iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 11 **Issue:** II **Month of publication:** February 2023

DOI: https://doi.org/10.22214/ijraset.2023.47947

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 11 Issue II Feb 2023- Available at www.ijraset.com

Production of A-Amylase from *Aspergillus Tamarii*

Renu A[1], Satheesh Kumar B[2], Rinaldin Aroma S[3]
[1, 2, 3]*Bala Sarada Institute for Science and Technological Research, Vellamcode, Chitharal – 629 151, Kanyakumari District, Tamil Nadu*

Abstract: Biotechnology is the exploitation of biochemical potential of the microorganisms for the medical, agricultural and industrial purposes. In processing, either the microorganisms are eliminated to prevent spoilage or they are encouraged to grow resulting in the production of fermented products. This study deals with the operating condition and comparison of enzyme production and purification of α-amylase from mutated and non- mutated Aspergillus tamarii. α- Amylase is an extracellular enzyme which can be produced by Aspergillus tamari using solid state and submerged fermentation. In the submerged fermentation, the α-amylase production was started after 24hrs and the maximum production was achieved after 7 days, but in the solid state fermentation the production was initiated after 3 days and the maximum production is observed after 12 days. The commercial medium used for the α-amylase production is sweet potato soluble starch. The pH for the solid state fermentation should be maintained at 4±0.2 and for the submerged process the pH should be at 7.0±0.2. The maximum yield of enzymes was achieved using submerged fermentation.

Keywords: Aspergillus tamarii, α- Amylase, Submerged fermentation, Solid state Fermentation

I. INTRODUCTION

Production of α-Amylase with *Aspergillus flavus* on Amaranthus grains by solid state fermentation was done by Viswanathan (2001). Use of response surface methodology for optimizing process parameters for the production of α-Amylase by *Aspergillus oryzae* was carried out by Francis, in the year 2003. Production and properties of an extra cellular α-Amylase from thermophillic Bacillus sp. was done by Martins, (2002). α- Amylase production by *Penicillium fellutanium* isolated from mangrove rhizosphere soil was carried out by Kathiresan, (2006).

Production and characterization of lpha-amylase from Aspergillus niger JGI 24 isolated in Bangalore was studied by Varalakshmi, *et al*,. 2009. Improved production of a heterologous amylase in *Saccharomyces cerevisiae* by inverse metabolic engineering was done by Liu, *et al*., 2014. Optimization of alkaline-amylase production by thermophilic *Bacillus subtilis* was published by Al-Johani, *et al*., 2017.

Enhanced hypocrellin production of *Shiraia* sp. SUPER-H168 by overexpression of α-amylase gene was carried out by Gao, *et al*., 2018. Feeding the combination of essential oils and exogenous α-amylase increases performance and carcass production of finishing beef cattle was studied by Meschiatti, *et al*., 2019. Effects of high-amylase corn on performance and carcass quality of finishing beef heifers was done by Horton, *et al*., 2020. Production and partial characterization of α-Amylase enzyme from marine Actinomycetes was published by Al- Agamy, *et al*., 2021. Optimization and scale-up of α-amylase production by *Aspergillus oryzae* using solid state fermentation of edible oil cakes was carried out by Balakrishnan *et al*., 2021. Production of extracellular α-amylase by single stage steady-state continuous cultures of Candida wangnamkhiaoensis in an airlift bioreactor was done by Chavez-Camarillo, *et al*., 2022.

II. MATERIALS AND METHODS

Aspergillus tamarii was collected from the nearby laboratory. Slide culture is the technique which was used for the visualization of *Aspergillus tamari* under the microscope without causing damage to the mycelium. Prepare PDA agar, and sterilize the medium. *Aspergillus tamarii* was inoculated over the piece of agar medium under sterile condition. Then the plate was kept for incubation at 28°C for 4 days. After 4 days the slide is separated from the medium and is stained with lactophenol cotton blue under10X. Small scale fermentation of *Aspergillus tamarii* for the production of α-amylase was carried out in conical flasks. The 100ml of culture medium were prepared based upon their composition. The culture medium was then steam sterilized using autoclave at 121°C for 15minutes. After sterilization, the medium was then cooled to room temperature and inoculated with loopful of organism obtained from the stock. The culture was then inoculated at 28°C for 7 days in the rotary shaker at 250 rpm. Growth curve was performed in order to study the growth pattern of the organism under the submerged fermentation. For this work 100ml of culture media was prepared composition on the media steam sterilized using autoclave at 121°C for 15minutes.

After sterilization 10% *Aspergillus tamarii* inoculum was added. For the zeroth day the OD was detected using spectrophotometer using culture medium as a blank, followed 1st, 2nd etc…upto 14th day.

The separated α-amylase was place over the starch media. After 30mints of incubation period gently flood the surface of plates with 2-3ml of lugol solution. Gently rotate each plate until entire surface of the plate is covered with lugol solution wait for 1-2mints. The iodine in the lugol solution will reacts with starch after 2mints the color of the medium was changed, discard the lugol solution and absorb the result. The formation of purple color conform the presence α-amylase. The wild strain of *Aspergillus tamarii* was mass cultured in order to produce large amount of α-amylase. The *Aspergillus tamarii* was mutated under UV light in order to produce large amount of α-amylase. The UV treatment is given in order to increase the production on α-amylase from *Aspergillus tamarii. Aspergillus tamarii* spores were inoculated in Petri plate containing PDA and incubated at 30°C for 4days. Spores produced were harvested. Sample of 15ml of the spore suspension were pour into a sterile petri dishes. The UV radiation source was placed 10cm above the surface of the suspension spores. Samples of 1ml were taken every 2mints during 10mints, placed in an ice bath for 5mints. Kept in the darkness for another 30mints from this solution kept 0.5ml were inoculated into a conical flask containing 100ml of medium.

From the above treatment, the *Aspergillus tamarii* which have given best result was mass cultured in both submerged and solid state fermentation. (Both mutated and non-mutated organism). The sweet potato was fully heated and digested in the mortar and pestle was the insoluble starch is converted into soluble starch. Meantime the peanut was digested with mortar and pestle. The digested peanut was mixed with soluble starch to which added $MgSO_4$.$7H_2O$, beef extract and yeast extract for 1 liter of distilled water. The extract composition of the medium was given in the table. The prepared medium was sterilized using autoclave at 121°C for 15mints. After sterilization media was poured onto the steel trays. After solidification about 10% inoculum of *Aspergillus tamarii* was added in the medium. The culture was maintained at 28°C for 12 days.The sweet potato was fully heated and digested in the mortar and pestle was the insoluble starch is converted into soluble starch. Mean time the peanut was digested with mortar and pestle. The digested peanut was mixed with soluble starch to which added $(NH_4)_2SO_4$.$CaCO_3$, $NaCO_3$, NaCl and soya bean oil for 3 liter of distilled water in Erlenmeyer flask. The pH of the medium was adjusted to 7±0.2 using 1% HCl or NaOH. The exact composition of the medium was given in the table the prepared medium was sterilized using autoclave at 121°C for 15mints. After sterilization the medium was inoculated with 10% inoculum of *Aspergillus tamarii.* The culture was maintained at 28°C for 7days.

After the solid state fermentation the medium was extracted with four volumes of 1% NaCl solution with shaking of 1hr at 28°C. The cell free supernatant was precipitated with 30% ammonium nitrate. Then resultant precipitate was centrifuged at 10000rpm for 10mints. The ammonium nitrate concentration was increased to 75% saturation and it was left overnight at 4°C the supernatant was collected by centrifugation at 10000rpm for 10minutes. Then it was again dissolved in 50ml of 25mM Tris-HCl buffer pH 7.5 then again centrifuged at 10000rpm for 10minutes. Then take a supernatant as a α-amylase.The cell free supernatant was precipitated with 30% ammonium nitrate. Then resultant precipitate was centrifuged at 10000rpm for 10minutes. The ammonium nitrate concentration was increased to 75% saturation and it was left overnight at 4°C. The supernatant was collected by centrifugation at 10000rpm for 10minutes. Then it was again dissolved in 50ml of 25mM Tris-HCl buffer pH 7.5 then again centrifuged at 10000rpm for 10minutes. Then take a supernatant as a α-amylase.

III. RESULT AND DISCUSSION

Aspergillus tamarii in the slide culture was microscopically identified under 10X by staining with Lacto phenol cotton blue. The stock *Aspergillus tamarii* was successfully maintained in the medium. The growth curve of *Aspergillus tamarii* was prepared by taking optical density for 14 days. In the growth curve optical density of *Aspergillus tamarii* at 570nm was plotted against number of days. The liquid media was used as blank. *Aspergillus tamarii* shows maximum growth after 12th day of inoculation and hence the OD is maximum. After 12th day the OD starts to decrease.

The α-amylase was separated from small scale culture and presence of it is confirmed by starch plate assay in which the α-amylase convert to maltose which reacts with lugol solution to form purple colour. Thus purple colour was formed on the starch plate. The sample was prepared by adding 1% 1ml starch solution along with 1ml of α-amylase. To the above mixture add 2ml of DNS. 1ml of sodium potassium tartrate was added to this mixture. 3ml of distilled water was also added along with that the OD was measured at 570nm. Instead of amylase sample, distilled water was added to the remaining composition and it was taken as blank. The α-amylase sample preparation for the both the solid state culture and submerged culture.

By the comparing the above mutated organism (for 8min) produce large amount of α-amylase. The organism was mass cultured using solid state fermentation and submerged fermentation.

The solid state fermentation of *Aspergillus tamarii* was carried out in a steel trays at 28°C for 7 days. The mass culture produced *Aspergillus tamarii* was undergone downstream processing for the separation of α-amylase.

A. Non Mutated Strain

S.no	Enzyme Solution (ml)		1% starch (ml)	DNS (ml)	Dis. water (ml)	OD at 570nm
1	Solid state fermentation	1	1	2	3	0.15
2	Sunmerged fermentation	1	1	2	3	0.21

B. Solid State Fermentation

S.no	Mutation in (min.)	Enzyme sol.(ml)	1% starch (ml)	DNS (ml)	Dis. water (ml)	OD at 570nm
1	2	1	1	2	3	0.14
2	4	1	1	2	3	0.19
3	6	1	1	2	3	0.22
4	8	1	1	2	3	0.35
5	10	1	1	2	3	0.26

C. Submerged Fermentation Of Mutated Strain

S.no	Mutation in (min.)	Enzyme sol.(ml)	1% starch (ml)	DNS (ml)	Dis. water (ml)	OD at 570nm
1	2	1	1	2	3	0.22
2	4	1	1	2	3	0.31
3	6	1	1	2	3	0.38
4	8	1	1	2	3	0.44
5	10	1	1	2	3	0.43

REFERENCES

[1] Al- Agamy M.H., Alhuzani M.R., Kelany M.S., Hamed M.M. 2021. Prk.oduction and partial characterization of α-Amylase enzyme from marine Actinomycetes. *Biomed Res Int*. Doi: 10. 1155/2021/5289848. eCollection 2021.PMID: 34917683.

[2] Al-Johani N.B., Al-Seeni M.N., Ahmed Y.M. 2017. Optimization of alkaline-amylase production by thermophilic *Bacillus subtilis*. *Afr J Tradit Complement Altern Med*. 14(1): 288-301.doi: 10.21010/ajtcam. V14i1.31. eCollection 2017.PMID: 28480407.

[3] Balakrishnan M., Jeevarathinam G., Kumar S.K.S., Muniraj I., Uthandi S. 2021. Optimization and scale-up of α-amylase production by Aspergillus oryzae using solid state fermentation of edible oil cakes. *BMC Biotechnol*. 21(1): 33.doi:10.1186/s12896-021-00686-7.

[4] Chavez-Camarillo G.M., Lopez-Nunez P.V., Jimenez-Nava R.A., Aranda-Garcia E., Cristiani-Urbina E. 2022. Production of extracellular α-amylase by single stage steady-state continuous cultures of Candida wangnamkhiaoensis in an airlift bioreactor. PLoS One. 17(3): e0264734. Doi: 10.1371/journal.pone.0264734.PMID: 35231077.

[5] Fransis F., Sabu A., Madhavan N. 2003. Use of response surface methodology for optimizing process parameters for the production of α-Amylase by *Aspergillus oryzae*. *Journal of Biochemical Engineering*, DOI:10.1016/S1369-703X(02)00192-4.

[6] Gao R., Xu Z., Deng H., Guan Z., Liao X., Zhao Y., Zheng X., Cai Y. 2018. Enhanced hypocrellin production of Shiraia sp. SUPER-H168 by overexpression of α-amylase gene. PLoS One. 13(5): e0196519. Doi: 10.1371/ journal. Pone. 0196519. eCollection 2018. PMID: 29723233.

[7] Horton L.M., Van Bibber-Krueger C.L., Müller H.C., Drouillard J.S. 2020. Effects of high-amylase corn on performance and carcass quality of finishing beef heifers. *J Anim Sci*. 2020 Oct 1;98(10):skaa302. doi: 10.1093/jas/skaa302.PMID: 32910163.

[8] Kathiresan K., Manivannan S. 2006. α- Amylase production by *Penicillium fellutanium* isolated from mangrove rhizosphere soil. *African Journal of Biotechnology*, Vol. 5 (10), pp. 829-832.

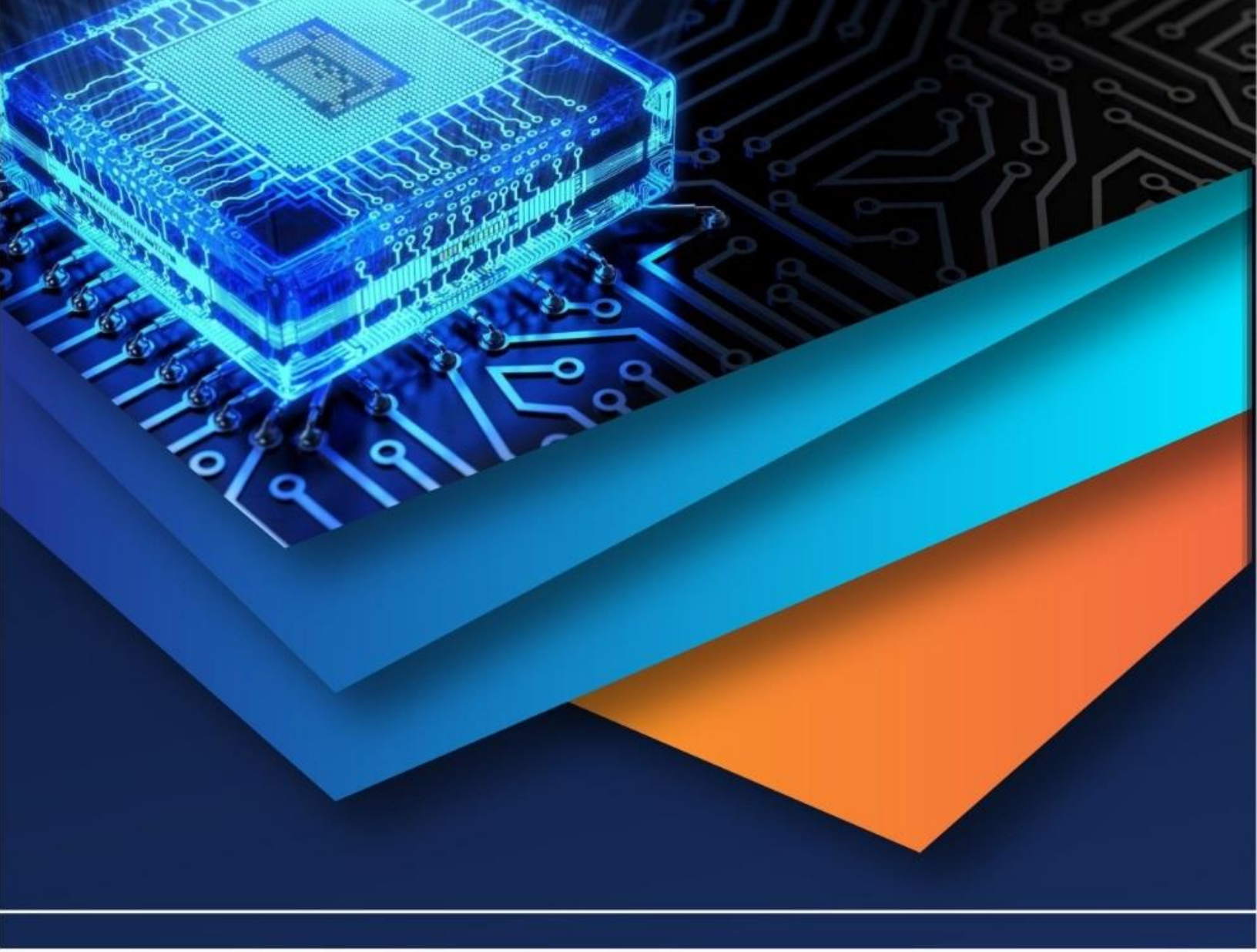
[9] Liu Z., Liu L., Osterlund T., Hou J., Huang M., Fagerberg L., Petranovic D., Uhlen M., Nielsen J. 2014. Improved production of a heterologous amylase in *Saccharomyces cerevisiae* by inverse metabolic engineering. *J. Appl Environ Microbiol*. 80(17): 5542-5550. Doi: 10.1128/AEM.00712-14. Epub 2014. PMID: 24973076.

[10] Martins M. L. L., Nascimento W. C. A. 2002. Production and properties of an extra cellular α-Amylase from thermophillic Bacillus sp. *Brazilian Journal of Microbiology*, 33(1), DOI:10.1590/S1517-83822002000100012.

[11] Meschiatti M.A.P., Gouvea V.N., Pellarin L.A., Batalha C.D.A., Biehl M.V., Acedo T.S., Dorea J.R.R., Tamassia L.F.M., Owens F.N., Santos F.A.P. 2019. Feeding the combination of essential oils and exogenous α-amylase increases performance and carcass production of finishing beef cattle. *J. Anim. Sci*. 2019 Jan 1; 97(1):456-471. doi: 10.1093/jas/sky415. PMID: 30351389.

[12] Varalakshmi K.N., Kumudini B.S., Nandini B.N., Solomon J., Suhas R., Mahesh B., Kavitha A.P. 2009. Production and characterization of lpha-amylase from Aspergillus niger JGI 24 isolated in Bangalore. Pol. *J. Microbiol.* 2009. 58(1):29-36.PMID: 19469283.

[13] Viswanathan P., Surlikar N.R. 2001. Production of α-Amylase with *Aspergillus flavus* on Amaranthus grains by solid state fermentation. *J. Basic Microbiol.*, 41(1): 57-64.



10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY