HAYVONLARDAN AJRATILGAN IZOLYATLAR VA *B.SUBTILIS* SHTAMMLLARI B-GLYUKOZIDAZA GENLARINING PCR AMPLIKATSIYASI

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Annotatsiya: *β-glyukozidaza fermenti sellyuloza va boshqa glikanlarni parchalaydigan muhim fermentlardan biridir. Ushbu fermentning asosiy vazifasi β-1,4-glikozid bog‘larini gidroliz qilish orqali glyukoza molekulalarini ajratib olishdir. β-glyukozidaza fermenti ko‘plab mikroorganizmlar, shu jumladan bakteriyalar va zamburug‘lar tomonidan sintezlanadi. Ushbu fermentning genetik asoslarini o‘rganish, biotexnologiya va sanoat sohalarida katta ahamiyatga ega bo‘lib, sellyuloza asosidagi biomassa konversiyasini optimallashtirish imkonini beradi. Tadqiqotimizda, Enterococcus faecium, Chryseobacterium anthropi, Proteus vulgaris, Alcaligenes faecalis va Bacillus subtilis ning 7 ta shtammlarida β-glyukozidaza fermenti genining mavjudligi aniqlangan va ushbu genning PCR amplifikatsiyasi orqali tasdiqlangan.*

Kalit so‘zlar: *B. subtilis, E.faecium, Ch.anthropi, β-glyukozidaza, sellyuloza, β-1,4-glyukozid, PCR, marker.*

Аннотация: *Фермент β-глюкозидаза является одним из важных ферментов, расщепляющих целлюлозу и другие гликаны. Основная функция этого фермента – выделение молекул глюкозы путем гидролиза β-1,4-гликозидных связей. Фермент β-глюкозидаза синтезируется многими микроорганизмами, включая бактерии и грибы. Исследование генетической основы этого фермента имеет большое значение в области биотехнологии и промышленности и позволяет оптимизировать переработку биомассы на основе целлюлозы. В нашем исследовании наличие гена фермента β-глюкозидазы было обнаружено у 7 штаммов Bacillus subtilis, Enterococcus faecium, Chryseobacterium anthropi, Proteus vulgaris и Alcaligenes faecalis и подтверждено методом ПЦР-амплификации этого гена.*

Ключевые слова: *B. subtilis, E. faecium, Ch. anthropi, β-глюкозидаза, целлюлоза, β-1,4-глюкозид, ПЦР, маркер.*

Abstract: *The β-glucosidase enzyme is one of the important enzymes that breaks down cellulose and other glycans. The main function of this enzyme is to isolate glucose molecules by hydrolyzing β-1,4-glycosidic bonds. β-glucosidase enzyme is synthesized by many microorganisms, including bacteria and fungi. The study of the genetic basis of this enzyme is of great importance in the fields of biotechnology and industry, and allows to optimize the conversion of cellulose-based biomass. In our study, the presence of β-glucosidase enzyme gene was detected in 7 strains of Bacillus subtilis, Enterococcus faecium, Chryseobacterium anthropi, Proteus vulgaris and Alcaligenes faecalis by PCR amplification of this gene.*

Keywords: *B. subtilis, E. faecium, Ch. anthropi, β-glucosidase, cellulose, β-1,4-glucoside, PCR, marker.*

Sellyuloza parchalanuvchi organizmlar uchta fermentdan iborat ko‘p fermentli kompleksni o‘z ichiga olgan: ekzoglyukanaza (selobiogidrolazalar), endoglyukanaza va β-glyukozidaza. Sellyulozaning to‘liq gidrolizi uchun bosqichma-bosqich ta’sir ko‘rsatadi [1]. Ushbu fermentativ tizimda β-glyukozidaza hal qiluvchi rol o‘ynaydi, bunda ular sellyuloza gidrolizining oraliq mahsuloti bo‘lgan sellobiozani glyukozaga aylantirish orqali gidrolizning oxirgi bosqichini yakunlaydi [2]. Kampos va sigir go‘ngidan ajratilgan *B. subtilis RA10* shtammida β-glyukozidaza geni kuchaytirilib, o‘sish sharoitlari optimallashtirilganda va b-glyukozidaza fermenti ishlab chiqarilishi 19,8 barobarga oshirilgan. *B. subtilis RA10* dan olingan β-glyukozidaza faolligi o‘rganilgan, 80 °C haroratda faolligi 78% ni, 50 °C haroratda 48 soat inkubatsiyadan keyin ferment faolligining 68,32% gacha saqlab qolgan [3]. *Enterococcus faecalis (EF85)* va *E. faecium (EF83)* shtammlari makkajo‘xori o‘simligini silos bostirishda foydalanilganda sut kislota miqdori sirka kislotaga qaraganda sezilarli darajada oshganligi aniqlangan [4]. *Pediococcus acidilactici PA204* shatammining sellyulozani parchalashi va sut kislota hosil qilish qobiliyati o‘rganilgan. Tadqiqotda 5 L bioreaktorda fermentatsiya mahsuldorligi 92,01 g/L, 0,77 g/g ni tashlil qilganligi qayd etilgan [5].

Ushbu tadqiqodning maqsadi, tut ipak qurti *(Bombyx mori)* va ot *(Equus caballus*) ovqat hazim qilish sistemasidan ajratib olingan, bakteriya izolyatlarining sellyulozani gidrolizida qatnashadigan ferment genini aniqlashdan iborat. Tadqiqotda hayvonlardan ajratib olingan *Bacillus subtilis, Enterococcus faecium*, *Chryseobacterium anthropi*, *Proteus vulgaris*, *Alcaligenes faecalis* va β-glyukozidaza (bglP) praymerlaridan foydalanildi.

Ozuqa muhiti tarkibi: Standart peptonli ozuqa muhiti (PB) (g/l): Pepton-10 g/l; glyukoza- 6 g/l; NaCl- 5 g/l; K_2HPO_4 – 0,5 g/l; $MgSO_4$-0,2 g/l, (pH-7,0). Lizogenli bulon (LB): trypton 10g/l, yeast extract 5g/l, NaCl 5g/l, (pH 6,8-7,2). De Man–Rogosa–Sharpe agar (MRS): pepton-1,0%, mol go‘shti ekstrakti-1,0%, xamirturush ekstrakti-0,4%, glyukoza-2,0%, natriy asetat uchgidrat-0,5%, polisorbat 80 – 0,1% (Tween 80), kaliy gidrofosfat-0,2%, triammoniy sitrat-0,2%, $MgSO_4 \cdot 7H_2O$ -0,02%, $MnSO_4 \cdot 4H_2O$-0,005%, agar-1,0% (pH – 6,2) [6].

Hayvonlardan ajratib olingan izolyatlar PB agarli ozuqa muhitida (sterilizatsiya 121 ^oC 20 daqiqa) 24 soat davomida 37 ^oC haroratda o‘stirilib, MALDI-TOF bilan idintifiatsiya qilindi (1-jadval). Bakteriya shtammlari suyuq ozuqa muhitida o‘stirildi va epindoflarga 1.5 ml dan olinib 1400 rpm/min, 10 daqiqa davomida sentrifuga qilib cho‘ktirildi. Lizis buffer, Proteinaza, RNaza, Fenol: Xloroforim: izoamil spirti (25:24:1), isopropanol, Etanol 96% ushbu ketma-ketlikda genom DNK ajratildi [7]. TE buferida eritilgan DNK namunalarining konsentratsiyasi Implen NanoPhotometer® yordamida o‘lchandi (2-jadval).

1-jadval

Ajratib olingan izolyatlarning MALDI-TOF yordamida idintifikatsiyasi

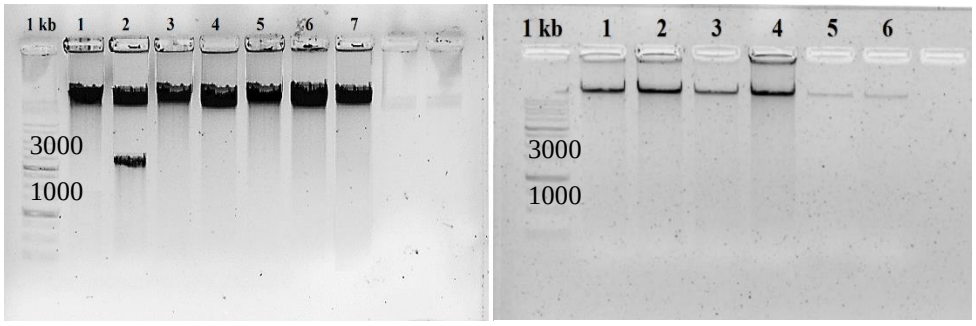
Spot	Ajratib olingan manba		Organizm	Score
A1	Ot so‘lagi		*Enterococcus faecium*	1.80
A2	Tut ipak qurti lichinkasi		*Chryseobacterium anthropi*	1.75
A3	Tuzlangan karam		*Pediococcus acidilactici*	1.78
A4	Ot ichagi		*Proteus vulgaris*	2.40
A5	Ot oshqozoni		*Alcaligenes faecalis*	2.20
A6	Ot ingichka ichagi		*Alcaligenes faecalis*	2.33

2-jadval

Hayvonlardan ajratib olingan izolyatlar va *B.subtilis* shtammlarining nuklein kislotalar konsentratsiyasi

№	Namunalar	DNK Kons	Ed	№	Namunalar	DNK Kons	Ed
1.	TE buferi	0,000	ng/ul	**8**	*B.subtilis ot 1*	271,20	ng/ul
2.	*E.faecium*	69,00	ng/ul	**9**	*B.subtilis ot 2*	207,40	ng/ul
3.	*Ch.anthropi*	61,45	ng/ul	**10**	*B.subtilis ot 3*	272,70	ng/ul
4.	*P.acidilactici*	30,65	ng/ul	**11**	*B.subtilis ot 4*	91,10	ng/ul
5.	*P.vulgaris*	102,75	ng/ul	**12**	*B.subtilis ot 5*	290,90	ng/ul
6.	*A.faecalis*	475,85	ng/ul	**13**	*B.subtilis ot 6*	204,40	ng/ul
7.	*A.faecalis*	7,15	ng/ul	**14**	*B.subtilis ot 7*	150,0	ng/ul

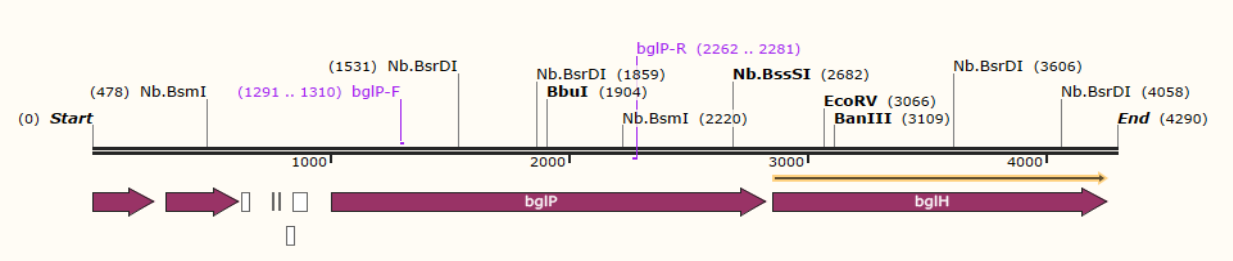
Etidum bromid bilan bo‘yalgan 0.8% li agaroza gelida DNK namunalari elektroforez (Bio-rad, USA) qilindi (1-rasm). Ushbu rasmdan shuni ko‘rishimiz mumkinki, *B.subtilis ot 2* shtammida genom DNK dan tashqari tahminan 3000 juft nukleotidli halqasimon plazmid ham ajralgan.



A **B**

1-rasm. A) *B.subtilis* ning 7 ta shtammlari, B) 1) *E.faecium*, 2) *Ch.anthropi*, 3) *P.acidilactici*, 4)*P.vulgaris*, 5) *A.faecalis*, 6) *A.faecalis* genom DNKsi 1kb markerda ko‘rinishi.

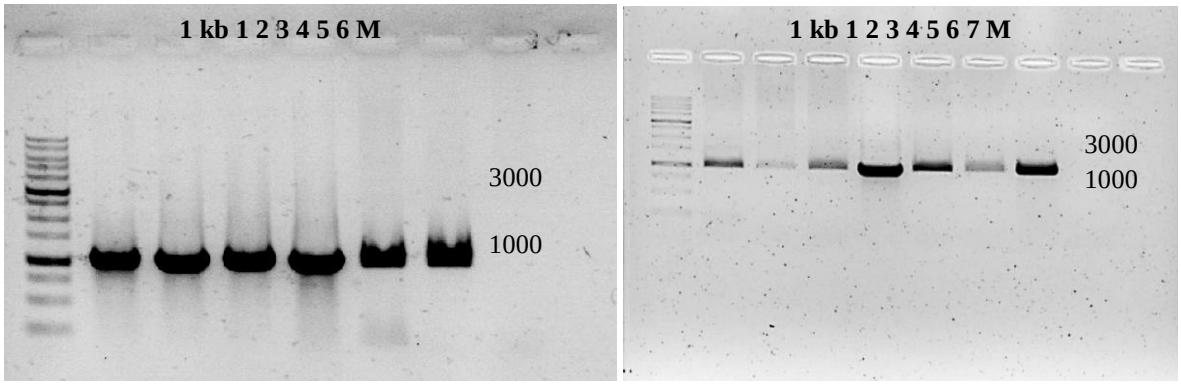
Sellyulozani parchalashda qatnashadigan β-glyukozidaza genlariga praymer dizayn qilindi (2-rasm). Ushbu praymer Integrated DNA Technologies (IDT) firmasi tomonidan sintez qilindi [8].



2-rasm. β-glyukozidaza (4290 bp) genida dizayn qilingan praymerlarning joylashgan o‘rni

Praymer	Sekvinslangan nukleotidlar	Gen nomi	PCR mahsuloti o‘lchami
bglP-F	5′ TGC TGA GTG CGG TCT TTG AT 3′	β-glyukozidaza	991 bp
bglP-R	5′ AGG CCG ATCA TCG CGT AAA T 3′		

GenPak® PCR MasterMix yordamida ajratib olingan DNK namunalariga PCR amplifikatsiyasi amalga oshirildi. Har bir PCR reaksiyasi 20 mkl hajmda tayyorlandi, bunda 10 mkl masterMix, 8.2 mkl distillangan suv (dis H2O), 0.4 mkl Forward praymer (Praymer-F), 0.4 mkl Reverse praymer (Praymer-R) va 1 mkl DNK namunasi ishlatildi. Denaturatsiya 94°C da 5 daqiqa, 58°C da 50 soniya, va 72°C da 2 daqiqa davomida 30 sikl bajarildi. Amplifikatsiyadan so‘ng, PCR mahsulotlari 0.8% li agaroza gelida ko‘rildi. (2-rasm).



A **B**

4-rasm. β-glyukozidaza genning PCR amplikatsiyasi:
A) Otlardan ajratilgan izolyatlar **B)** *B.subtilis*ning 7 ta shtammlari

Tadqiqot natijalari shuni ko‘rsatdiki, *E.faecium, Ch.anthropi, P.acidilactici, P.vulgaris*, va *A.faecalis* shtammlarida sellyulozani parchalashda qatnashadigan β-glyukozidaza fermenti geni mavjud. Ushbu gen uchun dizayn qilingan

bglP paymeri 991 bp uzunlikda bo‘lib, PCR amplifikatsiya mahsuloti ham aynan shu o‘lchamda ekanligi aniqlandi. Tajribada olingan natijalar asosida, turli manbalardan ajratib olingan bakteriyalarning sellyulozani parchalashda ishtirok etuvchi β-glyukozidaza genlarining PCR amplifikatsiyasi usuli samarali skrining imkoniyatlarini yaratadi.

Foydalanilgan adabiyotlar ro‘yxati:

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