Review of Uricase and Other Key Metabolites Produced by Microorganisms through Submerged Fermentation

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ABSTRACT:

Uricase is the clinically important enzyme that can control uric acid levels in the blood serum. Uric acid usually produced inside our body as a part of purine metabolism. If the concentration of uric acid increases in the body, that will lead to its deposition in the tissues and joints, which results in inflammation. This condition is call Gouty arthritis. At present 9,20,000 people in United states which is about 3.9% are living with gout. The normal uric acid levels alter with age and sex. As a part of evolutionary changes in the human beings lost their ability to express the enzyme uricase, but microbes, plants and other animals are capable of producing or expressing this enzyme. In general, uric acid is less soluble in water, but by the action of uricase enzyme, it is converted into more soluble allantoin which is easily excreted from the body. Not only uricase but also various important metabolites which are useful commercially, therapeutically and various other purposes are also produced from microbes, plant and animal cells with the advent of improved techniques in fermentation technology. Among all the mentioned production factories microbes are cheapest sources as their nutritional requirements are less when compared to plant and animal cell culturing.

INTRODUCTION:

For more than thirty years Allopurinol is the only drug of choice for the treatment of Gout [1, 2, 3]. In addition, Hydroxychloroquine and other NSAIDS are used for partial pain relief [4, 5]. With the advancements in 21st century Febuxostat and Lesinurad are the recently approved and used drugs for the treatment of Gout. Beside effective in maintaining the uric acid levels these drugs also pose some serious adverse effects [6]. Therefore, enzymatic treatment becomes a game changer in treating Gout.

Urateoxidase is an enzyme which helps in reducing the uric acid levels [7]. Specific biochemical reactions are catalyzed by the enzymes in a very efficient manner. Initially enzymes were produced by solid state fermentation process, but most of the current production of enzymes is based on submerged fermentation methods which are effectively aerated. Commercial enzymes are produced from plants, animals and microorganisms. Still enzymes from microbial source find superiority in terms of production by established fermentation techniques.

Utilizing microbiology, biochemistry, and engineering together with the aim of creating usable products from microbes, cell and tissue cultures, or their components, is known as biotechnology. There are two subcategories of biotechnology that are sometimes referred to as classic (conventional) biotechnology and new (modern) biotechnology [8]. The main goods produced by the classical biotechnology sector include citric acid, industrial alcohol, antibiotics, and additives for food and flavor. On a global scale, these products generate around $300 billion in sales a year. The current output of the new biotechnology, which uses the most recent techniques of genetic engineering and cell fusion to create organisms capable of producing useful products, is worth less than a billion dollars. The biotechnology sector will represent a significantly larger portion of the overall biotechnology market.

Microorganisms are capable of producing a large range of novel or unique chemicals owing to their extensive metabolic diversity. Creating methods for getting novel microbial metabolites is one of the key responsibilities of industrial microbiologists. There are five different methods.

- ✓ Screening for the production of novel chemical compounds with new isolated strains or tests or methods. It's the only way to obtain different kinds of compounds.
- ✔ Chemical alteration of known organisms that live.
- ✔ Biotransformation, in which a chemical molecule is altered by an enzymatic or microbial reaction.
- ✔ Interspecific protoplasm fusion, a technique for recombining the genetic material from producer strains that are relatively closely related. The method is widely used in the antibiotic industry and is believed to produce new or hybrid substances.
- ✔ Gene cloning is the process of transferring genes between unrelated strains with the aim of producing known compound. As an alternative, transfer could occur to non-producers with silent genes, resulting in the production of altered or even brand-new substances. Undoubtedly, in the future, this approach will be preferred.

MATERIAL AND METHODS:

Screening for new metabolites:

There is no one-size-fits-all screening technique. The right tests and microbes must be chosen for the screening programme in order for it to be successful. A commercial screening team can isolate and thoroughly test between 1000 and 2000 types of microorganisms annually. Nowadays, the majority of screening programs concentrate on drugs that are useful for treating cancer, tumors, and viruses, as well as looking for chemicals that are pharmacologically active and enzyme inhibitors. Better food industry starting cultures and microbes that can break down dangerous and persistent pollutants are both sorted. The success of a screening technique is dependent on both the organisms used and the methods for detecting activity. Currently, the selection of strainhas a 30–40% impact on the outcome, while the testing procedure has a 60–70% impact. Only about 1% of the world's microorganisms have been thoroughly studied. Above all, the approximately 1 lakhs known fungi have received insufficient attention, implying that a large number of new natural products can be expected from this group in the future.

Strains used in screening:

Investigators try to identify organisms from severe or unconventional conditions with the belief that these variants are able to generate unique compounds. Microbes from different altitudes, aquatic ecosystems, saline water, deep Ocean, glaciers, springs, or hydrocarbon plants, for instance, have been researched. Particular groups of organisms may be isolated depending on the inoculum source and enrichment procedure. Strain isolation can be accomplished using the following method.

- ✔ The soil or water sample is suspended in a definite amount of sterile water to which the tween has been added as an emulsifying agent. The sample is vigorously agitated.
- ✔ The supernatant is diluted 10^{-1} to 10^{-10}.
- ✔ Samples from these dilution series are plated on various culture media and then incubated.
- ✔ Single colonies from the plates are picked and purified by re streaking.
- ✔ The pure strains are maintained as agar cultures in test tubes.

Enzymes and other important metabolites of microbial origin:

Enzymes occur in every living cell, hence in all microorganisms every single strain of microorganism produces a large number of enzymes (hydrolyzing, oxidizing, reducing, and metabolic in nature).Even though they are major contributors of useful primary and secondary metabolites yields less product hence to overcome this problem we need novel approaches to accelerate the productivity of metabolites from actionomycetes.

1. High- through put whole cell screening of terrestrial and marine actionomycetes.

✔ Global soil sampling.

✔ Pooling of soils and extraction of spores.

✔ Miniaturized fermentations starting with spores.

✔ Screening organism resistant to common antibiotics.

✔ Improved throughput with automation.

2. Enrichment and selections for uncommon terrestrial and marine actinomycetes

✔ Antibiotics and taxon selective media.

✔ Untapped random and exotic soils.

✔ Untapped marine sediments.

3. Genome mining

✔ Sequencing multiple common actinomycetes.

✔ Sequencing rare and slow growing actinomycetes.

✔ Expression of new pathways in robust *Streptomyces* host.

4. Combinatorial biosynthesis

✔ NRPS (Non ribosomal peptide synthesase) pathways.

✔ PKS (Polyketide synthease) pathways.

✔ Glycosylation and other modifications.

Microbes from which uricase are produced:

Uricase is obtained prom different types of microbes but most researcher are targeted for screening of novel isolate produce uricase enzyme in industrial scale in economically.

Necessity for uricase production:

Uric acid is formed by metabolic breakdown of purines. Purines perform many functions such as controlling cell growth, contributing to sugar transport and donating phosphate groups in phosphorylation reactions.

Purine metabolism pathway[9]**:**

Fig 3: Enzymatic degradation of purines in humans

Uricase activity:

Uric acid is an aromatic heterocyclic compound with a 5- membered imidazole ring. Uricase E.C: 1.7.3.3 is an essential enzyme involved in purine metabolism pathway [10]. It is responsible for the formation of allantoin from uric acid, thus catalyzing the oxidative ring opening of the purine ring in the degradation pathway [11, 12, 13].

The catalytic activity of the uricase is seen in the following reaction:

Uric acid Allantoin

The solubility of allantoin is ten folds more when compared to uric acid, therefore it is easily excreted through urine.

Quantification of uricase enzyme:

Enzyme activity determines the concentration of the enzyme. So uricase activity can be determined in two ways, they are:

i) Measurement of uric acid concentration at 293 nm which usually decreases in the presence of uricase enzyme [14, 15].

ii) Measurement of product (allantoin) whose absorbance increases in the presence of uricase enzyme.

Basic operations involved in fermentation process

For the production of enzyme or any other metabolite from microbial source, some basic operations involved are

1.Upstream process:

Upstream processing encompasses all activities connected to the development of microorganisms, medium preparation, sterilization of the culture medium as well as the fermenter, and eventually harvesting.

2.Downstream process:

This process involves isolation and purification of fermentation products (enzymes, antibiotics) from their associated unwanted metabolites.

The entire procedure is broken down into three simple steps: the preparation of the growth substrate (often in sterile form), the fermentation process itself, beginning with the inoculation of the following the harvest of the product (in this case, enzymes), which may be concentrated and purified from the medium and production organisms and then either recovered in a liquidor dried, and iii. Downstream processing, this involves recovering the product in a liquid or drying it after it has been enriched and purified from the production medium and organisms.

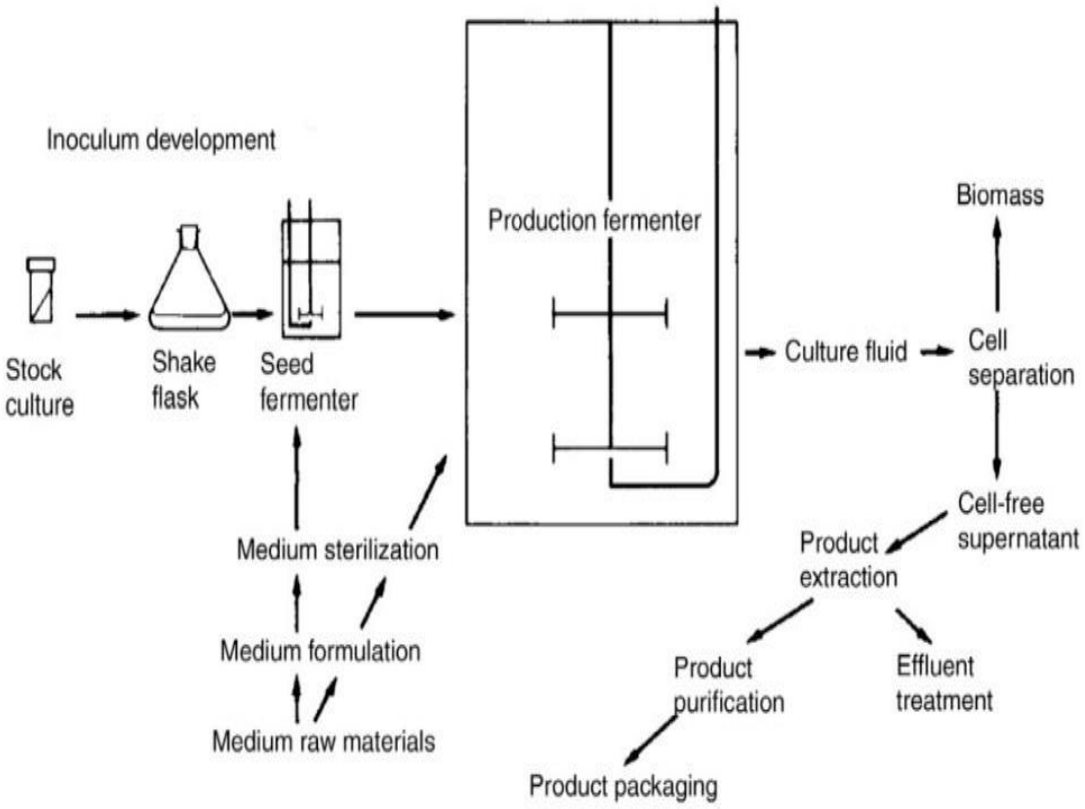

Figure:1, Schematic representation of fermentation process [16]

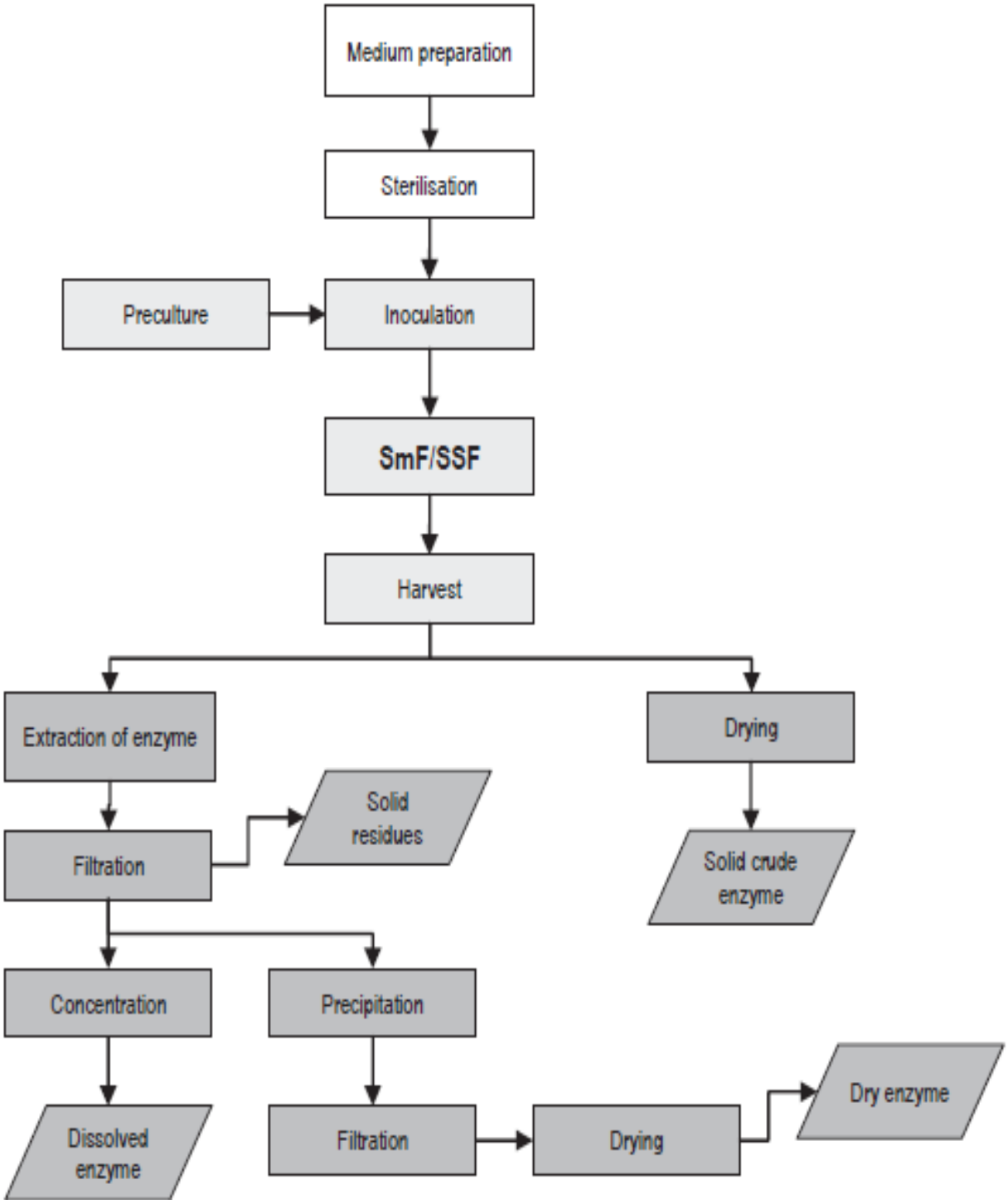

Figure 2: Enzyme synthesis process flow diagram: stages are shown in dark green boxes are upstream- processing, steps shown in light green boxes are fermentation steps whereas steps shown in white boxes are downstream processing **[17].**

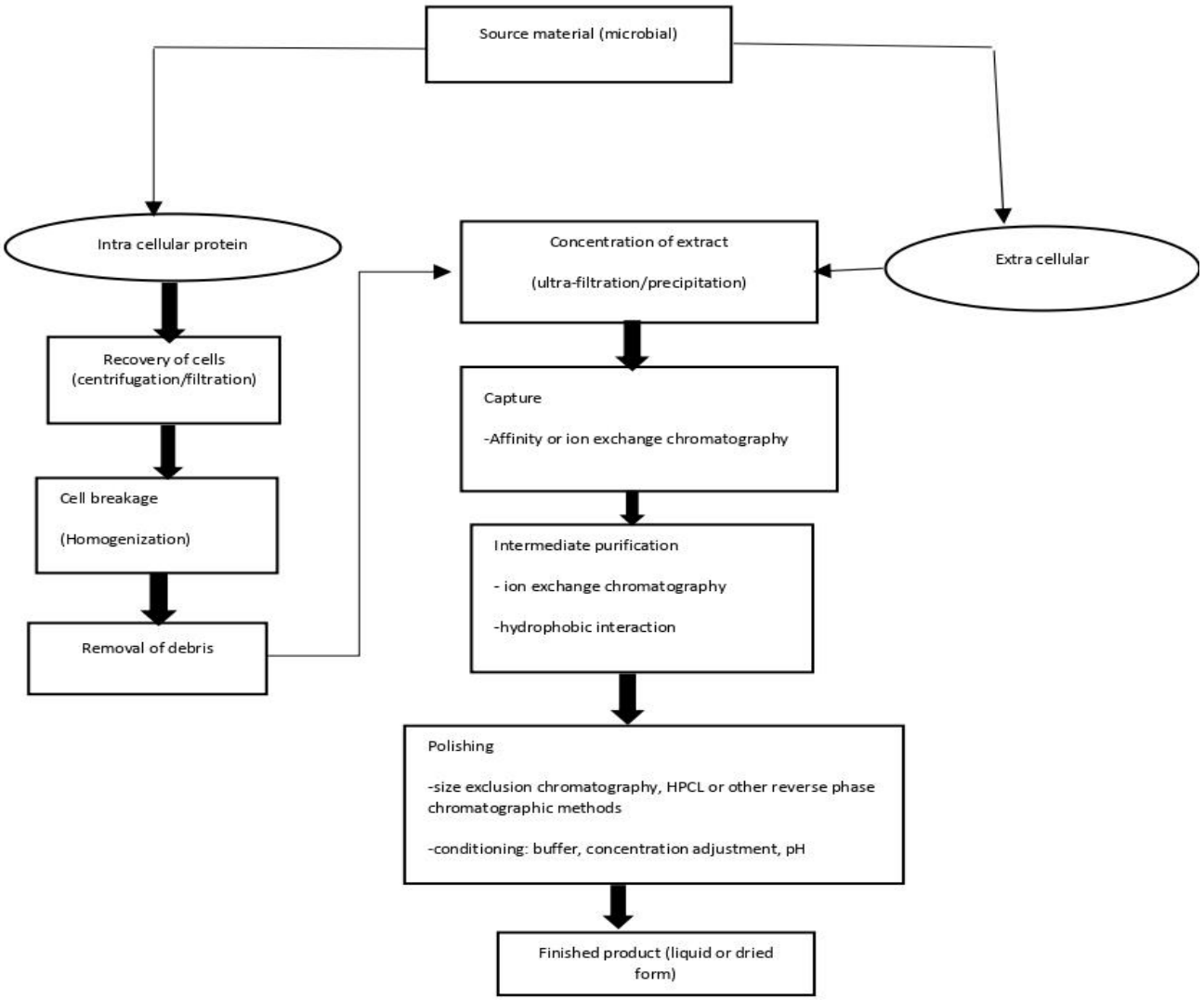

Fig 3 Brief outline of protein production from microbial source [18].

Protein purification:

The microbes secrete many proteins acquired by fermentation into the culture medium. In comparison to intracellular proteins, such extracellular proteins are typically considerably easier to purify in the subsequent downstream processing. Therefore, for extracellular produced proteins, there is considerably less need to separate the target product from foreign proteins and cellular components.

Fractionation strategies:

The most cost-effective step involved in the production of proteins through fermentation is the purification step, as it involves various steps. In the fermentation process the proteins (enzymes) are released as 1^0 metabolites whereas antibiotics are released as 2^0 metabolites. Proteins produced by microbes during fermentation are available as extracellular as well as intra cellular. When compared to the later one, extracellular proteins are easier to purify as these are available in the culture medium.

Different steps/strategies involved in protein purification are:

a) Initial fractionation step.

b) Intermediate purification step.

c) Final polishing step.

d) Finished product.

RESULT AND DISCUSSION:

Screening for new metabolites:

Actinomycetes create 67% of the 10,000 biologically active antimicrobial metabolites discovered in the late 1980s, with 9% produced by other bacteria and 15% by fungi).Marine actinomycetes producing novel metabolites during the period 2003-2015 is given in table 1. Among all the microbes (i.e., bacteria, fungi, actinomycetes) majority of metabolites are produced from actinomycetes strains.

Table 01: Marine actinomycetes producing novel metabolites during the period 2003-2015[19, 20].

Compound	Source	Activity
Abyssomicins	*Verrucosispora*sp. [21]	Antibacterial
Auroeoverticillactam	*Streptomyces aureoverticillatus*	Anticancer
Bonactin	*Streptomyces* sp BD21-2 [22]	Antibacterial, Anticancer
Chandhrananimycins	*Actinomadura*sp.	Antimicrobial, Anticancer
Choloro-dihydroquinones	Novel actinomycetes	Antibacterial, Anticancer
Diazepinomycin	*Micromonospora*sp. B0006 [23]	Antibacterial, Anticancer, anti-inflammatory
Frigocyclinone	*Streptomyces griseus*	Antibacterial
Glaciapyrroles	*Streptomyces* sp.[24]	Antibacterial
Helquinoline	*Janibacterlimosus*	Antibacterial

Himalomycins	*Streptomyces* sp. [25]	Antibacterial
Indimycins	*Streptomyces* sp.	Cytotoxicity
Indole alkaloid	*Serinicoccusprofundi*sp.	Antibacterial
Komadoquinone A	*Streptomyces* sp.	Neurogenetic activity
Lajollamycin	*Streptomyces nodosus*	Antibacterial
Marinomycins	*Marinospora*	Antibacterial, Anticancer
Mechercharmycins	*Thermoactinomyces*sp.	Anticancer
Methoxyneihumicin	*Nocardiopsis alba* [26]	Anticancer
Salinosporamide A (NPI0052) B & C	*Salinosporatropica*	Anticancer, Antimalarial
Spiroindimicins A- D	*Streptomyces* sp.	Anticancer
Strepsesquitriol	*Streptomyces* sp. SCSIO 10355	Inhibition of lipopolysaccharide induced TNFα production

Enzymes and other important metabolites of microbial origin:

Some of the industrial and therapeutic enzymes from marine actinomycetes are given in table 2.

Table 02: Industrial and therapeutic enzymes from marine actinomycetes.

Enzyme	Source
Amylase	*Streptomyces gancidicus*ASD_KT852565[27] *S. rochei* KR108310 strain HMM 13[28]
β-Lactamase inhibitor	*Streptomyces* ap. PM49[29,30]
Cellulase and xylanase	*S. albidoflavus*strain SAMRCUFH5 [31] *Streptomyces thermocoprophilus* Strain TC13W[32]
Chitinase	*Streptomyces albus*FS12 [33] *Streptomyces speibonae*324]
Collagenase	*Streptomyces* sp. *Strain* 3B [35] *Streptomyces violaceoruber*[36]
Keratinase	*Streptomyces albogriseolus*NGP [37], *Streptomyces* sp. G11C[38].

L-aspariginase	*Streptomyces* sp. [39]
Lipase	*Streptomyces sp*. Strain W007 [40]
Protease	*Streptomyces albidoflavus*[41] *Streptomyces sp*. GS-1[42]
Ribonuclease	*Streptomyces aureofaciens9* [43]

Table 03: Microbial sources of Uricase enzyme [44]

Among the three sources fungal sources are more efficient in uricase production, while actinomycetes strains produce less yield based on the literature.

Bacterial source	**Fungal source**	**Actinomycetes**
*Bacillus subtilis***[45]**	*Aspergillus*welwitschiae**[51]**	*Streptomyces* exfoliates[56]
*Pseudomonas aeruginosa***[46]**	*Candida* tropicalis[52]	Streptomyces rochei[57]
*Bacillus cereus***[47]**		Streptomyces albosrieolus[58]
AlcaligenesFaecalis[48]	*Gliomastixgueg*[53] *Gliocladiumviride*[54]	Streptomyces graminofaciens[59]
Pseudomonas aeruginosa[49] *Escherichia marmotae*[50]	Aspergillusniger[55]	Streptomyces aureofaciens[60]

Purines metabolic pathway:
Normal uric acid concentration in serum is 6.8mg/dl. It varies with age and sex.

Factors that increase the serum uric acid concentration are:

a) Specific enzyme defects (primary cause)

b) Alcohol abuse

c) Use of certain drugs such as Diuretics -Ethambutol

d) Heavy weight

e) Haemoglobinopathies

f) Thalassemia

g) Lisch-nyhan syndrome

Factors that decrease the serum uric acid concentration are:

1) Drugs such as Xanthine oxidase inhibitors, Coumarins and Anticoumarins, uricase drugs (Rasburicase).
Elevated levels of uric acid causes Gout disease, it of two types as follows, primary gout which is most common type and causes are not known. Secondary gout is not much prevalent when compared to primary gout, mostly seen in people with renal failure.
Diagnostic techniques for Gout disease

The diagnosis of the gout disease can be done by the following techniques:

2) Blood test

3) Joint fluid test

4) X-ray imaging

5) Ultra sound scanning

6) DECT (Dual Energy Computerized Tomography)

Generalized physicochemical properties of uricase enzyme obtained from various microbial sources:
After researching the physiological and chemical characteristics of enzymes derived from different organisms, including bacteria, fungus, and actinomycetes.
The generalized physio chemical properties of uricase includes the following

Table:4Physico-chemical properties of Uricase[44]

Molecular weight	145000-150000 Daltons
pI	4.3
Optimum pH	9.1
Optimum temperature	30-35^{o}C
Denaturation temperature	40-60^{o}C

Quantification of uricase enzyme:

Based on the previous literature fungal species shows highest uricase activity followed by bacteria and *Streptomyces* species**.** The fungal isolate *Gliomastix gueg crude* extract showed 1340.99, 876.74, 535.15 U/ mL of uricase activity with uric acid media followed by, Czapekdox and Yeast extract sucrose media. Other isolates *Gliocladium viride and Aspergillus welwitschiae* crude extract showed 84.21 U/ mL and 60.03U/ mL respectively [51][54][53]. Whereas Bacterial species such **as** *Escherichia marmotae*, *B. subtilis* SP6, *Pseudomonas aeruginosa*, *Pseudomonas otitidis* crud extract showed 27.47 U/mL, 13.58U/mL, 11 U/mL, 4.18 U/mL of uricase activity respectively [50][61][62][63] .The Streptomyces isolates such as *Streptomyces exfoliates*, *Streptomyces rochei NEAE-25* showed 0.5 U/mL, 47.49 U/mL uricase activity respectively after central composite design [56] [57].

Protein purification:

The crude extract of culture supernatant of *Escherichia marmotae* 27.47 U/mL of uricase activity but after purification by ammonium sulphate activity was increased to 40.02U/ml [50]. *B. subtilis* SP6 crude enzyme showed activity 13.58U/mL of uricase activity where as ammonium sulphate precipitation showed 58.35 U/mL uricase activity [61]. *Pseudomonas aeruginosa* crude enzyme shows 11 U/ml but70% ammonium sulphate 40 U/ml as maximum uricase activity, further increasing concentration leads to complete loss of activity[62].When crude extract of *Pseudomonas otitidis was subjected to* 70% ammonium sulphate precipitation, it showed 4.18 U/mL as the highest uricase activity while no enzyme activity was detected in 20%, 50% and 60%[63].

Conclusion:

Details on the fundamental procedures of fermentation technology are provided in this review article, which aids in the large-scale manufacture of uricase or any other metabolite. The generated uricase has demonstrated its capacity to metabolize insoluble uric acid. Solvable allenton and subsequent illness treatment Gout and other connected conditions. In comparison to other medications used to treat gout and other associated illnesses, this enzyme has also demonstrated less side effects. Hence, the uricase alter the serum uric acid levels and may be used as a healing drug. According to earlier research, bacterial and Streptomyces species had the lowest uricase activity, followed by fungal species. According to earlier research, the maximum uricase activity was found in the crude extract of isolates (fungi, bacteria, and actinomycetes) after 70% ammonium sulphate precipitation, while nil or little enzyme activity was found in the 20%, 50%, and 60% samples.

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