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DEVELOPMENT OF TECHNOLOGY FOR PILOT-SCALE PRODUCTION, DRYING, AND STORAGE OF EXTRACELLULARLY EXPRESSED RECOMBINANT AMY1974 ALPHA-AMYLASE

Tigran M. Soghomonyan
Scientific and Production Center (SPC) «Armbiotechnology»
National Academy of Sciences of Republic of Armenia (NAS RA)
14 Gyurjan, 0056, Yerevan
tigran.soghomonyan10@gmail.com
ORCID iD: 0009-0004-6486-0541
Republic of Armenia (RA)

Lev T. Khoyetsyan
Scientific and Production Center (SPC) «Armbiotechnology»
National Academy of Sciences of Republic of Armenia (NAS RA)
14 Gyurjan, 0056, Yerevan
levkhoetsyan@gmail.com
ORCID iD: 0000-0002-8040-8043
Republic of Armenia

Ani M. Paloyan
Scientific and Production Center (SPC) «Armbiotechnology»
National Academy of Sciences of Republic of Armenia
14 Gyurjan, 0056, Yerevan
anipaloyanm@gmail.com
ORCID iD: 0000-0003-0279-2104
Republic of Armenia

Artur A. Hambardzumyan
Scientific and Production Center (SPC) «Armbiotechnology»
National Academy of Sciences of Republic of Armenia (NAS RA)
14 Gyurjan, 0056, Yerevan
arthambardzumyan@gmail.com
ORCID iD: 0000-0001-9526-7768
Republic of Armenia

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Abstract

The α-amylase gene from *Bacillus amyloliquefaciens* MDC1974 was cloned into the pBE-S shuttle vector and expressed extracellularly using *Bacillus subtilis* RIK1285.

Following the optimization of fermentation conditions, a pilot-scale production of 100 liters was conducted, achieving a maximum volumetric activity of 1969 U/ml, which is 1.4 times higher than the maximum activity obtained in flask fermentation. Additionally, enzyme concentration, drying, and preservation conditions were studied and optimized.

Keywords: Bacillus amyloliquefaciens, α-amylase, spray drying, liophyl drying, ammonium sulphate fractionation

Introduction

α-Amylase (1,4-α-D-glucan glucanohydrolase, [E.C.3.2.1.1]) belongs to the family 13 of the glycosyl hydrolases and cleaves the 1,4-α-d-glucosidic linkages between adjacent glucose units in the linear amylose chain in an endo-acting manner [1, 2].

Specifically, α-amylase plays a significant role in the detergent, pharmaceutical, fermentation, food, paper, textile, and fine-chemical industries, making up 30% of the world's enzyme market. With the advent of new frontiers in biotechnology, the spectrum of amylase application has widened in many other fields. The increasing demand in various industries requires enzymes with suitable characteristics; therefore, the discovery of new α-amylases and the improvement of existing ones are of great interest [3, 4, 5]. Alpha-amylases can be isolated from a wide range of sources, with those from microbial sources receiving increasing attention due to their low production costs, good fermentation stability, and short production time [6]. Among these, α-amylases from Bacillus species are the most widely used, with those from *B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens*, and *B. stearothermophilus* having important industrial applications [7].

B. subtilis is a model of gram-positive bacteria that has been broadly applied in the industrial production of recombinant protein, such as proteases, α-amylases, and lipases [8, 9] Compared to other repeatedly used hosts, *B. subtilis* has an obvious advantage in terms of its reduced endotoxin contamination risk and has also been verified by the Food and Drug Administration as a generally regarded as safe (GRAS) organism; hence, *B. subtilis* is a safe production strain used in various genetic engineering methods, such as food-grade and pharmaceutical protein production. The capacity of various *Bacillus* strains to produce and secrete large quantities (10–15 g/L) of extracellular enzymes has placed them among the most important industrial enzyme producers. Indeed, they produce about 60 % of the commercially available enzymes [10, 11, 12]. Furthermore, *B. subtilis* strains are considered to serve as an important cellular factory for the production of α-amylases. In contrast, previous studies have suggested that other expression hosts (e.g., *E. coli*) exhibit exceptionally low-level secretory production. *B. subtilis*, however, has a powerful ability to secrete large amounts of foreign proteins into the surrounding medium. More than half of commercial and industrial enzymes are produced in *Bacillus* species.

Industrial production processes are designed to meet the demands of industries and economic considerations. Generally, amylases are produced by submerged cultures due to easy handling and greater control of environmental factors such as temperature and pH [13].

Among the major types of reactors for submerged fermentation, the stirred tank reactor, which provides a high kLa (volumetric mass transfer coefficient), is commonly used in many bioprocesses as it allows efficient contact among three phases: gas, liquid medium, and solid cells. Gas under pressure is supplied to the sparger, and the size of the gas bubbles and their dispersion throughout the tank are critical for reactor performance. Agitating the fermentation broth normally satisfies the oxygen demand of the fermentation process.

Among other factors impacting the operating conditions during fermentation in bioreactors are agitation and mixing. Agitation is important for adequate mixing, mass transfer, and heat transfer. It benefits the growth and performance of microbial cells by improving the mass transfer characteristics with respect to substrate and product/by-product [14]. Multiple impellers on a single shaft with appropriate combination and spacing have been suggested as optimum. In such cases, mixing and mass transfer are dependent on the gas flow rate, type of agitator, its speed and the properties of liquids. Power consumption per impeller decreases with an increase in the number of impellers and this increases the uniformity of energy dissipation [15]. The dissolved oxygen (DO) concentration becomes a limiting nutrient in the processes of high oxygen demand [16]. The supply of oxygen can be the controlling step in industrial bioprocesses and in the scale-up of aerobic biosynthesis systems [17, 18, 19]. The oxygen transfer rate could be affected by several factors, such as geometry and characteristics of the vessels, liquid properties (viscosity, superficial tension, etc.), the dissipated energy in the fluid, biocatalyst properties, concentration, and morphology of microorganisms, and it also depends on the air flow rate, the stirrer speed, mixing, etc. Mechanically agitated aerated vessels are widely used rather than vessels with aeration only, which can be inadequate to promote the liquid turbulence necessary for small air bubble generation. Although agitation can maintain the dissolved oxygen in the fermenter available, the inappropriate speed of agitation results in poor oxygen transfer, especially in highly viscous broths. The production of amylase by *B. amyloliquefaciens* is generally considered to be an aerobic process. The aeration rate needed to maintain an adequate dissolved oxygen (DO) level is often around one volume of air per volume of fermenter per minute (vvm) at the laboratory scale, with a gas hold-up as high as 20%. [20].

Conflict Setting

When developing an industrial fermentation, designing a fermentation medium is of critical importance because medium composition can significantly affect product yield. For commodity products, the cost of the medium can substantially affect the overall economics of the process [21]. The optimization of fermentation conditions, particularly physical and chemical parameters, is of primary importance in the development of any fermentation process owing to their impact on the economy and practicability of the process.

In this study the technology for recombinant Amy1974 α -amylase, which is secreted from RIK 1285 cells, is described, along with pilot-scale technology including: enzyme production, concentration by ultrafiltration and development of different variants of long time storage.

Materials and Methods

Microorganism, production medium and cultivation conditions

The process of obtaining and storing the recombinant AMY1974 α-amylase producing strain was described earlier [22]. The conserved bacteria were initially activated in LB broth and subsequently cultivated on LB agar medium with the following composition (g.L-1): peptone, 10.0; yeast extract, 5.0; NaCl, 5.0; and agar, 20.0. The pH of the medium was adjusted to 7.0 before sterilization. The seed culture was prepared by inoculating 50 ml of LB broth in a 250 ml Erlenmeyer flask with 1 ml of bacterial suspension obtained from fresh LB agar slants. The inoculated flasks were incubated on a rotary shaker at 200 rpm and 33°C (Senova, China). After 24 hours, the grown cells were used to inoculate either a shake flask or a bioreactor at a final concentration of 5% (v/v).

The initial enzyme production medium (fermentation medium) was composed of ($g.L^{-1}$): sucrose: 10, starch: 10, $(NH_4)_2SO_4$: 3, NH_4Cl: 2, KH_2PO_4: 5, $MgSO_4$: 0.25. The media also contained 40 mg/l of L-lysine and L-tryptophan and 10 mg/l of kanamycin.

Pilot production, concentration and drying

Pilot-scale production, concentration, and development of variants for long-term storage of Amy1974 were conducted at "Vipeco" LLC [https://w ww.vipeco.am/hy] The diagram below illustrates the hardware-technology scheme of the process.

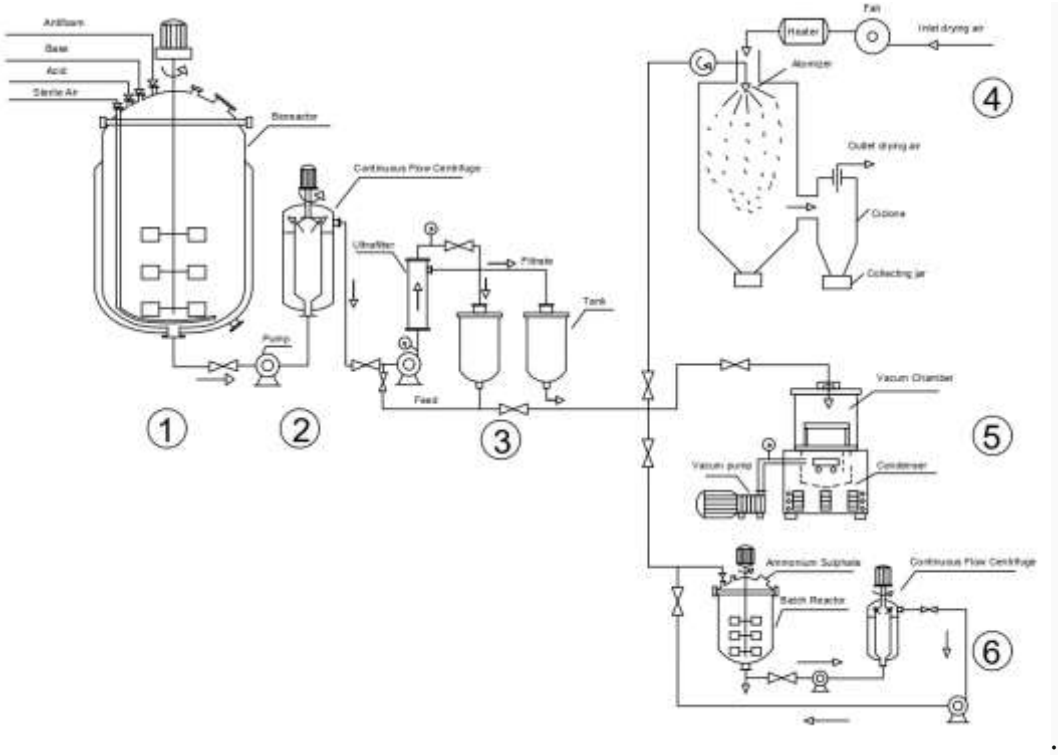

Fig 1. Technological scheme for the production, concentration, and drying of recombinant alpha-amylases. 1 – Bioreactor for the production of α-amylase, 2 – Continuous Flow Centrifuge for the sedimentation of biomass and insoluble matter, 3 – Ultrafiltration system for enzyme concentration, 4 – Spray drying system for amylase drying, 5 – Vacuum lyophilizer for amylase drying, 6 – Fractionation with ammonium sulfate

All bioreactor experiments were conducted in a 250-L stirred tank bioreactor (Artlife Techno, Russia) as depicted in node 1 of the scheme. Agitation was achieved using two 4-bladed Rushton turbines with the following dimensions: di (impeller diameter) = 220 mm; dt (tank diameter) = 800 mm; di/dt = 0.275. The agitation speed was set at 350 rpm, and the aeration was adjusted to 1.5 $v.v^{-1}.min^{-1}$.

The pH of the culture was continuously monitored using a sterilizable pH electrode (Mettler-Toledo AG, Switzerland). Throughout all bioreactor cultures, the pH was controlled and maintained at 7.0 by the continuous addition of 2N Ammonium hydroxide or 1N Phosphoric acid. Dissolved oxygen levels in the culture were assessed during cultivation using a polarographic electrode (Gold electrode, Mettler-Toledo AG, Switzerland). Foaming was managed using Hi/Lo foam sensors and connected to an antifoam pump controlled by the antifoam agent (Silicone antifoam A, Sigma-Aldrich, USA).

After the end of the process, the fermentation liquid is centrifuged with a Continuous Flow Centrifuge (GQ-145 Open-type tubular clarifier, China) at 14,000 rpm (2 node of the scheme).

At the subsequent stage (node 3 of the scheme), the supernatant containing the extracellular enzyme undergoes ultrafiltration using an AP-2 ultrafiltration membrane (NPK "Biotest", Russia) with a cutoff value of 15 kDa (which is a surface-modified membrane). The ultrafiltration process was conducted under conditions of 0.15 MPa pressure and a volumetric flow rate of 20 l/min. Consequently, the supernatant was concentrated by a factor of 6.3.

At technological node 4, the concentrated liquid underwent spray drying using a Lab Spray Dryer YC-018 (China). The amylase was spray-dried with inlet air temperatures set at 55 °C and outlet temperatures at 45 °C. To enhance the enzyme's stability against temperature and pressure, 2% starch was added to the enzyme solution concentrated 6.3 times. For comparison purposes, the enzyme was also dried without starch [23].

At technological node 5, the concentrated enzyme solution undergoes lyophilization using equipment from Iney 3-2 (Russia). The ultimate vacuum pressure is 0.065 mPa, and the temperature in the vacuum chamber is -40 °C. To enhance the stability of the enzyme, 2% sucrose and 2% starch were added to the liquid [24].

At technological node 6, fractionation with Ammonium Sulfate is conducted. This process enables the separation of proteins based on their solubility in various concentrations of ammonium sulfate, aiming to purify and concentrate the desired enzyme. Fractionation of the concentrated crude enzyme solution with ammonium sulfate is performed at 2 degrees of saturation. Initially, solid ammonium sulfate is slowly added to the batch reactor (100 L) with constant stirring of the enzyme solution to achieve a final saturation of 30%, which is then kept for 1 hour at temperature 4 °C. The precipitated proteins are separated by centrifugation using a Continuous Flow Centrifuge for 14000 rpm at temperature 4 °C and then dissolved in a minimal volume of 0.02 M phosphate buffer (pH 7.5). Subsequently, the supernatant is saturated with ammonium sulfate up to 60% and maintained for 1 hour at 4°C. Further, the precipitated proteins are separated by centrifugation and dissolved in a minimal volume of 0.02 M phosphate buffer (pH 7.5). Following this, the amylase activities of all fractions are measured.

Enzyme Assay and Total Protein Determination

Enzyme activity in the culture fluid was assessed by estimating the number of reducing groups formed during starch hydrolysis using our modification of Miller's modified [25] Sumner's 3,5-dinitrosalicylic acid (DNS) reagent method [26]. The reaction medium with a final volume of 0.2 mL contained 1% (w/v) starch, 50 mM acetate buffer at pH 6.0, 1 mM CaCl2, and the required amount of enzyme preparation. After 10 minutes of incubation at 55°C, 1 mL of DNS reagent (Miller's modified) supplemented with 15 mM 2-mercaptoethanol (to neutralize the dissolved oxygen effect on the stoichiometry of DNS interaction with reducing groups of carbohydrates) was added, followed by 15 minutes of incubation in a boiling water bath. The optical absorption of the solutions was measured at a wavelength of 546 nm.

One unit of enzyme activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol of reducing groups in one minute under the specified conditions. The amount of protein was estimated using the method of Groves and Davis [27].

Research Results

Kinetics of cell growth and α-amylase production in 250-L batch bioreactor

The bioreactor with a working volume of 250 L was inoculated and operated as described in the Materials and Methods section. The agitation speed was set to 350 rpm, aeration was adjusted to 1.5 $v.v^{-1}.min^{-1}$, and the pH was maintained at 7.2 ± 0.2 during the cultivation. For the cultivation of recombinant *B. subtilis* Rik1285_amy1974, 5 L of inoculum was added to a working volume of 95 L of fermentation medium and fermented until reaching the maximum volumetric activity (1969 U/ml) at 25 hours, in contrast to the flask fermentation where the maximum activity (1410 U/ml) was achieved at 48 hours.

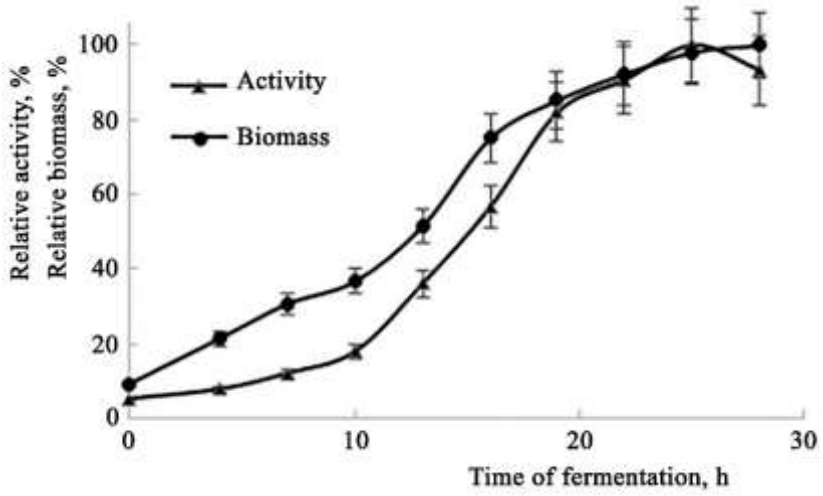

Fig. 2 Kinetics of cell growth and volumetric α-amylase production. For biomass, 100% corresponds to 8.77 g/L of dry mass, and for activity, 100% corresponds to 1969.3 U/mL of volumetric activity

Cell growth and enzyme production parameters were monitored over the culturing time to better understand the kinetics of cell growth and α-amylase production. From the results obtained (refer to Fig. 2), it is evident that the maximum rate of cell growth is

observed at the 16th hour, reaching a value of 0.153 g/L/h for dry weight. Subsequently, following this peak, the cell growth rate began to gradually decrease, while the rate of α-amylase volumetric production continued to rise, reaching a maximum volumetric enzyme production of 167 U/mL/h at 19 hours. At the conclusion of the process, the culture liquid was centrifuged, resulting in the obtention of 4.64 kg of wet bacterial biomass and 177.2 million units of α-amylase activity. Sampling was conducted every 3 hours during the process to assess bacterial biomass and amylase activity.

Drying and storage of alpha amylases

The supernatant of B. subtilis RIK 1285_amy1974, grown in the bioreactor for 28 hours, yielded 90 L of culture liquid, which was concentrated 6.3 times via ultrafiltration, resulting in a volume of 14.3 L. The extracellular α-amylase concentrated by ultrafiltration was subjected to various conservation procedures (different drying techniques and ammonium sulfate precipitation).

Subsequently, 2 L of the concentrated enzyme preparation underwent lyophilization (1 L with 2% sucrose, 1 L with 2% starch), while another 2 L was subjected to spray drying (1 L of concentrated enzyme preparation with and 1 L without 2% starch). Additionally, 10 liters of the enzyme solution were fractionated with ammonium sulfate. The detailed analysis of the various conservation procedures is summarized in Tab. 1.

Table 1

Comparison of various recovery and preservation methods for recombinant Amy1974 α-amylase synthesized extracellularly by *B. subtilis* Rik1285_amy1974 cells.

Step	V, L	Proteine concentration, mg/ml	Specific activity, U/mg	Volum-metric activity, U/ml	Activity.10^6, U	Recovery of activity at the stage, %	Yeald of total activity, %
Crude enzyme	90.0	9.6	205	1969	177.2	100.0	100.0
Ultrafiltration	14.3	32.4	370	11977	171.3	96.6	96.6
Liophyl. (2% sucrose)	1.0	37.1	290	10748	10.7	89.7	86.7
Liophyl. (2% starch)	1.0	40.3	308	12415	12.4	103.7	100.2
Spray drying	1.0	51.6	170	8794	8.8	73.4	70.9
Spray drying (2% starch)	1.0	36.6	281	10287	10.3	85.9	83.0
A.S. saturation 0-30 %	2.0	48.0	124	5950	11.9	9.9	-
A.S. saturation 30-60%	2.0	31.0	1344	41650	83.3	69.5	67.1
A.S. 60% Supernatant	10.0	18.4	162	2975	29.8	24.8	-

As seen in Table 1, the ultrafiltration step achieves a total activity recovery of 96.6%, yielding 171.3 million units of total activity.

In the lyophilization step, the step yields were 89.7% and 103.7% in the presence of 2% sucrose and starch, respectively. The respective overall yields of total activity were 86.7% and 100.2%.

In the spray drying step, the yields were 73.4% without starch and 85.9% with 2% starch. And the respective overall yields of total activity were 70.9% and 83.0%.

Ammonium sulfate fractionation was performed in two steps: 0% to 30% saturation and 30% to 60% saturation. The sediment obtained after each step was resuspended in 2000 ml of 0.02 M phosphate buffer (pH 7.5). The main portion of enzyme activity was observed in the 30% to 60% ammonium sulfate saturation, resulting in step and total activity yields of 69.5% and 67.1%, respectively. As a result of this procedure, Amy1974 was also significantly purified, reaching a specific activity of 1344 U/mg.

Conclusion

This Acquiring new recombinant microorganisms for production is crucial for enhancing alpha-amylase production. Scaling up production, concentrating, optimizing drying, and storage conditions using the Bacillus subtilis RIK1285 strain with the amy1974 gene cloned in the pBE-S shuttle vector demonstrates its potential for large-scale production.

The fermentation conducted in the bioreactor showed that the peak of maximum enzyme production follows the peak of maximum biomass production. This phenomenon will be utilized in the future to develop fed-batch technology for enzyme production.

It can also be mentioned that in enzyme drying, both by lyophilization and spray drying, the presence of 2% starch significantly enhanced the α -amylase recovery.

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References

1. *Tonkova A., Enzyme.* Bacterial cyclodextrin glucanotransferase, Enzyme and Microbial Technology, Volume 22, Issue 8, 1998, Pages 678-686 https://doi.org/10.1016/S0141-0229(97)00263-9.
2. *Macgregor E.A., Janecek S.*, Relationship of sequence and structure to specificity in the alpha-amylase family of enzymes, Biochim. Biophys. Acta 1546 (2001)1–20. https://doi.org/10.1016/s0167-4838(00)00302-2.
3. *Van der Maarel, Uitdehaag B.V.,* Properties and applications of starch converting enzymes of the α-amylase family. Journal of Biotechnology 94:137–155 (2002). https://doi.org/10.1016/S0168-1656(01)00407-2
4. *Francis F., Sabu A.*, Use of response surface methodology for optimizing process parameters for the production of α-amylase by Aspergillus oryzae. Bioch Eng J 15:107–115 (2003). https://doi.org/10.1016/S0168-1656(01)00407-2
5. *Farooq, M.A., Ali, S., Hassan, A., Tahir H.M., Mumtaz, S., Mumtaz, S.*, Biosynthesis and industrial applications of α-amylase: A review. Arch. Microbiol. 2021, 203, 1281–1292. https://doi.org/DOI: 10.1007/s00203-020-02128-y

6. *Hussain I, Siddique F, Mahmood MS, Ahmed SI.* A review of the microbiological aspect of alpha-amylase production. Int J Agric Biol. 2013;15:1029 34. https://doi.org/10.5897/AJMR2014.7069
7. *Gopinath S.C., Anbu P., Arshad MKM, Lakshmipriya T, Voon CH, Hashim U, Chinni S.V,* Biotechnological processes in microbial amylase production. Biomed Res Int. 2017. https://doi.org/10.1155/2017/1272193
8. *Schallmey M., Singh A., Ward O.P.*, Developments in the use of Bacillus species for industrial production, Can. J. Microbiol. 50 (2004) 1–17, https://doi.org/10.1139/ w03-076
9. *Takata H., Kuriki T., Okada S., Takesada Y., Iizuka M., Minamiura N., Imanaka T.* Action of neopullulanase: Neopullulanase catalyzes both hydrolysis and transglycosylation at a-(1-4)- and a-(1-6)-glucosidic linkages. J. Biol. Chem., 267, 18447-18452, 1992. https://doi.org/10.1016/S0021-9258(19)36983-2
10. *Schallmey M., Singh A., Ward O.P*., Developments in the use of Bacillus species for industrial production, Can. J. Microbiol. 50 (2004) 1–17. https://doi.org/10.1139/w03-076
11. *Dong H., Zhang D.W.*, Current development in genetic engineering strategies of Bacillus species, Microb. Cell Fact. 13 (2014) 63–74, https://doi.org/10.1186/ 1475-2859-13-63
12. *Fu G., Liu J.L, Li J.S., Zhu B.W., Zhang D.W*., Systematic screening of optimal signal peptides for secretory production of heterologous proteins in Bacillus subtilis, J. Agr. Food. Chem. 66 (2018) 13141–13151, https://doi.org/10.1021/acs. jafc.8b04183.
13. *Harshemi, I., Ali S., Javed M. M., Hameed U., Saleem, A., Adnan, F*., Introduction of alpha amylase from a randomly induced mutant strain of Bacillus amyloliquefaciens and its application as a desizer in textile industry. Pak J Bot., 2011, 42 (1): 473-484. https://doi.org/10.1186/2193-1801-2-154
14. *Kongkiattikajorn J., Rodmui A., Dandusitapun Y.*, Effect of agitation rate on batch fermentation of mixture culture of yeasts during ethanol production from mixed glucose and xylose, Thai J. Biotechnol. 5 (2007) 1–4 https://doi.org/10.4314/gjer.v12i1.2
15. *Nienow A.W., Lilly M.D.*, Power drawn by multiple impellers in sparged agitated vessel, Biotechnol. Bioeng. 21(1979) 2341–2345. https://doi.org/10.1016/0032-9592(92)87014-8
16. *Lo Y.M., Hsu C.H., Yang S.T., Min D.B.*, Oxygen transfer characteristics of a centrifugal, packed-bed reactor during viscous xanthan fermentation, Bioprocess. Biosyst. Eng. 24 (2001) 187–193 https://doi.org/10.1007/s004490100250
17. *Al-Masry W.A.*, Effect of scale-up on average shear rates for aerated non-Newtonian liquids in external loop airlift reactors, Biotechnol. Bioeng. 62 (1999) 494–498 https://doi.org/10.1002/(sici)1097-0290(19990220)62:4<494::aid-bit14>3.0.co;2-6

18. *Elibol M., Ozer D.*, Influence of oxygen transfer on lipase production by Rhizopus arrhizus, Process Biochem. 36 (2000) 325–329 https://doi.org/10.1016/S0032-9592(00)00226-0
19. *D. Weuster-Botz, E. Hьnekes, A. Hartbrich*, Scale-up and application of a cyclone reactor for fermentation processes, Bioprocess Eng. 18 (1998) 433–438 https://doi.org/10.1007/978-3-031-27811-2_8
20. *Milner J.A., Martin D.J., Smith A.*, Oxygen transfer conditions in the production of alpha-amylase by Bacillus amyloliquefaciens, Enzyme Microb. Technol. 18 (1996) 507–512. https://doi.org/10.1016/0141-0229(95)00155-7
21. *Chen Q.H., He G.Q., Mokhtar A.M. Ali*, Optimization of medium composition for the production of elastase by Bacillus sp. EL31410 with response surface methodology, Enzyme and Microbial Technology, Volume 30, Issue 5,2002, Pages 667-672 https://doi.org/10.1016/S0141-0229(02)00028-5.
22. *Soghomonyan T.*, Extracellular expression of the alpha-amylase gene from *Bacillus amyloliquefaciens mdc1974* strain using *Bacillus subtilis* RIK1285 cells, Bulletin of high technology , 2024, N 1 (29) 2024, 15-24, https://doi.org/10.56243/18294898-2024.1-15
23. *Samborska K., Witrowa R., Dorota G.*, Spray-Drying of α-Amylase - The Effect of Process Variables on the Enzyme Inactivation. Drying Technology, (2005). *23(4), 941–953*. doi:10.1081/DRT-200054243
24. *Van Beek, Hugo L.; Beyer, Nina; Janssen, Dick B.; Fraaije, Marco W.* Lyophilization conditions for the storage of monooxygenases. Journal of Biotechnology, (2015) 203(), 41–44. https://doi.org/10.1016/j.jbiotec.2015.03.0
25. *Miller G.L.* Use of DinitrosaIicyIic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry 31, 426-428, 1959. https://doi.org/10.1021/ac60147a030
26. *Sumner J.B.* The Estimation of Sugar in Diabetic Urine, Using Dinitrosalicylic Acid. J. Biol. Chem., 62(2), 287-290, 1954 https://doi.org/10.1016/S0021-9258(18)85062-1
27. *Peterson G.*The amount of protein was estimated using the method of Groves and Davis, Determination of total protein. Meth. Enzymol., 91, 95-119, 1983 https://doi.org/10.1016/s0076-6879(83)91014-5

ՌԵԿՈՄԲԻՆԱՆՏ ԱՐՏԱԲՋՋԱՅԻՆ ԷՔՍՊՐԵՍՎՈՂ AMY1974 ԱԼՖԱ ԱՄԻԼԱԶԻ ՊԻԼՈՏԱՅԻՆ ԱՐՏԱԴՐՈՒԹՅԱՆ, ՉՈՐԱՑՄԱՆ ԵՎ ՊԱՀՊԱՆՄԱՆ ՏԵԽՆՈԼՈԳԻԱՆԵՐԻ ՄՇԱԿՈՒՄ

Տ.Մ. Սողոմոնյան, Լ.Տ. Խոյեցյան, Ա.Մ.Պալոյան, Ա.Ա. Համբարձումյան

ՀՀ ԳԱԱ «Հայկենսատեխնոլոգիա» Գիտաարտադրական կենտրոն

Bacillus amyloliquefaciens MDC1974-ից α-ամիլազային գենը կլոնավորվել է pBE-S մաքոքային վեկտորի մեջ և արտաբջջային ձևով էքսպրեսվել՝ օգտագործելով *Bacillus subtilis* RIK1285 շտամը: Ֆերմենտացիայի պայմանների օպտիմալացումից հետո

իրականացվել է 100 լիտր պիլոտային արտադրություն՝ ֆերմենտի ծավալային ակտիվությունը հասցնելով 1969 Միավոր/մլ-ի, որը 1,4 անգամ գերազանցում է կոլբայում ֆերմենտացման ժամանակ ստացված առավելագույն ակտիվությանը: Բացի այդ, ուսումնասիրվել և օպտիմալացվել են ֆերմենտի խտացման, չորացման և պահպանման պայմանները:

Բանալի բառեր. *Bacillus amyloliquefaciens,* α-ամիլազ, հեղուկացրային չորացում, լիոֆիլ չորացում, ամոնիումի սուլֆատով ֆրակցիոնացում

РАЗРАБОТКА ТЕХНОЛОГИИ ОПЫТНОГО ПРОИЗВОДСТВА, СУШКИ И ХРАНЕНИЯ ВНЕКЛЕТОЧНО ЭКСПРЕССИРУЕМОЙ РЕКОМБИНАНТНОЙ АЛЬФА-АМИЛАЗЫ AMY1974

Т. М. Согомонян, Л. Т. Хоецян, А. М. Палоян, А.А. Амбарцумян
Научно-производственный Центр (НПЦ) «Армбиотехнология» НАН РА

Ген α-амилазы из Bacillus amyloliquefaciens MDC1974 был клонирован в челночный вектор pBE-S и экспрессирован внеклеточно с использованием Bacillus subtilis RIK1285. После оптимизации условий ферментации было проведено опытно-промышленное производство объемом 100 литров, в результате чего была достигнута максимальная объемная активность 1969 Ед/мл, что в 1,4 раза превышает максимальную активность, полученную при ферментации в колбе. Кроме того, были изучены и оптимизированы концентрация фермента, условия сушки и консервации.

Ключевые слова: *Bacillus amyloliquefaciens,* α-амилаза, распылительная сушка, лиофильная сушка, фракционирование сульфатом аммония

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