Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [9] August 2021 : 77-84

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Optimization of Growth parameters for Uricase enzyme activity of *Bacillus subtilis* MM13 enumerated from birds fecal contaminated soil

V. Manimekalai[1] and B. Anandharaj[2]
[1]Research Scholar, Department of Microbiology, M.R. Government College (Affiliated to Bharathidasan University), Mannargudi - 614001, TamilNadu, India.
[2]Department of Microbiology, M.R. Government College (Affiliated to Bharathidasan University), Mannargudi - 614001, TamilNadu, India

ABSTRACT
This research was designed to enumerate the uricase producing bacterial isolate from bird's fecal contaminated soil and optimize the growth parameters suitable for uricase enzymatic activity. Isolation done by dilution plate method using selective medium. In this study, a potent uricase producing organism was isolated by a thorough screening and identified as Bacillus subtilis strain by using 16s rDNA sequencing. Optimization of various factors influencing maximum enzyme coproduction by Bacillus sp is performed. The statistical experimental design method was further applied to obtain optimal concentration of significant parameters such as pH, temperature, uric acid concentration, urea concentration, carbon, nitrogen, substrate are tested. Totally ten isolates were recovered and one uricase producing bacterial isolate selected for optimal studies and characterized by 16SrDNA sequencing. The optimal essential growth factors required to obtain maximum uricase enzyme activity by the tested isolate were found as pH 7.0, 40°C, 1% glucose, 0.2% yeast extract, and 0.32% of uric acid. The B. subtilis MM13 yielded uricase ranged from 1.25 to 2.54 U/ml in a 48 h submerged fermentation process under these optimized conditions. None of the tested carbon and inorganic nitrogen sources had significant stimulatory effect on uricase productivity except Sucrose and yeast extract This predominant uricase producing bacterial isolate Bacillus subtilis MM13 sequence was submitted to NCBI-GenBank and received accession number as MK503710. Use of statistical optimization upsurges uricase yield from 1.25 U/ml to 15.87 U/ml enhancing the overall production by 13.23 fold; which confirms that the model is effective for process optimization. These results conclude that the B. subtilis MM13 enumerated from pigeon fecal contaminated soil may be considered for further purification and biomedical applications related in-vitro and in-vivo studies.
Keywords: *Fecal matter; endospore; Uricase; Optimization; Submerged fermentation*

Received 21.07.2021 Revised 01.08.2021 Accepted 21.08.2021

INTRODUCTION

Hyperuricemia is a condition that is characterized by an increased quantity of serum urate that usually precedes the emergence of monosodium crystals [1-2]. This monosodium crystal accumulates in distal tendons and peripheral tissues, causing gout, an inflammatory arthritic disease[3-4]. Overconsumption of fructose-enriched corn syrup, purine-rich foods, red meat, seafood, organ meat, beans, and excessive alcohol consumption may contribute to hyperuricemia development [5-6]. Uric acid is the major byproduct of purine metabolism. Hyperuricemia can occur as a result of uric acid excess production or poor excretion in humans. According to a recent report, hyperuricemia affects approximately 14.4% of adolescents (aged 35 to 49) worldwide [7-8]. According to human evolutionary theory, elevated uric acid quantities might well have offered a survival benefit during periods of starvation in the previous era [9-10]. Even though evidence that hyperuricemia can be both an effective protection and a causative factor in non-communicable disorders that encompasses cardiovascular, neurological disorder, gout, leukemia, toxemia of pregnancy, severe renal impairment, and idiopathic calcium urate nephrolithiasis[11-12]. The main levels of serum uric acid (SUA) have been observed in recent years, and hyperuricemia seems prominent in developed and emerging nations[13-14]. The global prevalence of non-communicable diseases is rising as a result of industrialization, urbanization, and ageing [15-17]. The uricase is a hepatocellular and urate oxidase enzyme that reduces uric acid to allantoin, a water-soluble molecule that is more rapidly eliminated by kidneys [18-19].

In some cases, though, mutations in missense and frame shift after evolution resulted in an inactive

uricase gene [20-22]. Hence the uricase enzyme has been used to treat hyperuricemia and related disorders[23]. Many species, encompassing higher plants and microbes, may synthesize uricase, influenced by culture medium composition. All these organisms' capability to break down uric acid and utilize this for development is an inducible feature [24]. Although this enzyme is found in abundance in most animals, it is not found in humans. Humans as well as other higher mammals are unable to generate uricase and are hence prone to uric acid-related illnesses [25-26]. Among various organisms, the microbes, especially bacteria are preferably used for uricase fabrication, and bacterial species such as *Bacillus pasteurii*, *Proteus mirabilis*, and *Escherichia coli* produce intracellular enzyme and while *Streptomyces albosriseolus*, *Microbacterium* sp., *Bacillus thermocatenulatus*, *Candida tropicalis*, and *Pseudomonas aeruginosa* [27]. However, the existing uricase production is unable to meet the global requirement, thus finding potential bacterial strains are timely needed [28]. Urate oxidase in most species converts uric acid to 5-hydroxyisourate. Depending on species, 5-hydroxyisourate is degraded further and eliminated from the body as allantoin and ammonia[29]. Uricase is just a non - soluble crystalloid that comprised with peroxisomes in vertebrate animals [30]. In most microbes and aquatic vertebrates, uricases are dissolvable as well as present in either the cytosol (bacteria) or the peroxisome (yeast). In general the researchers prefer the enzymes production from bacterial culture due to simple, cost-effective and reliable production. Bacterial enzyme production is primarily determined by growth factors such as pH, temperature, nutritional sources such as carbon and nitrogen sources, and so on[31]. As a result, optimizing the growth parameters is the most important factor in determining a bacterial strain's uricase producing maximum potential. This is the first report about enumerating uricase producing predominant bacterial species from rose garden soil and optimized the suitable growth conditions for the uricase producing bacterial strain. Since, under the optimal growth conditions, the cell growth, metabolic activity, and reproduction rate will be significantly increased and yielded considerable enzymes. Hence, this research was designed to isolate the uricase producing predominant bacterial strain from various birds' fecal contaminated soil and optimize the suitable growth conditions for uricase producing bacteria to produce maximum yield.

MATERIAL AND METHODS

Collection and processing of fecal contaminated soil sample

The fecal contaminated soil samples were collected from pigeons, turkey, parrot, and poultry farms/nests in Rayakota, Berigai, Perandapalli, and Kumudepalli. The samples were collected in sterile zip-lock cover and immediately transferred to the laboratory, and subjected to a standard serial dilution process for each sample individually.

Enumeration and confirmation of uricase producing predominant bacterial isolates

From the standard serial dilution, 0.1 mL of 10^{-6} dilution of each sample was individually inoculated on sterilized uric acid agar medium containing plates by spread plate method. The inoculated plates were incubated at 37˚C for 24 h. After incubation, the clearance zone formed around the colonies was measured, and colonies that showed maximum clearance zone were enumerated. The uricase secreting potential of enumerated bacterial isolates was confirmed by performing uric acid or uricase assay using Amplex™ uricase assay kit (Thermo Fisher Scientific: Cat. log. No: A22181) and standard Lowry's method.

Preliminary growth parameters optimization[32]

To evaluate the basic optimal growth parameters required for the better growth and synthesis of uricase were studied as follows . The one-factor-at-a-time method was applied for each factor such as pH 5-9, temperature 30-70˚C under static submerged condition. 5 mL (%) of 24 h old *B. subtilis* is inoculated on 100 ml of uric acid broth medium and incubated in a shaker incubator with 150 rpm for 48 h. The standard uricase assay activity was performed after incubation of each growth parameter

Secondary growth parameter optimization[33]

Iinfluence of various carbon and nitrogen sources on growth and uricase production was assessed with 2% of carbon source: glucose, sucrose, maltose, fructose, and lactose, 1% of nitrogen source: yeast extract, peptone, ammonium nitrate, ammonium chloride, and casein. 100 ml of uric acid broth medium enriched with above carbon and nitrogen independently and autoclaved. the various concentration of uric acid (0.12, 0.22, 0.32, 0.42, and 0.52%), and 5% of natural uric acid enriched with dried and sterilized powdered form of wheat bran, beans, cauliflower, and pigeon fecal with standard growth conditions in 100 mL of uric acid broth medium containing 250 mL conical flask individually. About 5 mL (%) of 24 h old *B. subtilis* MM13 (OD_{600}: 1.5) was inoculated on each flask and incubated in a shaker incubator with 150 rpm for 48 h. The standard uricase assay activity was performed after incubation of each growth parameter

Effect of Sucrose and yeast extract

Production of enzyme at different concentrations of sucrose (0.25, 0.5, 0.75, 1.0, and 1.5%) and yeast extract (0.2, 0.3, 0.4, 0.5, and 0.6%) required for attaining maximum yield from *B. subtilis* MM13 were optimized through submerged fermentation process one-factor-at-a-time approach in 100 mL of uric acid broth in 250 mL of the conical flask. The culture inoculated flask was incubated in a shaker incubator at 150 rpm for 48 h, and a uricase assay was performed to calculate the uricase activity

Uricase activity assay [34]

Uric acid at a concentration of10µg/ml was dissolved in 2 ml of 200mM borate buffer (pH 8.5), mixed with 0.8 ml water and 0.1 ml of crude enzyme (CFCS). The mixture was incubated at 35°C for 10 min and then the reaction was stopped by adding 0.2 ml of 100 mM potassium cyanide solution (PCS). The PCS added to the mixture before the CFCS addition was served as the reference. The absorbance was measured at 293 nm. The difference in the absorbance between the test and the reference is equivalent to the decrease in uric acid during the enzyme reaction. One unit of uricase enzyme was equivalent to the amount of enzyme that converts 1µmol of uric acid to allantoin per min.

Molecular characterization of test isolates [35-36]

The preliminary screening (based on clear zone formation) and uricase assay results declared that 1 isolate out of 10 has an outstanding uricase enzyme activity, and that isolate was termed as MM13. The molecular characterization (16S rRNA sequencing) study was performed to identify the genus and species of test isolate MM13. The bacterial total genomic DNA extraction kit (gDNA extraction kit, from ThermoFisher Scientific). The thermo cycler (ProFlex – Thermo Fisher Scientific) was used to perform the 16S rRNA sequencing amplification process using forward: 5′-CCAGTAGCCAAGAATGGCCAGC-3′ (EN1F) and reverse:5′-GGAATAATCGCCGCTTTGTGC-3′ (EN1R). The standard operating conditions (denaturation, annealing, and extension) were followed, with 25 cycles of amplification were performed. The amplified PCR product was purified using a readymade PCR product purification kit (GenElute™ PCR clean up kit (NA1020)-Sigma -Aldrich), and 518F/800R sequencing system was performed and submitted at NCBI-GenBank and obtained accession number (MK503710). To confirm the genus and species of test isolate. The MEGA X was applied to construct the phylogenetic tree and circular sequence analyses to understand the evolutionary relationship and possible restriction sites and GC percentile analyses.

RESULT AND DISCUSSION

The demand for uricase enzymes has been increasing recently, especially in the medical sector, to treat hyperuricemia and related diseases. Hence, finding a suitable and potential uricase producing bacterial isolate promptly is required to meet global demand. A total of 10 bacterial isolates enumerated from a pigeon (5 nos.), turkey (2 nos.), parrot (2 nos.), and poultry fecal (1 no.) contaminated soil was found as possessing uricase activity. One isolate out of 10 isolates named MM13 enumerated from pigeon fecal contaminated soil showed outstanding uricase enzyme activity (30 mm). It was determined by the zone of clearance around the colony in millimeters. The zone of clearance formed around the colony is due to the conversion of uric acid into 5-hydroxyisourate by uricase with the aid of O_2 and H_2O and yielded H_2O_2 and subsequently reduced as allantoin. The size of zone of clearance formed around the colony from uric acid was considered a key factor in determining the uricase secreting potential of bacterial isolate [38].

Similarly, the *Bacillus cereus* DL3 enumerated from poultry farms had been reported to possess excellent uricase secreting activity. It was determined by measuring the zone of clearance around the colonies on uric acid media. The pigeon fecal matter may contain a significant quantity of uric acid than other bird's fecal matter. Thus the bacteria isolated (MM13) could possess the maximum potential to secrete uricase enzyme and utilize the uric acid in the pigeon fecal matter[37]. Thong et al. [38] enumerated uricase producing *Clostridia* sp. isolated from the gut region of various termites and identified by a zone of clearance around the colonies**.**

The optimal essential growth parameters are required to obtain the maximum uricase enzyme activity of *B. subtilis* MM13. The metabolic activity and growth rate of bacteria can be influenced by essential growth factors such as pH, temperature, carbon and nitrogen sources, etc. Thus table 1 represents the concept of the research to optimize the growth parameters like pH, temperature, carbon , nitrogen and uric acid for uricase production by *Bacillus* sp.

The optimal physical factors such as pH and temperature are required for producing maximum uricase enzyme activity. The obtained results showed that the maximum uricase enzyme activity was 1.85 and 1.25 U/ml at pH 7.0 and 40°C respectively at 48 h of the submerged fermentation process, and these were statistically significant at $P<0.05$ (Fig. 1a and Fig. 1b). Furthermore, the uricase enzyme activity was significantly reduced at pH 5, 6, 8, and 9 and at 30, 50, 60, and 70°C, respectively. The increased temperature (increasing kinetic energy) and optimal pH can speed up the reaction, however, increased

temperature and pH (breaking bonds) instantly reducing the enzyme and metabolic activities[39]. The optimum pH and temperature ranges for enzyme activity were reported as 4.0-9.0 and 30-50°C, respectively, and these ranges may be related to various strains and substrates using for enzyme production. Similarly, the optimal pH and temperature for uricase enzyme activity of *Saccharopolyspora* sp. PNR11 was reported as 7.0 and 30°C, respectively[40].

The availability of suitable carbon and nitrogen sources determines the enzymatic and metabolic activities and the growth rate of bacteria. In this study, various carbon sources such as glucose, maltose, lactose, sucrose, and fructose were subjected to identify the suitable carbon source for *B. subtilis* MM13 to secrete uricase enzyme activity. The obtained results state that the test isolate preferably used glucose as a major carbon source than other sugars and showed maximum uricase enzyme activity 2.54 U/ml. This value was statistically significant at $P<0.05$ (Fig. 1c). Similarly, the *B. subtilis* MM13 strain effectively utilized the yeast extract as the preferred nitrogen source and yielded the uricase enzyme activity as 2.37 U/ml in a 48 h period of incubation. This was statistically significant at $P<0.05$ (Fig. 1d). The uricase activity was considerably reduced in other nitrogen sources such as peptone, ammonium nitrate, ammonium chloride, and casein. The bacteria *Bacillus cereus* DL3 isolated from the poultry source was preferably utilizing the Carboxymethylcellulose and asparagine as a suitable carbon and nitrogen source and yielded around 15.43 U/mL of uricase activity. Another report stated that the *Bacillus subtilis* SP6 isolated from poultry wastes was showed maximum uricase enzyme activity (1.2 to 15.87 U/ml) using lactose and soya peptone as preferred carbon and nitrogen sources, respectively . The *Bacillus subtilis* RNZ-79 effectively utilize raw carbon source such as rice bran and uric acid as preferable carbon and nitrogen sources and yielded a significant volume of uricase activity .

The uric acid served as a primary inducer for uricase activity in bacteria. The optimal concentration of uric acid for the uricase enzyme activity of *B. subtilis* MM13 was 0.32%. It yielded about 1.17 U/ml of uricase enzyme in a 48 h of incubation period and this value was statistical significance at $P<0.05$ (Fig. 1e). Other strains of *B. subtilis*, such as RNZ-79, effectively produced maximum uricase enzyme activity using 0.4% of uric acid in a short period . Similarly, the other strain *Bacillus subtil**is*** SP6 showed maximum uricase activity using 2.55 g/L concentration of uric acid . Another report stated the *Streptomyces exfoliatus* UR10 enumerated from poultry form effectively showed maximum uricase enzyme activity at 0.2% uric acid. This uricase enzyme converts the uric acid into water soluble allantoin (Fig. 2) with the intermediate of 5-hydroxyisourate and byproduct of H_2O_2 . The dried and sterilized powdered form of uric acid enriched wheat bran, beans, cauliflower, and pigeon fecal were studied and interestingly, the *B. subtilis* MM13 effectively utilized the pigeon fecal matter and showed considerable uricase enzyme activity as 0.7 U/ml, however this was significantly lower than the readymade form uric acid (1.17 U/ml). This result suggests that the tested natural uric acid enriched sources have not significant influence on enzymatic activity in *B. subtilis* MM13. This might be due to the insufficient quantity of uric acid content in these natural sources[41].

The initial optimization study results revealed that the sucrose and yeast extract were found as suitable carbon and nitrogen sources for excellent uricase enzyme activity in *B. subtilis* MM13. Hence, the suitable concentration for these two energy sources for *B. subtilis* MM13 to produce maximum uricase enzyme activity were found as 1% for sucrose and 0.2% for yeast extract and yielded 2.54 and 2.37 U/ml, respectively. These values were statistically significant at $P<0.05$ (Fig. 2a and 2b). A report stated that the *Bacillus subtilis* SP6 isolated from poultry waste was found as a uricase producer and effectively showed outstanding uricase activity using lactose as carbon source and soya peptone as nitrogen source at the concentration of 12.2 and 12.79 g/L, respectively . The optimal concentration of carbon and nitrogen source can support the cell proliferation and cell metabolism process that enhances the enzyme secretion activity and chelate the metabolism process[42]. *Proteus vulgaris* enumerated from soil sample use 15 of glucose and 0.5% of ammonium phosphate as the preferable concentration of carbon and nitrogen source and produce significant uricase enzyme in short duration of time[43].

Molecular characterization of bacterial test isolates

The 16S rRNA sequencing and phylogenetic tree analysis results revealed that the uricase producing predominant test bacterial isolate MM13 was identified as *Bacillus subtilis* MM13 and obtained sequence was submitted to GenBank and received accession number for the sequence as MK503710. The sequence of *Bacillus subtilis* MM13was 96% similarity matched with *Bacillus subtilis* 3691 strain (Fig. 3). The circular sequence analysis by BioLab software revealed the GC and AT content of genomic DNA and possible restriction sites. The results state that the Bacillus subtilis MM13 contains about 56% of GC and 44% AT (Fig. 3). Pustake et al. enumerated the uricase producing *Bacillus subtilis* SP6 from poultry wastes, and it was characterized and confirmed using 16*S* rRNA sequencing[44]. Another bacterial strain isolated from poultry form, which possesses uricase producing potential, was identified as Bacillus cereus DL3 using molecular characterization (16*S* rRNA sequencing). Apart from *Bacillus* sp., the *Streptomyces*

exfoliatus UR10 enumerated from farm wastes was recognized as uricase producer and characterized by 16S rRNA molecular sequencing.

Table 1 Enzyme activity on different substrate

Carbon	**U/mL**	**Nitrogen**	**U/mL**	**% Uric acid**	**U/mL**
Glucose	0.45	**Yeast extract**	**0.72**	0.12	0.14
Sucrose	**0.69**	Peptone	0.49	0.22	0.26
Maltose	0.27	Ammonium Nitrate	0.34	**0.32**	**0.63**
Fructose	0.19	Ammonium Chloride	0.27	0.42	0.36
Lactose	0.49	Casein	0.16	0.52	0.33

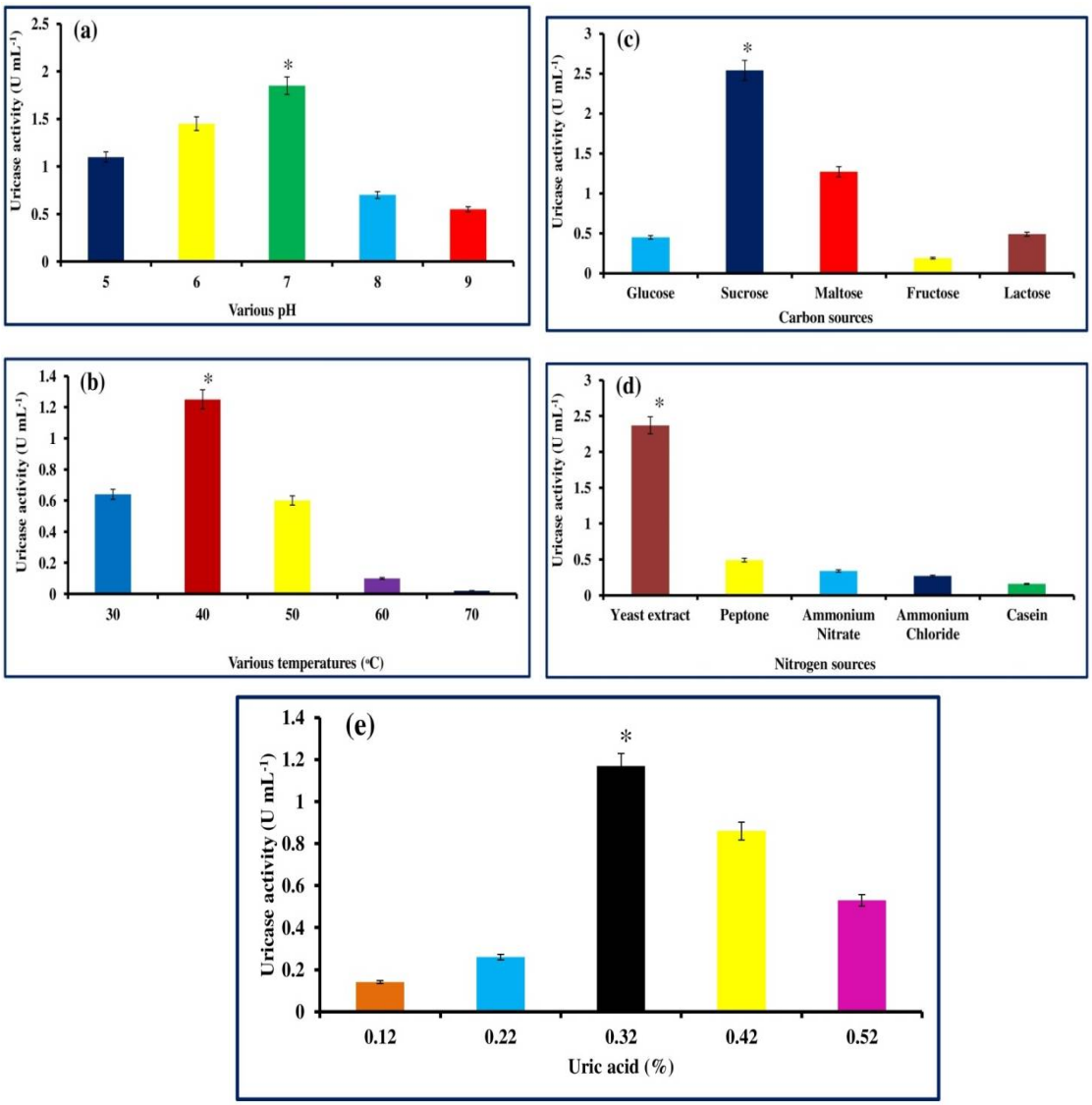


Figure 1. Optimization of growth parameters for uricase enzyme activity in *B. subtilis* MM13. **(a)** various pH **(b)** various temperature **(c)** various carbon sources **(d)** various nitrogen sources **(e)** various concentration of uric acid * indicates statistical significance at P<0.05

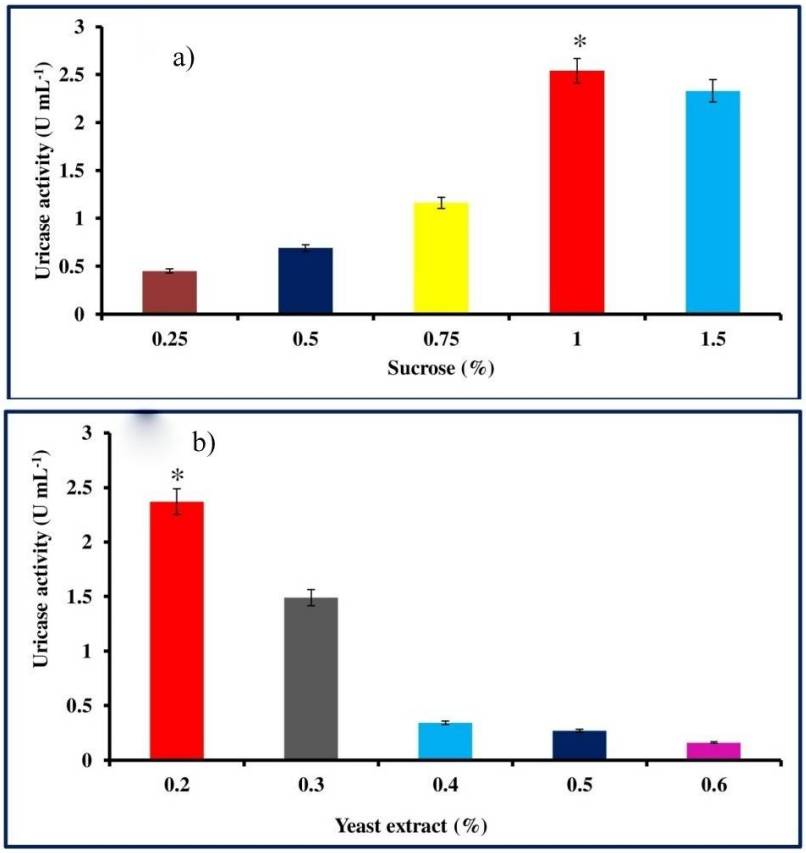
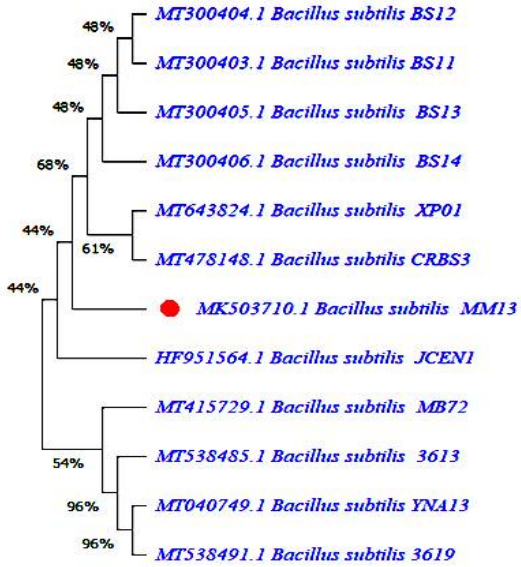


Figure 2(a) various concentration of sucrose **(b)** various concentration of yeast extract.

Figure.2 Phylogenetic tree analysis of *Bacillus subtilis* MM13



CONCLUSION

Among various bird's fecal contaminated soil, the pigeon fecal contaminated soil possesses excellent uricase producing bacterial isolate than other birds fecal contaminated soil. This bacterial isolate was identified as *Bacillus subtilis* MM13 through molecular characterization. The growth parameters required for this strain to produce uricase enzyme were optimized as pH 7.0, 40°C, 1% glucose, 0.2% yeast extract, and 0.32% of uric acid. Under these optimized conditions, the uricase enzyme activity was ranged from 1.25 to 2.54 U/ml in 48 h of the submerged fermentation process. These results suggest that the *B. subtilis* MM13 isolated from pigeon fecal contaminated soil can produce a significant quantity of uricase enzyme

under optimal conditions. The purification and application related studies are needed to understand their maximum biomedical potential and commercialization possibilities.

REFERENCES

1. Butler F, Alghubayshi A, Roman Y (2021) The Epidemiology and Genetics of Hyperuricemia and Gout across Major Racial Groups: A Literature Review and Population Genetics Secondary Database Analysis, Journal of Personalized Medicine. 11: 231.
2. Mohanasundari C, Anbalagan S, Srinivasan K, Chinnathambi A, Salmen S.H, Meganathan V, Narayanan M (2021) Evaluation of antibacterial efficacy of various solvent extracts of Evolvulus alsinoides and Mucuna pruriens against multidrug resistant (MDR) pathogenic bacteria, Applied Nanoscience.5: 1-11.
3. Erickson T.D, Assylbekova B, Chong A.C (2021) Multiple Subcutaneous Gouty Tophi Even with Appropriate Medical Treatment: Case Report and Review of Literature, Kansas Journal of Medicine. 14: 12.
4. Pugazhendhi A, Vasantharaj S, Sathiyavimal S, Raja R.K, Karuppusamy I, Narayanan M, Kandasamy S, Brindhadevi K. (2021) Organic and inorganic nanomaterial coatings for the prevention of microbial growth and infections on biotic and abiotic surfaces, Surface and Coatings Technology. 127739.
5. Jamnik J, Rehman S, Mejia S.B, de Souza R.J, Khan T.A, Leiter L.A, Wolever T.M, Kendall C.W, Jenkins D.J, Sievenpiper J.L (2016) Fructose intake and risk of gout and hyperuricemia: a systematic review and meta-analysis of prospective cohort studies, BMJ open. 6: e013191.
6. Narayanan M, Deepika M, Ma Y, Nasif O, Alharbi S.A, Srinivasan R, Natarajan D (2021) Phyto-fabrication, characterization, and biomedical activity of silver nanoparticles mediated from an epiphytic plant Luisia tenuifolia Blume, Applied Nanoscience. 1-11.
7. Chen L, Han S, Liu F, Chen S, Chen X, Chen H. Global prevalence of hyperuricemia in adolescents from 2000 to 2019: A meta-analysis, in, Research Square. 2020.
8. Subramaniam S, Kumarasamy S, Narayanan M, Ranganathan M, Rathinavel T, Chinnathambi A, Alahmadi T.A, Karuppusamy I, Pugazhendhi A, Whangchai K (2021) Spectral and structure characterization of Ferula assafoetida fabricated silver nanoparticles and evaluation of its cytotoxic, and photocatalytic competence, Environmental Research. 111987.
9. Johnson R.J, Stenvinkel P, Martin S.L, Jani A, Sánchez-Lozada L.G, Hill J.O, Lanaspa M.A (2013) Redefining metabolic syndrome as a fat storage condition based on studies of comparative physiology, Obesity. 21: 659-664.
10. Narayanan M, Jayashree T, Kandasamy S, Natarajan D, Liu G, Elesawy B.H, Elfasakhany A, Pugazhendhi A. (2021) An in vitro investigation of the antidermatophytic, antioxidant, and nephroprotective activity of Solanum surattense, Process Biochemistry. 109: 178-185.
11. Jin M, Yang F, Yang I, Yin Y, Luo J.J, Wang H, Yang X-F (2012) Uric acid, hyperuricemia and vascular diseases, Frontiers in bioscience: a journal and virtual library. 17: 656.
12. Narayanan M, Krishnan L, Natarajan D, Kandasamy S, El Askary A, Elfasakhany A, Pugazhendhi A. (2021) Evaluation of antibacterial, antioxidant, and nephroprotective proficiency of methanol extract of Aerva lanata, Process Biochemistry. 109: 98-103.
13. Sun D.-Q, Wu S.-J, Liu W.-Y, Lu Q.-D, Zhu G.-Q, Shi K.-Q, Braddock M, Song D, Zheng M.-H (2016) Serum uric acid: a new therapeutic target for nonalcoholic fatty liver disease, Expert opinion on therapeutic targets. 20: 375-387.
14. Anusha P, Narayanan M, Natarajan D, Kandasamy S, Chinnathambi A, Alharbi S.A, Brindhadevi K. (2021) Assessment of hexavalent chromium (VI) biosorption competence of indigenous Aspergillus tubingensis AF3 isolated from bauxite mine tailing, Chemosphere. 131055.
15. Khorrami Z, Rezapour M, Etemad K, Yarahmadi S, Khodakarim S, Hezaveh A.M, Kameli M, Khanjani N (2020) The patterns of non-communicable disease multimorbidity in Iran: a multilevel analysis, Scientific reports. 10: 1-11.
16. Egbuna C, Awuchi C.G, Kushwaha G, Rudrapal M, Patrick-Iwuanyanwu K.C, Singh O, Odoh U.E, Khan J, Jeevanandam J, Kumarasamy S. (2021) Bioactive Compounds Effective Against Type 2 Diabetes Mellitus: A Systematic Review, Current Topics in Medicinal Chemistry..
17. Kandasamy S, Narayanan M, He Z, Liu G, Ramakrishnan M, Thangavel P, Pugazhendhi A, Raja R, Carvalho I.S. (2021) Current strategies and prospects in algae for remediation and biofuels: An overview, Biocatalysis and Agricultural Biotechnology. 102045.
18. Chen C, Lü J.-M, Yao Q. (2016) Hyperuricemia-related diseases and xanthine oxidoreductase (XOR) inhibitors: an overview, Medical science monitor: international medical journal of experimental and clinical research. 22: 2501.
19. Narayanan M, Vigneshwari P, Natarajan D, Kandasamy S, Alsehli M, Elfasakhany A, Pugazhendhi A. (2021) Synthesis and characterization of TiO2 NPs by aqueous leaf extract of Coleus aromaticus and assess their antibacterial, larvicidal, and anticancer potential, Environmental Research. 111335.
20. Marchetti M, Liuzzi A, Fermi B, Corsini R, Folli C, Speranzini V, Gandolfi F, Bettati S, Ronda L, Cendron L. (2016) Catalysis and structure of zebrafish urate oxidase provide insights into the origin of hyperuricemia in hominoids, Scientific reports. 6: 1-13.
21. Shivakumar S, Ramkumar G (2021) Eco-friendly approach to control mosquitos (A. stephensi, C. quinquefasciatus, and A. aegypti) using silver nanoparticle, Journal of Environmental Treatment Techniques. 9: 203-210.
22. Whangchai K, Van Hung T, Al-Rashed S, Narayanan M, Kandasamy S, Pugazhendhi A (2021). Biodegradation competence of Streptomyces toxytricini D2 isolated from leaves surface of the hybrid cotton crop against β cypermethrin, Chemosphere. 276: 130152.

23. Gliozzi M, Malara N, Muscoli S, Mollace V (2016). The treatment of hyperuricemia, International journal of cardiology. 213: 23-27.
24. Londoño-Hernández L, Ramírez-Toro C, Ruiz H.A, Ascacio-Valdés J.A, Aguilar-Gonzalez M.A, Rodríguez-Herrera R, Aguilar C.N (2017). Rhizopus oryzae–ancient microbial resource with importance in modern food industry, International journal of food microbiology. 257: 110-127.
25. Liu N, Xu H, Sun Q, Yu X, Chen W, Wei H, Jiang J, Xu Y, Lu W. (2021) The Role of Oxidative Stress in Hyperuricemia and Xanthine Oxidoreductase (XOR) Inhibitors, Oxidative Medicine and Cellular Longevity. 2021.
26. Saranyadevi S, Suresh K, Mathiyazhagan N, Muthusamy R, Thirumalaisamy R. (2021) Silver nanoparticles Synthesized using Asafoetida resin, Characterization of their broad Spectrum and Larvicidal Activity, Annals of the Romanian Society for Cell Biology.; 15035-15049.
27. Hafez R.M, Abdel-Rahman T.M, Naguib R.M. (2017) Uric acid in plants and microorganisms: Biological applications and genetics - A review, Journal of advanced research. 8; 475-486.
28. Daisley B.A, Pitek A.P, Chmiel J.A, Al K.F, Chernyshova A.M, Faragalla K.M, Burton J.P, Thompson G.J, Reid G (2020). Novel probiotic approach to counter Paenibacillus larvae infection in honey bees, The ISME journal. 14L 476-491.
29. Iwadate Y, Kato J.-i. (2019)Identification of a formate-dependent uric acid degradation pathway in Escherichia coli, Journal of bacteriology. 201: e00573-00518.
30. Hafez R.M, Abdel-Rahman T.M, Naguib R.M (2017). Uric acid in plants and microorganisms: Biological applications and genetics-A review, Journal of advanced research. 8; 475-486.
31. Aly M, Tork S, Al-Garni S, Allam R. (2013) Production and characterization of uricase from Streptomyces exfoliatus UR10 isolated from farm wastes, Turkish Journal of Biology. 37; 520-529.
32. Fu X, Yan Q, Wang J, Yang S, Jiang Z. (2016) Purification and biochemical characterization of novel acidic chitinase from Paenicibacillus barengoltzii, International journal of biological macromolecules. 91; 973-979.
33. Kotb E. (2016) Improvement of uricase production from Bacillus subtilis RNZ-79 by solid state fermentation of shrimp shell wastes, Biologia. 71 229-238.
34. Nanda P, Babu P.E. (2014) Isolation, screening and production studies of uricase producing bacteria from poultry sources, Preparative biochemistry & biotechnology. 44; 811-821.
35. Spennemann D.H, Watson M.J (2017) Dietary habits of urban pigeons (Columba livia) and implications of excreta pH–a review, European Journal of Ecology. 3: 27-41.
36. Ashe S, Maji U.J, Sen R, Mohanty S, Maiti N.K. Specific oligonucleotide primers for detection of endoglucanase positive Bacillus subtilis by PCR, 3 Biotech. 4 (2014); 461-465.
37. Lorenz R, Bernhart S.H, Höner zu Siederdissen C, Tafer H, Flamm C, Stadler P.F, Hofacker I.L. ViennaRNA Package 2.0, Algorithms for Molecular Biology, 6 (2011); 26
38. Thong-On A, Suzuki K, Noda S, Inoue J.-i, Kajiwara S, Ohkuma M. Isolation and characterization of anaerobic bacteria for symbiotic recycling of uric acid nitrogen in the gut of various termites, Microbes Environ. 27 (2012); 186-192.
39. Rollin J.A, del Campo J.M, Myung S, Sun F, You C, Bakovic A, Castro R, Chandrayan S.K, Wu C.-H, Adams M.W (2015) High-yield hydrogen production from biomass by in vitro metabolic engineering: mixed sugars coutilization and kinetic modeling, Proceedings of the National Academy of Sciences. 112: 4964-4969.
40. Khucharoenphaisan K, Sinma K (2011).Production and partial characterization of uric acid degrading enzyme from new source Saccharopolyspora sp. PNR11, Pakistan journal of biological sciences: PJBS. 14: 226-231.
41. RoyChoudhury S, Umasankar Y, Hutcheson J.D, Lev-Tov H.A, Kirsner R.S, Bhansali S. Uricase based enzymatic biosensor for non-invasive detection of uric acid by entrapment in PVA-SbQ polymer matrix, Electroanalysis. 30 (2018); 2374-2385.
42. Jacob J.M, Karthik C, Saratale R.G, Kumar S.S, Prabakar D, Kadirvelu K, Pugazhendhi (2018) A. Biological approaches to tackle heavy metal pollution: a survey of literature, Journal of environmental management. 217: 56-70.
43. Azab E.A, Ali M.M, Fareed M.F. (2005) Studies on uricase induction in certain bacteria, Egyptian Journal of Biology. 7.
44. Pustake S.O, Bhagwat P.K, Dandge P.B (2019) Statistical media optimization for the production of clinical uricase from Bacillus subtilis strain SP6, Heliyon. 5; e01756-e01756.

CITATION OF THIS ARTICLE

V. Manimekalai and B. Anandharaj. Optimization of Growth parameters for Uricase enzyme activity of *Bacillus subtilis* MM13 enumerated from birds fecal contaminated soil. Bull. Env.Pharmacol. Life Sci., Vol10[9] August 2021 : 77-84