found to be producing the beta amylase in 1st time in the microbial kingdom.

ROLE:

Its activation also helps promote polyamine synthesis B. megaterium is a bacterium that can live on different types of substrates such as carbon, meat, and waste from the petrochemical industry, and usually survive in extreme conditions by producing spores. Arginase is used to lower blood serum levels of arginine to starve cancer cells that are auxotrophic to arginine. In some recent studies it's been seen that it contributes to the vascular remodeling in the PAH, all the forms of both them been found in lungs of the mice which been initially exposed to the chronic hypoxia but the increased level of arg 2 been detected in the arteries.

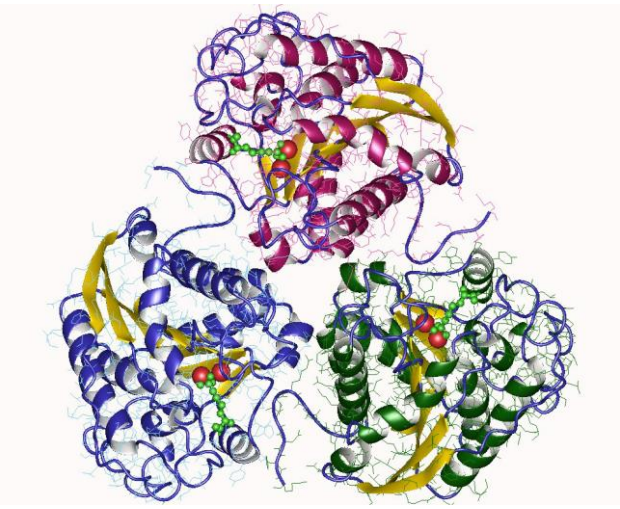
PATHOLOGY

Arginase deficiency generally refers to decreased function of arginase I, the hepatic isoform of arginase. This deficiency is commonly known as hyperargininemia or arginemia. The disorder is inherited and is autosomal recessive. It is characterized by decreased arginase activity in liver cells. It is considered the rarest of the hereditary defects of ureagenesis. Symptoms of the condition include neurological impairment, dementia, growth retardation, and hyperammonemia. While some symptoms of the disease can be controlled through dietary restrictions and pharmaceutical developments, there is currently no completely effective cure or therapy. Due to its immense size of about 60 cubic microns, B. megaterium has been used since the 1950s to study the structure, protein localization, and membranes of bacteria. B. megaterium in particular is the organism they used. Lwoff and Guttman in the studies that discovered lysogenesis. It considered as nonpathogenic; this species is usually a good

cloning host because it's usually able to host the numerous plasmid vectors while staying stable.

STRUCTURE:

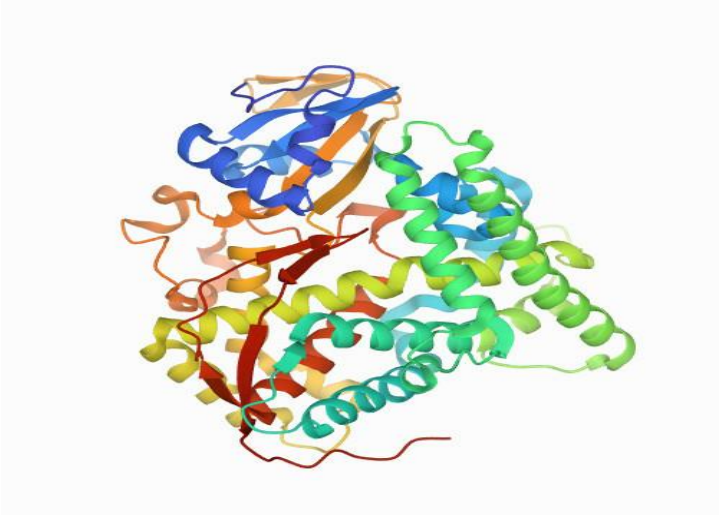
- Arginase + 3 Arg analogue (green) + 6 Mn (red), Rattus norvegicus



molecular weight of approximately 1,500-fold purified arginase from rat liver (specific activity = 19,500 pmoles , rat liver arginase, a manganese-metalloenzyme crystallized from polyethylene glycol, is a trimer with three 35,000 Da monomers in the asymmetric unit.

- Crystal structure of bacillus megaterium

(https://www.rcsb.org/structure/3KX3)



MATERIAL AND METHODS

MATERIAL:

- Autoclave machine
- Flask
- Pipette
- Spoon
- Measuring machine
- Petri plates
- Gloves
- Microscope
- Glass plates
- Measuring cylinder
- Test tube
- Micropipettes
- LAF

CHEMICAL:

- Agar agar
- Nutrient broth
- Water
- Crystal violet dye
- Safranin
- Ethanol

General lab safety measures rule:

- Wear the lab every time you working in the lab
- Use gloves
- Wear glasses while working with chemical
- Sterilize your working space and clean it after using
- Use autoclave carefully
- Wash hands every time you leave the lab

PROCEDURE:

Initially we will start with sterilizing our equipment's and clean our working space so that any type of contamination will not take place then we start with autoclaving our equipment for decontamination then we have to prepare agar plates of nutrient broth and for the broth we firstly measure the amount of agar agar and nutrient broth mix into a flask and mix it with 100 ml of distilled water.

Then we put a cotton plug on top of the flask and cover it properly and to protect it from steam we put paper covering outside it, then we use autoclave and do it for around 20-25 minutes after all it done we take it out carefully and bring it to the room temperature to cool it down.

After that when all is done, we then inoculate Bacillus MEGATERIUM in the flask properly, then again after inoculation we put the cotton plug to cover it and move it into the shaker where it will rotate at a Constant speed, which helps to show growth, which usually lasts about few days after continuous shaking.

Next step, then we pour the agar broth into the petri plates and keep it in under UV lights and fan of LAF to settle it down, when all the plates have been made the take bacterial growth inoculum and streak it into the plates.

keep it for night and next day when we see the growth in the plates we will use those growth colonies for the staining procedure, streaking process is a crucial if a proper streaking is not done the growth of the bacteria will not be seen easily and will not be used for further process.

In this step we take colonies and streak it a bit in glass plates which includes three steps,

Firstly, we will use the crystal violet dye (in this the dye is being used as an intercalating dye which then be used as a quantification of DNA) on the cells then in second step we will use grams iodine solution (this is being used to differentiate between the gram positive and gram negative)

we put it for a while after that lastly we will use decolorizer such as ethyl alcohol or acetone and then a secondary strain safranin for the red color and then we perform arginase plate assay for the final process.

- *Figure 0-1 colony growth of Bacillus .megaterium(Colonies form in chains due to sticky polysaccharides on the cell wall, and maximum growth of B. Megaterium was observed after six hours.)*

Figure 0-2 nutrient broth (a liquid medium used to cultivate a wide range of organisms from clinical specimens and other materials for specific purposes, this medium can be enriched with other ingredients such as blood, serum, sugars, and so on.)

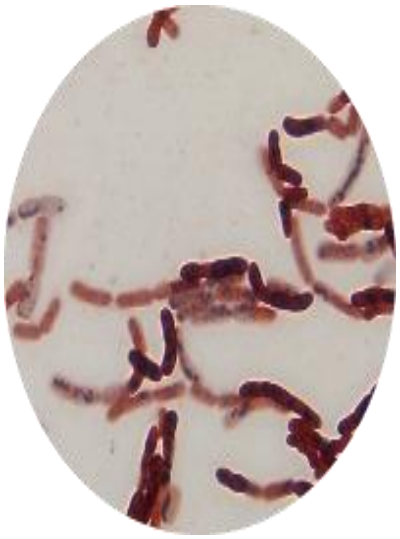


Figure 0-3 gram positive cells under microscope(Gram-positive, rod-like,)

MORPHOLOGICAL CHARACTERISTICS:

The basic staining process been done on the strains of bacillus megaterium and when we observed under the microscope. it shows the presence of the growth of the bacteria and then being stained after a while to see the presence of the gram-positive bacteria as in red color.

Arginine deprivation for the treatment of cancer:

1. Due to the low incidence of serious side effects such as myelosuppression and gastrointestinal or other serious organ toxicity, arginine deprivation can easily be combined with other anticancer drugs to increase the response rate.
2. Food restriction, transport inhibition and enzymatic degradation are three main strategies used for therapeutic arginine depletion.
3. The arginine deprivation process can be roughly divided into early and late stages: (a) Early stage of arginine deprivation Autophagy: in the early stage of arginine deprivation (0-48 h in cancer cells in vitro)

APPLICATIONS:

Arginase activity has 2 main homeostatic purposes. Remove ammonia from the body by urea synthesis to produce ornithine, which is a precursor of polyamines and prolines. Arginase plays an important role in health a disease, and it's involved in diseases and injuries affecting the peripheral cardiovascular system and (CNS). B.megaterium have a very efficient protein secretion system, grows in different carbon system Increased the richness of soil bacteria and most of the fungal communities.

CONCLUSION:

In this whole project experiment, we conclude that the final growth and the viability is being visible and after staining process all the rod-shaped cells in chains of bacillus megaterium being seen which were gram positive and then we did arginase plate assay for the examining of state of cell. But work on bacillus megaterium still going on and many researches being started but not yet being much noticed so far with the arginase so maybe in future there will be more researches will be done but as far now there is none such as being done. By my knowledge.

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