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Effects of Temperature, pH, and Agitation Rate on the Production of Microbial L-Arginase

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Abstract: *The enzyme arginase metabolizes L-arginine to L-ornithine and urea. Besides its fundamental role in the hepatic urea cycle, arginase is also expressed the immune system of mice and man. While significant interspecies differences exist regarding expression, subcellular localization and regulation of immune cell arginase, associated pathways of immunopathology are comparable between species. Minimal Arginine Agar (MAA) medium was prepared and used for the screening of bacterial isolates for the production of L-arginase. Colonies that formed highest pink zones were picked and maintained on the agar slants at 4°C. The zone of colour change from the agar medium that produced a colour change from yellow to pink. The highest zone was recorded from SB6 (42.00 ± 0.09 mm) and SB10 (33.00 ± 0.30 mm). The effect of temperature, the effect of agitation rate and the effect of pH on the activity of L-arginase was examined for SB6 and SB10. The highest enzyme activity of 121±0.03U/ml was recorded at temperature 35°C for SB6, the highest enzyme activity of 120±0.01U/ml was recorded at agitation rate 200rpm for SB6 and the highest enzyme activity of 114±0.03 was recorded at pH 7.0 for SB6.Two of the isolates screened in this project work showed remarkable L-arginase activity. They could be promising microbes for industrial production of L-arginase. Isolate SB6 was identified as Alcaligenes faecalis Strain ZB.*

Keywords: *Alcaligenes, Arginine, faecalis, L-arginine, L-ornithine, urea*

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I. Introduction

L-Arginine (Arg) is a basic amino acid (AA) in physiological fluids. Its content is relatively high in seafood, watermelon juice, nuts, seeds, algae, meats, rice protein concentrate, and soy protein isolate [1-3], but low in the milk of most mammals (including cows, humans, and pigs) [4,5]. Results of the third National Health and Nutrition Examination Survey indicate that mean Arg intake for the US adult population is 4.4 g/day, with 25, 20 and 10% of people consuming <2.6 (suboptimal), 5–7.5, and >7.5 g/day respectively [2]. In addition, preterm infants, who represent 10–12% of newborns, exhibit Arg deficiency [6]. Arginine nutrition remains a significant concern in both human and animal health, as well as livestock production. Substantial amounts of orally administered Arg do not enter the systemic circulation in adults (humans, pigs, and rats), because 40% of dietary Arg is degraded by the small intestine in first pass metabolism [7,8].

In contrast, there is little L-arginase activity in absorptive epithelial cells (enterocytes) of the neonatal small intestine and nearly all of the absorbed dietary Arg that is not utilized locally for protein synthesis can enter the portal vein of suckling infants [3]. Because Arg is the nitrogenous precursor for the synthesis of nitric oxide (NO; a key signalling molecule in virtually every cell type) by NO synthase (NOS) and regulates vital metabolic pathways [9], there is growing interest in Arg nutrition and physiology beyond protein synthesis. Therefore, this article will review the versatile roles of Arg in multiorgan functions, as well as the prevention and treatment of major problems related to developmental biology and nutrient metabolism [10-12].

Arginase was discovered in mammalian liver [13] who showed that the products of its action are ornithine and urea. Since then, the enzyme has attracted a great deal of interest from many points of view. The enzyme has been found to exist in two forms that have evolved with differing tissue distributions, metabolic functions and subcellular locations in mammals [13]. The cytosolic form, *Arginase I* is found predominantly in the liver or hepatic cells and is important in ureogenesis. *Arginase II* is a mitochondrial enzyme that is extrahepatic and more widely distributed in numerous tissues, for example, kidney, and skeletal muscle [14].

It may be found at lower levels in macrophages, lactating mammary glands, and brain. Important roles of *Arginase II* have been reported to be the biosynthesis of polyamines, the amino acid ornithine, proline and glutamate and in the inflammatory process. Genetic "knockout" experiments suggest that *arginase II* functions in L- arginine homeostasis by regulating L-arginine concentrations for cellular biosynthetic reactions such as nitric oxide biosynthesis. The human type I and type II *arginases* are related by 58% sequence identity and are

immunologically distinct [15].The arginases catalyse the divalent cation-dependent hydrolysis of L-arginine to form the non-protein amino acid L-ornithine and urea [15,16]. L-Arginase is widely distributed in the plants, animals, human beings and the microorganisms. Arginase extracted from the livers of the animals exhibits low recovery, huge cost and contamination with the viruses. Other bacterial sources showed the presence of huge number of inclusion bodies, hence difficult to purify [15].

II. Literature Review

Microbial enzymes are of great importance in the development of industrial bioprocesses. Current applications are focused on many different markets including pulp and paper**,** leather, detergents, and textiles, pharmaceuticals, chemical, food and beverages, biofuels, animal feed and personal care, among others. Today there is a need for new, improved or/and more versatile enzymes in order to develop more novel, sustainable and economically competitive production processes. Microbial diversity and modern molecular techniques, such as metagenomics and genomics, are being used to discover new microbial enzymes whose catalytic properties can be improved/modified by different strategies based on rational, semi-rational and random directed evolution. Most industrial enzymes are recombinant forms produced in bacteria and fungi [17].

Microbial enzymes are known to play a crucial role as metabolic catalysts, leading to their use in various industries and applications. The end use market for industrial enzymes is extremely wide-spread with numerous industrial commercial applications. Over 500 industrial products are being made using enzymes [17]. The demand for industrial enzymes is on a continuous rise driven by a growing need for sustainable solutions. Microbes have served and continue to serve as one of the largest and useful sources of many enzymes. Many industrial processes, including chemical synthesis for production of chemicals and pharmaceuticals, have several disadvantages: low catalytic efficiency, lack of enantiometric specificity for chiral synthesis, need for high temperature, low pH, and high pressure [18].

Also, the use of organic solvents leads to organic waste and pollutants. Enzymes are more useful for these applications as they work under mild reaction conditions (e.g, temperature, pH, atmospheric conditions), do not need protection of substrate functional groups, have a long half-life, a high stereo-selectivity yielding stereo- and region-chemically-defined reaction products at an acceleration of 10 to10- fold, and, in addition, they work on unnatural substrates. Furthermore, enzymes can be selected genetically and chemically-modified to enhance their key properties: stability, substrate specificity and specific activity. There are drawbacks however, to the use of enzymes, e.g., certain enzymes require co-factors. However, various approaches such as cofactor recycling and use of whole cells can solve this problem. About 150 industrial processes use enymes or whole microbial cell catalysts [18].

The global industrial enzymes market is very competitive with Novozymes being the largest player in the industry, followed by DSM, and DuPont (after it acquired a majority stake in Danisco and its Generator division), among others. The companies mainly compete on the basis of product quality, performance, use of intellectual property rights, and the ability to innovate, among other such factors. North America and Europe are the largest consumers of industrial enzymes although the asia pacific region will undergo a rapid increase in enzyme demand in China, Japan and India, reflecting the size and strength of these country's economies [18].

2.1 Sources of Enzyme

Enzymes occur in all living organisms and catalyze biochemical reactions necessary to support life [19]. A wide array of enzymes is extracted from plant sources; they have many advantages including cost of production and stability of products. An ample range of sources are used for commercial enzyme production from a broad spectrum of plant species. Non-microbial sources provide a larger proportion of these, at the present time [20]. Microbes are preferred to plants and animals as sources of enzymes because microbial enzymes are more stable than their corresponding plant and animal enzymes and their production is more convenient and safer about fifty years ago, enzymes were being extracted strictly from animals like pig and cow from their pancreas [21,22]. Animal enzymes were multifold, they were not very stable at low pH environment so the enzyme product was destroyed before doing the job. To overcome this problem plant enzymes were discovered, most important one is extraction of peroxidase from horseradish roots occurs on a relatively large scale because of the commercial uses of enzyme [23].

Peroxidase can also be extracted from soybean, it is also having the common features with horseradish peroxidise [15]. Some plants like *Cruciferous* vegetables, including *broccoli*, cabbage, kale and collard and turnip greens and papaya are rich in catalase [24] heat sprouts contain high levels of catalase and vegetarian source of catalase include apricots, avocados, carrots. Catalase is also present in some microbes and bacteria, *Aspergillus niger* culture alsomproduces catalase enzyme [25].

2.2 Sources of L-Arginase

L-Arginase apart from being ubiquitously present in mammalian tissues has also been characterized from various worms, molluscs, fishes, bacteria, fungi, yeast, actinomycetes, algae and plants.

2.2.1 Bacteria

Among bacteria producing L-Arginase, the prominent ones include many bacilli, the *mycobacteria* [26], *T.aquaticus* [27], *Agrobacterium-Rhizobium* group, [28] and cyanobacterium *Aphanocapsa* 6308 reported by [29] and other *cyanobacteria*, [30]. Among the bacilli, *Bacillus licheniformis* arginase has been purified by [31]. L-Arginase production was studied closely in *Bacillus subtilis* 168 by [32]. L-Arginase activity was reported in *Streptomyces* spp. [33] and *Streptomyces calvuligerus* by [34]. L-Arginase from extreme thermophile *Bacillus caldovelox* has been purified and characterized by [35]. [36] have described arginase production and characterization in the phototrophic bacterium *Rhodobacter capsulatus* E1F1.

Arginase activity in cephamycin producers *Streptomyces clavuligerus* and *Nocardia lactamdurans* was reported. *Bacillus brevis* Nagano - gramicidin S- producing bacteria was shown to be a source of arginase. The enzyme produced from bacteria is said to be highly specific for L-arginine and inducible by the addition of L-arginine to the glutamate medium. *Helicobacter pylori*- the gastric human pathogen has been reported to have rocF gene that encodes arginase. In pathogens such as *Helicobacter*, by consumption and outcompeting the host for the limited arginine available, arginase pathway is a major escape mechanism helping to evade the toxic antimicrobial effects of host nitric oxide. In *Cyanobacteria* L-arginase is useful for the utilization of arginine into urea and subsequently into ammonia which is taken up by the bacteria as a nitrogen source. The spore-forming, gram positive bacterium *Bacillus anthracis*, the causative agent of anthrax has shown arginase activity. Mainly *B. anthracis* bacilli and endospores exhibit arginase activity [37]. An engineered L-arginine sensor (arginine-regulated transgene, ART) of *Chlamydia pneumoniae* enables arginine-adjustable transcription control in mammalian cells and mice [38].

2.2.2 Protozoa

Species of *Leptomonas*, *Leishmania*, *Crithidia* and *Blastocridhidia* have been reported by [39] to posess arginase. *Entamoeba histolytica* has been shown to have arginase called *Entamoeba histolytica* arginase (EhArg). Its cloning and expression has been discussed by [40]

2.2.3 Fungi

Two forms of L-Arginase expressed by *Neurospora crassa* as reported by [41] is the only reported example of multiple forms of arginase in a microbial organism. Mycelial extracts of *Trichoderma sp.* were reported to be a source of arginase by [42]. An extensive survey of higher fungi carried revealed that the presence of L-arginase in members of family Agaricaceae including *Agaricus bisporus* that led to the accumulation of urea in its fruit bodies [43].

2.2.4 Lichens

[44] reported the purification of three forms of L-arginase from thallus of *Evernia prunastri* incubated at different time intervals. The third form was obtained by culture grown in presence of cycloheximide Secreted L-arginases from *Evernia prunastri* and Xanthoria *parietina* thalli have been reported by [45] to utilize arginine in a Mn^{2+} dependent manner and show lectin function by to utilize arginine in a Mn^{2+} dependent manner and show lectin function by binding to the cell wall of both homologous and heterologous algae thus helping in further algal-lichen associations.

The secreted arginase acts as a lectin by binding to the surface of *Nostoc* cells through a specific receptor which develops urease activity. *Leptogium corniculatum*, a cyanolichen containing *Nostoc* as photobiont, has been reported to produce and secrete arginase to culture medium containing arginine [46].

2.2.5 Yeast

In *Saccharomyces cerevisiae*, arginase has been reported to form a multienzyme complex with ornithine transcarbamoylase in which L-arginase acts as a negative allosteric effector of ornithine transcarbamoylase [47]. The purification and characterization of arginase from a plasmid-containing, enzyme-overproducing, protease deficient yeast strain (*S.cerevisiae*) was reported by [48]. L-Arginase was purified from *Schizosaccharomyces pombe* by [49].

2.2.6 Sea-organisms

Larva of *Phoronis pallida* was shown to possess arginase activity [50]. The giant African snail-*Achatina fulica* has been shown to posess 3.5 fold increased L-arginase activity after an injection of ammonium chloride [51]. L-Arginase has been reported in African lungfishes- *Protopterus aethiopicus* and *Protopterus*

annectens in the cytosolic fractions of their livers [52]. South American fish pacu (*Piaractus mesopotamicus*) when supplemented with arginine utilized the supplementation efficiently with normal hepatic L-arginase activity as reported by [53].

L-Arginase activity was found to be highest in to juveniles of sea bream *Sparus aurata* fed with yeast diets. [54] reported that basal arginase I expression was found in carp *Cyprinus carpio* mid kidney while arginase II was found to be distributed in all organs examined with highest levels in liver. Pacific spiney dogfish shark *Squalus acanthias* showed enhanced activity of arginase with elevated nitrogen intake [55]. The effect of protein in diet was directly related to increase in L-arginase activity in liver of juveniles of *Rhamdia quelen*. L-arginine metabolism by L-arginase in mitochondria isolated from the liver of Antarctic fish *Notothenia rossii* and *Notothenia neglecta* was studied by [56].

2.3 L-Arginase in the Human Immune System

In humans, L-arginase was detected in the peripheral blood mononuclear cell (PBMC) fraction after injury [57], inflammatory synovial fluid macrophages (due to arginase II) of patients with arthritis [58], inflammatory cells of bronchoalveolar lavage fluid of asthmatic patients [59], psoriatic lesions [60], in activated monocytes of patients with autoimmune diseases [61] and in the PBMC fraction of patients with active pulmonary tuberculosis [62].

Interestingly, *Saccharomyces cerevisiae* and *Candida albican* up-regulate genes of their endogenous L-arginine biosynthetic pathways upon phagocytosis by human neutrophils [63]. This transcriptional response likely reflects the L-arginine-deprived intraphagosomal micromilieu of PMN and is not detectable upon phagocytosis by human monocytes [63] which do not express L-arginase [64]. Another study confirmed the expression of arginase I in human PMN but localized the enzyme to the gelatinase granules [65]. The discrepancy in results is still unclear at the moment. *In vitro*, constitutive human PMN arginase activity was not modulated by a variety of pro- and anti-inflammatory stimuli, including cytokines that typically lead to arginase induction in murine myeloid cells [64].

In contrast, L-arginase is inducible in a variety of other human cell types such as endothelium, epithelial cells and smooth muscle. Arginase I shares this feature with other important constitutive PMN proteins or peptides involved in inflammation and microbial defense like human cationic anti-microbial protein of 18 kDa (hCAP18), neutrophil gelatinase-associated lipocalin (NGAL), bactericidal/ permeability-increasing protein (BPI) and the defensins [66].

The fundamental disrepancies of L-arginase expression and regulation between murine and human immune cells fit into a growing list of differences in the immune systems of both species [67]. This must be kept in mind when data from animal models are extrapolated to the human situation. Also, data on L-arginase expression in the human PBMC fraction without further purification need to be interpreted with caution. It remains to be analysed if L-arginase protein and activity is really induced in monocytes within the PBMC fraction. Alternatively, activated PMN are known to aberrantly co-purify within the PBMC fraction of patients with tumours or inflammation [68], so that *de novo* arginase activity in the PBMC population under conditions of inflammation might actually be confined to the neutrophil subset.

2.4 L-arginase and Cancer

Research over the last couple of years has convincingly demonstrated a crucial role for L-arginase in tumour immunobiology [69]. Earlier reports focused on the expression of L-arginase in murine or human primary cancer tissue as well as malignant cell lines [70] and emphasized its potential role in the promotion of tumour growth via polyamine synthesis or down-regulation of NO-mediated tumour cytotoxicity. It also became clear that malignant tumours have evolved strategies to evade an effective tumour-cytotoxic immune response by inducing pathways of inflammation-associated immunosuppression [69, 71, 72].

The fate of a developing tumour is dictated not only by the properties of the malignant cells but also by the phenotype of tumour-infiltrating and tumour-interacting myeloid cells [73]. Leukocyte-tumour interaction can result in tumour destruction as well as promotion of tumour growth, tissue invasion or metastasis [73]. A key mechanism of tumour evasion from immunemediated destruction is the induced impairment of T cell functions [74]. Alternatively, tumour progression can be enhanced by infiltrating CD4+ T cells and a reduced carcinogenesis interestingly correlated with decreased infiltration of neutrophils [75] recapitulating an earlier report on the tumour growth promoting potential of PMN [76].

III. Materials and Methods

3.1 Sterilization of Materials

Glassware such as conical flasks, test tubes, pipettes, measuring cylinder and Petri dishes were washed thoroughly with detergent, rinsed with water and sterilized in the oven at 140^{o}C for 180 minutes. Petri dishes, pipettes and measuring cylinders were wrapped properly with aluminium foil before sterilization [77].

3.2 Preparation of Culture Media

Nutrient agar and nutrient broth were used for the cultivation of bacteria. Sterilized glassware was used during the preparation of media. Appropriate weights of the nutrient agar and nutrient broth were transferred into separate conical flask, following the manufacturers' instructions. For the submerged fermentation and screening techniques the media were formulated following established procedures. The media were sterilized in the autoclave at 121^{o}C for 15 minutes. The media were poured in Petri dishes and allowed to set and broth was dispensed aseptically in the test tubes before autoclaving.

3.3 Procurement of Bacterial Isolates

The bacterial isolates used were procured from the Microbiology Laboratory of Al-Hikmah University, Ilorin, Nigeria. The isolates were originally isolated from the soil environment.

3.4 Purification of the Selected Bacterial Isolates

The bacterial isolates used were streaked on sterile nutrient agar plate and incubated for 24 hours at 37^{o}C. They were then subcultured severally in order to obtain pure cultures of the isolates. Purified distinct colonies were picked using sterile inoculating loop and transferred onto nutrient agar slant using streaking technique. The slants were incubated at 37^{o}C for 24 hours. After incubation the McCartney bottles containing the isolates were kept at refrigeration temperature for further use [77].

3.5 Screening of Selected Bacteria for the Production of L-arginase

Selected bacterial isolates were screened for the production of L-arginase. The screening was carried out following the method described by [78]. Minimal agar media containing the substrates (L-arginine) as the sole carbon and nitrogen source were used. A change from yellow to pink colour of the agar medium due to the growth of a particular microbial colony was an indication of a change in pH [79].

Minimal Arginine Agar (MAA) medium was prepared and used for the screening of bacterial isolates for the production of L-arginase. Components of the media (g/L) include 0.5 KCl, 0.5 MgSO4, 1.0 KH_2PO4, 0.1 FeSO4, 0.1 ZnSO4, 25 NaCl, 15 Agar and 10 L-arginine. L-arginine was used as carbon and nitrogen source. The media were supplemented with 0.012 (g/L) of 2.5 % of phenol red as pH indicator. After inoculation, all the plates were incubated at 37°C for 24 hours. L-arginase activity was identified by the formation of a pink zone around colonies. Colonies that formed highest pink zones were picked and maintained on the agar slants at 4°C [80-82].

3.6 Production of Enzymes

Production of enzyme from promising isolates was carried out in a medium containing 4% (w/v) of the additive (L-arginine for L-arginase), 0.2% KH_2PO4 (w/v), 4.0% KNO_3 (w/v), 4.0% NaCl (w/v), 0.005% $MgSO_4$.7H2O (w/v), 0.002% $CaCO_3$ (w/v), 0.001% $FeSO_4$.7H2O (w/v) with pH adjusted to 7.4. The submerged fermentation medium was maintained at 37°C for 72 hours at 200 rpm in a shaking incubator. At the end of the fermentation period, the broth was centrifuged at 10,000 rpm for 20 minute at 4°C. The cell free supernatant was recovered as crude enzyme preparation and subjected to purification for further studies [78, 83].

3.7 Purification of Enzymes

The clear supernatant containing crude enzyme was precipitated with ammonium sulfate. The precipitated enzyme was then dissolved in potassium phosphate buffer [78, 83].

3.8 Enzyme Assay

Activity of each enzyme was determined using 2.0% of each substrate (L-arginine for L-arginase). One milliliter (1 ml) of enzyme solution was mixed with 1 ml of the substrate (L-arginine for L-arginase) in 50 mM Tris-HCl (pH 8.5) and incubated at 30°C for 10 minutes. After incubation, the reaction was stopped by the addition of 2 ml of 0.4 M trichloroacetic acid. Then, the precipitate was removed by centrifugation at 10,000 rpm for 10 min, and 1 ml of each supernatant was neutralized with 5 ml of 0.4 M sodium carbonate and incubated with 1ml of 1 N Folin Ciocalteu's reagent solution at 40°C for 20 min. Subsequently absorbance at 340 nm was measured using spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme that caused absorbance at 340 nm to increase at a rate of 0.1 OD per min per ml of crude enzyme extract under the assay conditions [78, 83, 84].

3.9 Enzyme Kinetics (Optimization of Culture Conditions)

Each isolate was subjected to different culture conditions to derive the optimum conditions for enzyme production. Enzyme production was estimated at various temperatures, pH, and agitation rate. Experiments were conducted in triplicate and the mean values were recorded [78, 83, 84].

3.10 Identification of the Bacterial Isolates Using the Polymerase Chain Reaction (PCR) Amplification Technique

Promising bacteria were subjected to PCR analysis for appropriate identification. The molecular characterisation and identification of the bacterial isolates were carried out at IITA Laboratory, Ibadan, Nigeria. Polymerase chain reaction (PCR) can be used to identify microorganisms using a method that depends on knowledge of DNA sequence unique to the organism under study and provides a specific means of identifying that organism.

This method can also be used to identify a target organism even in small numbers in mixed cultures. Another method has an absolute requirement for a pure culture of the target organism but requires no knowledge whatsoever of the DNA sequence of that organism. This method depends on random priming of the PCR using an oligonucleotide primer (or pair of primers) of arbitrary sequence.

Sequence comparison with the databases was performed using Basic Alignment Search Tool (BLAST) through the National Centre for Biotechnological Information (NCBI).

IV. Results

The zone of colour change from the agar medium that produced a colour change of yellow to pink were shown in Table 1 below and the standard error of mean was computed. The highest zone was recorded from SB6 (42.00 ± 0.09 mm) and SB10 (33.00 ± 0.30 mm). The effect of temperature on the production of L-arginase was shown in Table 2 and 3; the effect of agitation rate on the enzyme is also shown in Table 4 and 5.

The effect of pH on L-arginase was also shown in Table 6 and 7 based on the two isolates respectively.

Identification of Bacterial Isolate

SB6 – *Alcaligenes faecalis*

Strain ZB

Accession Number: FJ151631.1

rRNA Sequence:

AGGCCATCCCTTTAAATTTGATATGGCTCTGGTGCGCGTGGGTCACCCCTCTATAGTTTTAGAGTGG
TCGAGGGTGAGGATATATCGGAACGTGCCCAGTAGCGGGGGATAACTACTCGAAAGAGTGGCTAA
TACCGCATACGCCCTACGGGGGAAAGGGGGGGATCGCAAGACCTCTCACTATTGGAGCGGCCGAT
ATCGGATTAGCTAGTTGGTGGGGTAAAGGCTCACCAAGGCAACGATCCGTAGCTGGTTTGAGAGG
ACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTT
TGGACAATGGGGGAAACCCTGATCCAGCCATCCCGCGTGTATGATGAAGGCCTTCGGGTTGTAAA
GTACTTTTGGCAGAGAAGAAAAGGTATCCCCTAATACGGGATACTGCTGACGGTATCTGCAGAAT
AAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATT
ACTGGGCGTAAAGCGTGTGTAGGCGGTTCGGAAAGAAAGATGTGAAATCCCAGGGCTCAACCTTG
GAACTGCATTTTTAACTGCCGAGCTAGAGTATGTCAGAGGGGGGTAGAATTCCACGTGTAGCAGT
GAAATGCGTAGATATGTGGAGGAATACCGATGGCGAAGGCAGCCCCCTGGGATAATACTGACGCT
CAGACACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGATAGTCCACGCCCTAAACGATG
TCAACTAGCTGTTGGGGCCGTTAGGCCTTAGTAGCGCAGCTAACGCGTGTATGTCGACCGCCTGGG
GAGTACGGTCGCAAGATTTAAAACTCAAAGAATTTGACGGGGACCCGCACAAGCCGGTGGATTGA
TGTGGATTAATTCGATGCAACGCGAAATACCTTACTACCTTTGACATGTCTGGAAGCGATATGATT
CGGGCCGTGCTCCGCAGAGATACTGAAACCAAGTGCTGCATGCTGCGGTCCAGCTCGTGTCCGGA
ATGTGATTATTCCCGCTACGAGCGACCTTGCATACATGGCTTACCTAGAACCCTAATGAACTGGCG
GTCAATCCGCAGAAGTGGGGAATATGAAGGTCT

IV. Figures and Tables

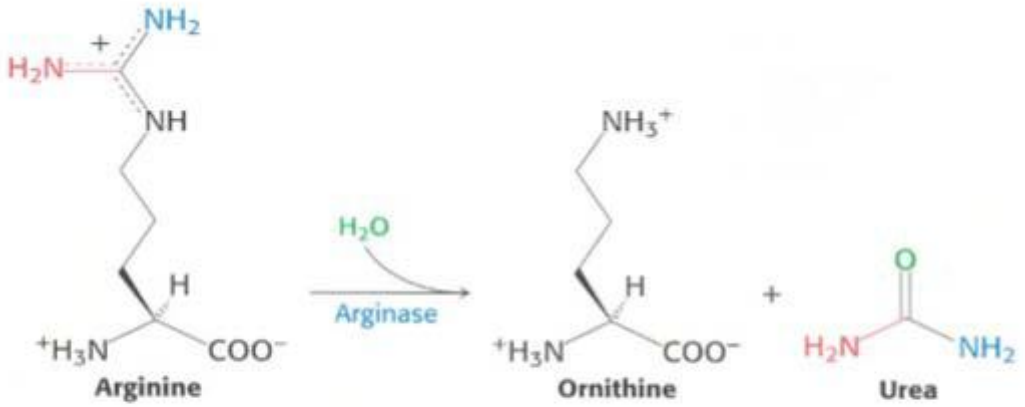

Figure 1 : The arginase reaction.

Figure 1: The arginase reaction

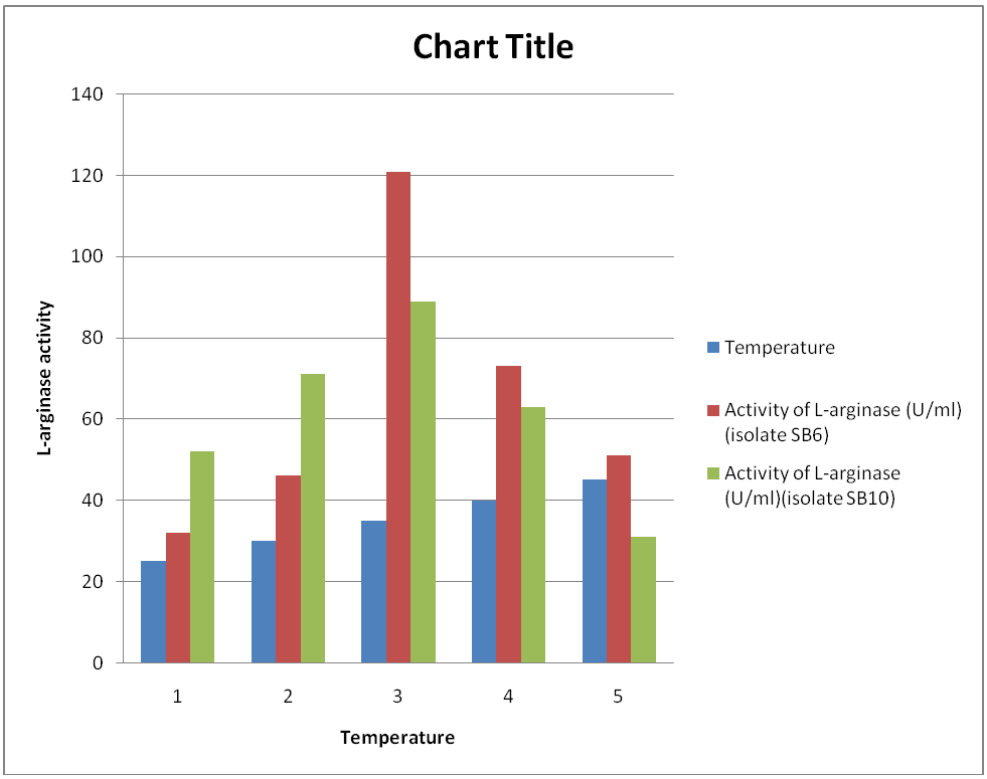

Figure 2: Comparison of the effect of temperature on the production of L-arginase between isolates SB6 and SB10 .

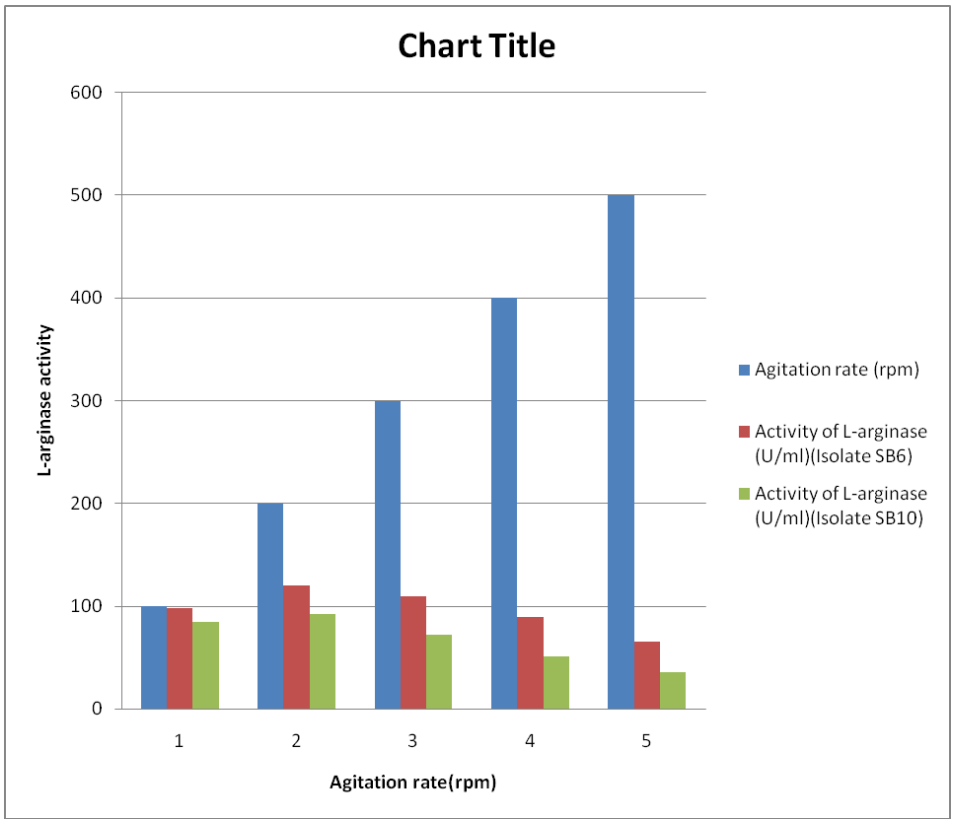

Figure 3: Comparison of the effect of agitation rate on the production of L-arginase between isolates SB6 and SB10 .

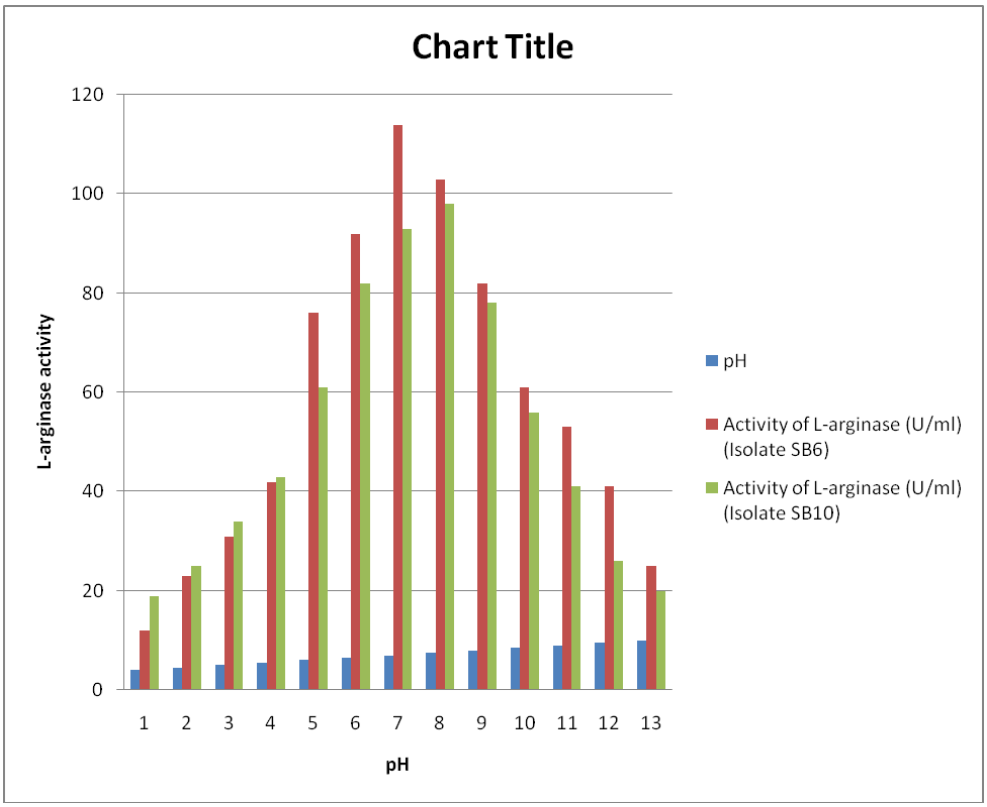

Figure 4: Comparison of the effect of pH on the production of L-arginase between isolates SB6 and SB10.

Table 1: Enzyme Screening Results (mm)

Bacterial Isolate	**L-arginase**
SB1	10.00 ± 0.04
SB2	0.00 ± 0.00
SB3	20.00 ± 0.04
SB4	0.00 ± 0.00
SB5	21.00 ± 0.09
SB6	42.00 ± 0.09
SB7	0.00 ± 0.00
SB8	17.00 ± 0.07
SB9	0.00 ± 0.00
SB10	33.00 ± 0.30
SB11	0.00 ± 0.00
SB12	25.00 ± 0.01
SB13	0.00 ± 0.00
SB14	0.00 ± 0.00
SB15	0.00 ± 0.00

Table 2: Effect of Temperature on the Activity of L-arginase (Isolate SB6)

S/N	**Temperature (°C)**	**Activity of L-arginase (U/mL)**
1	25	32 ± 0.06
2	30	46 ± 0.01
3	35	121 ± 0.03
4	40	73 ± 0.02
5	45	51 ± 0.01

Table 3: Effect of Temperature on the Activity of L-arginase (Isolate SB10)

S/N	**Temperature (°C)**	**Activity of L-arginase (U/mL)**
1	25	52 ± 0.01
2	30	71 ± 0.03
3	35	89 ± 0.05
4	40	63 ± 0.01
5	45	31 ± 0.07

Table 4: Effect of Agitation Rate on the Activity of L-arginase (Isolate SB6)

S/N	Agitation Rate (rpm)	Activity of L-arginase (U/mL)
1	100	98 ± 0.01
2	200	120 ± 0.01
3	300	109 ± 0.04
4	400	89 ± 0.03
5	500	65 ± 0.02

Table 5: Effect of Agitation Rate on the Activity of L-arginase (Isolate SB10)

S/N	Agitation Rate (rpm)	Activity of L-arginase (U/mL)
1	100	84 ± 0.03
2	200	92 ± 0.02
3	300	72 ± 0.02
4	400	51 ± 0.01
5	500	36 ± 0.01

Table 6: Effect of pH on the Activity of L-arginase (Isolate SB6)

S/N	pH	Activity of L-arginase (U/mL)
1	4.0	12 ± 0.09
2	4.5	23 ± 0.02
3	5.0	31 ± 0.05
4	5.5	42 ± 0.04
5	6.0	76 ± 0.03
6	6.5	92 ± 0.02
7	7.0	114 ± 0.03
8	7.5	103 ± 0.06
9	8.0	82 ± 0.09
10	8.5	61 ± 0.04
11	9.0	53 ± 0.09
12	9.5	41 ± 0.03
13	10.0	25 ± 0.01

Table 7: Effect of Ph on the Activity of L-arginase (Isolate SB10)

S/N	pH	Activity of L-arginase (U/mL)
1	4.0	19 ± 0.02
2	4.5	25 ± 0.01
3	5.0	34 ± 0.02
4	5.5	43 ± 0.01
5	6.0	61 ± 0.04
6	6.5	82 ± 0.01
7	7.0	93 ± 0.07
8	7.5	98 ± 0.03
9	8.0	78 ± 0.03
10	8.5	56 ± 0.01
11	9.0	41 ± 0.03
12	9.5	26 ± 0.02
13	10.0	20 ± 0.07

V. Conclusion

Two of the isolates screened in this project work showed remarkable L-arginase production. They could be promising microbes for industrial production of L-arginase. The highest enzyme activity was noticed from bacterial isolate *Alcaligenes faecalis* at temperature 35 ^{o}C, agitation rate 200 rpm and pH 7.0.

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