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Enhanced extracellular production of α -amylase in *Bacillus subtilis* by optimization of regulatory elements and over-expression of PrsA lipoprotein

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Abstract α -Amylase was used as a heterologous model protein to investigate the effects of promoters, signal peptides and over-expression of an extracytoplasmic molecular chaperone, PrsA lipoprotein, on enhancing the secretion of α -amylase in *Bacillus subtilis*. Four promoters and six signal peptides were compared, successively, and the highest yield of α -amylase was achieved under the promotion mediated by P_{APRE}, a strong constitutive promoter, and secretion by SP_{APRE}, a signal peptide from *B. subtilis*. Moreover, under conditions of overexpressed PrsA

lipoprotein, the secretion production and activity of α -amylase increased to 2.5-fold. The performance of the recombinant *B. subtilis* 1A751PL31 was evaluated with a fed-batch fermentation in a 7.5 l fermentor. Optimization of regulatory elements and over-expression of PrsA lipoprotein had a significant effect on enhancing the production of α -amylase in *B. subtilis*.

Keywords α -Amylase · *Bacillus subtilis* · Fed-batch fermentation · Promoter · PrsA · Regulatory elements · Signal peptide

Introduction

Production of heterologous proteins of interest at high levels is crucial for both basic research and practical applications. *Bacillus subtilis* is a widely used host for the expression of heterologous proteins. Compared to *Escherichia coli*, *B. subtilis* is considered as a generally recognized as safe (GRAS) organism (Westers et al. 2004). It has a naturally high secretory capacity and exports proteins directly into the extracellular medium (Simonen and Palva 1993). However, there are several bottlenecks in the *B. subtilis* expression system (i.e., transcription, protein folding, translocation across the membrane, signal peptide processing and proteolysis) that limit its application potential (Li et al. 2004).

To obtain large amounts of secreted protein, the use of strong promoters and optimal signal peptides are

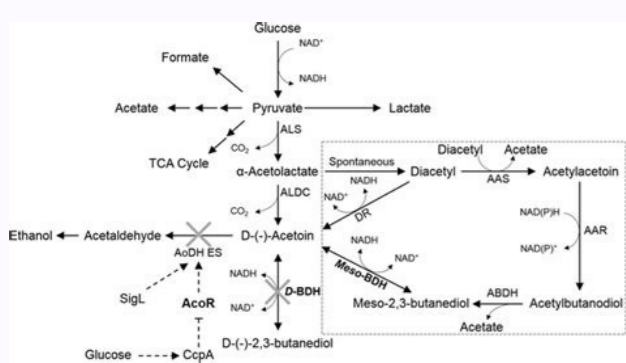
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Sl. No.	Author(s)	Year	Enzymes	Ref
1	Bacillus licheniformis	1913	2,3,6-amylase	[10]
2	B. subtilis	1913	2,3,6-amylase	[10]
3	B. licheniformis	1913	2,3,6-amylase	[10]
4	B. licheniformis	1913	2,3,6-amylase	[10]
5	B. licheniformis	1913	2,3,6-amylase	[10]

Where do α -amylase and β -amylase carry out their enzymatic functions. A-amylase from bacillus licheniformis. What is the function of alpha-amylase in saliva.

Vol. Ribonucleotide reductase activity in intact mammalian cells: stimulation of enzyme activity by MgCl₂, dithiothreitol, and several nucleotides. Hards R, Bajpai P, Bajpai P. 254 (5 Pt 1): G775–80. 39 (10): 1256–60. [Google Scholar]Page 2(A) Enzyme production by B.licheniformis MTCC strain 2617. Bioeng. USA: Academic Press; 1955. doi:10.1107/S0907444995014119. The catalytic domain has a structure consisting of an eight-stranded alpha/beta barrel that contains the active site, interrupted by a ~70-amino acid calcium-binding domain protruding between beta strand 3 and alpha helix 3, and a carboxyl-terminal Greek key beta-barrel domain.[19] Several alpha-amylases contain a beta-sheet domain, usually at the C terminus. Perry and colleagues speculated the increased copy number of the salivary amylase gene may have enhanced survival coincident to a shift to a starchy diet during human evolution. 239 (1): 104–21. 272 (23): 6145–53. Turk. "Starch and glucose oligosaccharides protect salivary-type amylase activity at acid pH". Biochem. Process Biochem. [Google Scholar]25. "Diet and the evolution of human amylase gene copy number variation". Acta Crystallographica D. PMID 19249160. Biochimie (2nd ed.). Carvalho R. Genes salivary - AMY1A, AMY1B, AMY1C pancreatic - AMY2A, AMY2B In grain α -Amylase activity in grain is measured by, for instance, the Hagberg-Perten Falling Number, a test to assess sprout damages.[12] or the Phadebas method. doi:10.1002/pro.5560070304. ^ Noto Y, Sato T, Kudo M, Kurata K, Hirota K (December 2005). "Amylase". 61 (2): AAC.01998-16. 2005. ^ a b Perry GH, Dominy NJ, Claw KG, Lee AS, Fiegler H, Redon R, Werner J, Villanea FA, Mountain JL, Misra R, Carter NP, Lee C, Stone AC (October 2007). Brazil. It is a member of glycoside hydrolase family 13. 3 (1): 49–63. ISSN 1380-3743. Eng. PMC 2377015. 2005;40(7):2401–2408. [Google Scholar]15. In contrast, a Biaka individual carried six copies (three copies on each allele). 1984;231(1):9–16. The starch iodine test, a development of the iodine test, is based on colour change, as α -amylase degrades starch and is commonly used in many applications. metaterium α -amylase. 2003;38(10):1397–1403. [PMC free article] [PubMed] [Google Scholar]9. In humans, all amylase isoforms link to chromosome 1p21 (see AMY1A). Properties of an Amylase from Thermophilic Bacillus sp. 1985;49(4):1091–1097. PDB 1SM01 [IdentifiersEC no.3.2.1.OCAS no.9000-90-2 DatabasesIntEnzIntEnz viewBRENDA BRENDA entryExPASyNiceZyme viewKEGGKEGG entryMetaCycmetabolic pathwayPRIMAprofilePDB structureRCSB PDB PDBe PDBsumSearchPMCarticlesPubMedarticlesNCBIproteins GH13 catalytic domainCyclodextrin glucanotransferase (e.c.2.4.1.19) (cglase) [IdentifiersSymbolAlpha-amylasePfamPF00128Pfam clanCL0058InterProIPR006047SCOP21 ppi / SCOPe / SUPFAMOPM superfamily1170PM proteinIwzaCAZyGH13CDcDc11338Available protein structures:Pfam structures / ECOD PDBRCSB PDB, PDBe, PDBjPDBsumstructure summary Alpha-amylase C-terminal beta-sheet domainCrystal structure of barley alpha-amylase isozyme 1 (amy1) inactive mutant d180a in complex with maltoheptaoseIdentifiersSymbolAlpha-amyl C2PfamPF07021InterProIPR012850Available protein structures:Pfam structures / ECOD PDBRCSB PDB, PDBe, PDBjPDBsumstructure summary Alpha-amylase, C-terminal all-beta domainmaltotriose complex of precondoned cyclodextrin glycosyltransferase mutantIdentifiersSymbolAlpha-amylase. CPFamPF02806Pfam clanCL0369InterProIPR006048SCOP21 ppi / SCOPe / SUPFAMAvailable protein structures:Pfam structures / ECOD PDBRCSB PDB, PDBe, PDBjPDBsumstructure summary Alpha-amylase (α -amylase) is an enzyme EC 3.2.1.1 that hydrolyses alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen, yielding shorter chains thereof, dextrins, and maltose.[2] It is the major form of amylase found in humans and other mammals.[3] It is also present in seeds containing starch as a food reserve, and is secreted by many fungi. ^ Gatehouse AM, Davison GM, Newell CA, Merryweather A, Hamilton WD, Burgess EP, Gilbert RJ, Gatehouse JA (1997). ^ "Falling Number - Introduction". "Salivary alpha-amylase in biobehavioral research: recent developments and applications". ^ Fleming D, Chahin L, Rumbaugh K (February 2017). Chem. D., Garriott O. Occurs in wheat.[13] Industrial use α -Amylase is used in ethanol production to break starches in grains into fermentable sugars. 278 (1): 205–17. 149. Archived from the original on 9 September 2009. Int. In contrast, 50% of amylase activity remained after 150 minutes of exposure to gastric juice at pH 4.3.[5] Both starch, the substrate for ptyalin, and the product (short chains of glucose) are able to partially protect it against inactivation by gastric acid. [PubMed] [Google Scholar]12. Santos E.O., Martins M.L.L. Effect of the medium composition on formation of amylase by Bacillus sp. The Biaka are rainforest hunter-gatherers who have traditionally consumed a low-starch diet. ^ Kadziola A, Abe J, Svensson B, Haser R (May 1994). The American Journal of Physiology. Kadziola A, Sogaard M, Svensson B, Haser R (April 1998). 1098: 122–44. J., Ng J. ^ Nater UM, Rohleder N (May 2009). Perten Instruments. Store Medisinske Leksikon. α -Amylase Production by B. PMID 17628263. B. [Google Scholar]10. 34 (4): 486–96. Inhibition of paraxonase activity in human liver: microsomes by exposure to EDTA, chloride ion in green. Sarikaya E., Gürgün V. The name derives from the Greek word *truxo* (l spit), because the substance was obtained from saliva.[4] It will break large, insoluble starch molecules into soluble starches (amylodextrin, erythrodestrin, and achrodestrin) producing successively smaller starches and ultimately maltose. 32 (10): 1097–103. 2004;47(2):309–317. 2001;8(1):61–81. Bernfeld P. 246 (4): 545–59. Determination Several methods are available for determination of α -amylase activity, and different industries tend to rely on different methods. PMID 9571044. PMC 5278739. The Japanese diet has traditionally contained large amounts of rice starch. doi:10.2174/092986608783489616. Wind R. PMID 27872074. Production and properties of α -amylase from thermophilic Bacillus sp. Alpha amylase from a fungal culture grown on oil cakes and its properties. This domain is organised as a five-stranded antiparallel beta-sheet.[20][21] Several alpha-amylases contain an all-beta domain, usually at the C terminus.[22] See also Digestive enzyme References ^ Ramasubbu N, Paloth V, Luo Y, Brayer GD, Levine MJ (May 1996). Antimicrobial Agents and Chemotherapy. They behave differently on isoelectric focusing, and can also be separated in testing by using specific monoclonal antibodies. Five to 10 times the ULN may indicate ileus or duodenal disease or kidney failure, and lower elevations are commonly found in salivary gland disease. Journal of Molecular Biology. External links The alpha-Amylase Protein alpha-Amylase at the US National Library of Medicine Medical Subject Headings (MeSH) This article incorporates text from the public domain Pfam and InterPro: IPR0012850 This article incorporates text from the public domain Pfam and InterPro: IPR006048 Portal:Biological Retrieved from " Ramaachandran S., Patel A. K., Nampootheri K. M., Chandran S., Szakacs G., Soccol C. R., Pandey A. Bernhardsdotter E. "Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research". PMID 15299664. O. This form of amylase is also called "ptyalin" /"taolin", which was named by Swedish chemist Jöns Jacob Berzelius. [Google Scholar]24. And Technol. Biol. Annals of the New York Academy of Sciences. "Crystal and molecular structure of barley alpha-chloride ion in green. Sarikaya E., Gürgün V. The name derives from the Greek word *truxo* (l spit), because the substance was obtained from saliva.[4] It will break large, insoluble starch molecules into soluble starches (amylodextrin, erythrodestrin, and achrodestrin) producing successively smaller starches and ultimately maltose. 32 (10): 1097–103. Bioconversion: pp. de S. cereus, Food Technol. "Crystal structures of the psychrophilic alpha-amylase from Alteromonas haloplanctis in its native form and complexed with an inhibitor". doi:10.1038/ng2123. G., Wright J. M., Magalhães P. Faculty of Engineering, Science and the Built Environment, London South Bank University, Prabhakaran G., Pugalvendhan R. PMID 2452576. "Crystal structure of calcium-depleted Bacillus licheniformis alpha-amylase at 2.2 Å resolution". 2002;33(1):57–61. PMID 17332070. M., Martins M. Mansur L. Culture Conditions for the Production of Thermostable Amylase by Bacillus. Interact. 2010;41:850–861. 5. V., Córrea T. I. p. 1583. Microbial α -amylases: a biotechnological perspective. Burhan A.I., Nisa U., Gokhan C., Omer C., Ashabil A., Osman G. ^ Abe A, Yoshida H, Tonozuka T, Sakano Y, Kamitori S (December 2005). PMID 16301277. "Binding of Tris to Bacillus licheniformis alpha-amylase can affect its starch hydrolysis activity". doi:10.1196/annals.1384.008. Çalik P., Özdamar T.H. Carbon sources affect metabolic capabilities of Bacillus species for the production of industrial enzymes. 15 (2): 212–4. doi:10.1111/j.1742-4658.2005.05013.x. PMID 16302977. If only pancreatic amylase is measured, an increase will not be noted with mumps or other salivary gland trauma. ^ Fried M, Abramson S, Meyer JH (October 1987). Brumm P. D., Buitelaar R.M.G., Huizing H.J., Dijkhuizen L, Gupta R., Gigras P., Mohapatra H., Goswami V.K., Chauhan B. 2008;39:102–107. Fogarty W.M., Kelly C.T. Vol. High-temperature alkaline α -amylase from Bacillus licheniformis TCRDC-B13. 1989;33(1):72–78. ^ J. L. (B) Enzyme production by B.licheniformis MTCC strain 2618Click on the image to see a larger version. doi:10.1152/ajpcel.1988.254.5.G775. 2003;46(1):129–134. Salivary amylase (ptyalin) Amylase is found in saliva and breaks starch into maltose and dextrin. Microorganisms. S2CID 41008169. ^ Machius M, Wiesgand G, Huber R (March 1995). Amylase, amyloglucosidase and related glucanases. [Google Scholar]7. 2000;24(2):299–308. Berzelius (Ms. Esslinger, trans.). Traité de Chimie (Paris, France: Firmin Didot Frères, 1833), vol. Archived from the original on 20 October 2009. Biotech. ^ Ghalanbor Z, Ghaemi N, Marashi SA, Amanlou M, Habibi-Rezaei M, Khajeh K, Ranjbar B (2008). Souza P. Digestive Diseases and Sciences. Technol. 2000;31:298–302. F., Villanuova E., Pia A. 115–170. The first step in the production of high-fructose corn syrup is the treatment of cornstarch with α -amylase, producing shorter chains of sugars oligosaccharides. R. 2010;53(1):35–45. [PubMed] [Google Scholar]3. ^ Aghajari N, Feller G, Gerday C, Haser R (March 1998). Psychoneuroendocrinology. An amylase producing maltotetrose and from maltopentose from B. Molecular Breeding. Method in Enzymology. Anesthesia and Analgesia. Microbiol. Nature Genetics. Salivary amylase is inactivated in the stomach by gastric acid. An α -amylase called "Termamyl", sourced from Bacillus licheniformis, is also used in some detergents, especially dishwashing and starch-removing detergents.[14] See amylase for more uses of the amylase family in general. Ptyalin acts on linear α (1,4) glycosidic linkages, but compound hydrolysis requires an enzyme that acts on branched products. Isolate ANT-6. PMID 9541387. Teodoro C.E., Martins M. 1991;43(8):319–323. 2003;5(1):36–39. Arch. S2CID 7564969. [Google Scholar]4. Part I. Purification & hydrolytic properties. doi:10.1006/jmbi.1994.0106. [Google Scholar]2. [Google Scholar]17. PMID 18289113. In human biology Although found in many tissues, amylase is most prominent in pancreatic juice and saliva, each of which has its own isoform of human α -amylase. ^ Granger DA, Kivilighan KT, el-Sheikh M, Gordis EB, Stroud LR (March 2007). J. Cordeiro C. doi:10.1128/AAC.01998-16. "Molecular structure of a barley alpha-amylase-inhibitor complex: implications for starch binding and catalysis". Protein and Peptide Letters. 2009;41(1):89–91. Takasaki Y. Application of Microbial α -Amylase In Industry—A Review. Retrieved 24 January 2021. PMID 8196040. The number of gene copies correlates with the levels of salivary amylase, as measured by protein blot assays using antibodies to human amylase. doi:10.1213/01.ANE.0000184196.60838.8D. Retrieved 21 November 2009. PMID 7877175. S2CID 22252878. "Structure of human salivary alpha-amylase at 1.6 Å resolution: implications for its role in the oral cavity". Amylase Production on Submerged

Fermentation by *Bacillus* spp. Gonzalvo M. [Google Scholar]22. 20 December 2004. New York: Academic Press Inc; 1980. Archived from the original on 14 October 2016. [^] Rosenblum JL, Irwin CL, Alpers DH (May 1988). C., Martins M. [Google Scholar]19. M., de O. Microbiol. PMC 5488086. C. 7 (3): 564-72. 1994;41(2):155-162. [PubMed] [Google Scholar]14. S2CID 24845837. J., Hebeda R.E., Teague W. Blood should be taken soon after a bout of pancreatitis pain, otherwise it is excreted rapidly by the kidneys. PMID 3652896. "Passage of salivary amylase through the stomach in humans". [Google Scholar]18. R., Silva J. Enzymic properties of an alkaline chelator-resistant amylase from an alkaliphilic *Bacillus* sp. PMC 2143949. Purification & characterization of commercialized, cloned B. isolate L1711. [Google Scholar]11. "Approaches to Dispersing Medical Biofilms". doi:10.1016/j.psyneuen.2009.01.014. Characterization of a new *Bacillus stearothermophilus* isolate: a highly thermostable α -amylase producing strain. L. Luciano A. Interpretation Increased plasma levels in humans are found in: Salivary trauma (including anaesthetic intubation) Mumps – due to inflammation of the salivary glands Pancreatitis – because of damage to the cells that produce amylase Kidney failure – due to reduced excretion Total amylase readings of over 10 times the upper limit of normal (ULN) are suggestive of pancreatitis. [Google Scholar]5. Medical laboratories will usually measure either pancreatic amylase or total amylase. 1983;47(10):2193-2199. Biotechnol. 2003;38(11):1599-1616. "The relationship between salivary biomarkers and state-trait anxiety inventory score under mental arithmetic stress: a pilot study". Amylases: α and β p. Ptyalin added to buffer at pH 3.0 underwent complete inactivation in 120 minutes; however, addition of starch at a 0.1% level resulted in 10% of the activity remaining, and similar addition of starch to a 1.0% level resulted in about 40% of the activity remaining at 120 minutes.[6] Optimum conditions for ptyalin Optimum pH - 7.0[7] Human body temperature[citation needed] Presence of certain anions and activators: Chloride and bromide – most effective Iodide – less effective Sulfate and phosphate – least effective Genetic variation in human salivary amylase Further information: Amylase § Salivary amylase The salivary amylase gene has undergone duplication during evolution, and DNA hybridization studies indicate many individuals have multiple tandem repeats of the gene. Starch/Stärke. "Complexes of *Thermoactinomyces vulgaris* R-47 α -amylase 1 and pullulan model oligosaccharides provide new insight into the mechanism for recognizing substrates with α -1,6 glycosidic linkages". Anto H., Trivedi U., Patel K. 2006;44(2):241-245. Agric. Alpha Amylase Production from Banana Peel. Shaisa, Kokab M., Rehman, Asghar K., Asad M., J., Adedoye O. 7, page 156. "Glycoside Hydrolases Degrade Polymicrobial Bacterial Biofilms in Wounds". The FEBS Journal. 5 (2): 15. doi:10.1007/b01300195. [^] "Amylase, Alpha - Worthington Enzyme Manual". "Transgenic potato plants with enhanced resistance to the tomato moth, *Lacanobia oleracea*: growth room trials". Agri. Salivary α -amylase has been used as a biomarker for stress[9][10] and as a surrogate marker of sympathetic nervous system (SNS) activity[11] that does not require a blood draw. Oliveira A.N., Oliveira L.A., Andrade J.S. Partial Characterization of Amylases of Two Indigenous Central Amazonian Rhizobia strains. doi:10.1006/jmbi.1994.1354. [^] Fleming D, Rumbaugh KP (April 2017). 1. World Journal of Chemistry.

α -Amylase : *Bacillus amyloliquefaciens*: Only α -1,4-oligosaccharide links are cleaved to give a-dextrins and predominantly maltose (G2), G3, G6, and G7 oligosaccharides: *B. licheniformis*: Only α -1,4-oligosaccharide links are cleaved to give a-dextrins and predominantly maltose, G3, G4, and G5 oligosaccharides: *Aspergillus oryzae*, *A. niger* Malt (α -amylase and β -amylase) from barley to hydrolyze starch. \$184.1583: Pancreatin (peptide hydrolase) from porcine or bovine pancreatic tissue used to hydrolyze proteins or polypeptides ... Thermostable α -amylases hold a very important place in commercial industrial applications in Sri Lanka. Therefore, the main aim of this study was to identify superior *Bacillus* strain and optimize growth conditions that could yield high α -amylase production. Three *Bacillus* strains, *B. amyloliquefaciens* ATCC 23350, *B. licheniformis* ATCC 14580 and *B. megaterium* ATCC 14581 ... *Bacillus subtilis*, known also as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium, found in soil and the gastrointestinal tract of ruminants, humans and marine sponges. As a member of the genus *Bacillus*, *B. subtilis* is rod-shaped, and can form a tough, protective endospore, allowing it to tolerate extreme environmental conditions. High-efficiency chromosomal integrative amplification strategy for overexpressing α -amylase in *Bacillus licheniformis*. Lonnie O'Neal Ingram (1947-2020) - Dedication and Determination . Inorganic phosphate self-sufficient whole-cell biocatalysts containing two co-expressed phosphorylases facilitate cellobiose production 04/02/2010 · *Bacillus subtilis*, *Bacillus stearothermophilus*, *Bacillus licheniformis* and *Bacillus amyloliquefaciens* are known to be good producers of thermostable α -Amylase. Thermostability is an important characteristic as enzymatic liquefaction and saccharification of starch are performed at high temperatures (100–110°C). Glucose is usually present in solid form as a monohydrate with a closed pyran ring (dextrose hydrate). In aqueous solution, on the other hand, it is an open-chain to a small extent and is present predominantly as α - or β -pyranose, which interconvert.From aqueous solutions, the three known forms can be crystallized: α -glucopyranose, β -glucopyranose and β -glucopyranose ... High-efficiency chromosomal integrative amplification strategy for overexpressing α -amylase in *Bacillus licheniformis*. Lonnie O'Neal Ingram (1947-2020) - Dedication and Determination . Inorganic phosphate self-sufficient whole-cell biocatalysts containing two co-expressed phosphorylases facilitate cellobiose production



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