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Mengturayev Jumanazar Chori o‘g‘li
(mengtorayevjumanazar90@gmail.com)
Magistr, TIQXMMI Milliy Tadqiqot Universiteti, Toshkent.
Sayidova Ra’no Bahodir qizi
sayidrano@gmail.com
Magistr, TIQXMMI Milliy Tadqiqot Universiteti, Toshkent.
Turdaliyev Dilmurodjon Burxonjon o‘g‘li
(dilmurodjonturdaliyev6@gmail.com)
PhD talabasi, “TIIAME” Milliy tadqiqot universiteti,
Fundamental va amaliy tadqiqotlar instituti,Toshkent.

O‘ZBEKISTON FARMASEVTIKA VA OZIQ-OVQAT SANOATIDA QO‘LLANISHI MUMKIN BO‘LGAN REKOMBINANT BETA-GALOKTOSIDAZA OQSILINI BAKULAVIRUS EKSPRESSIYA TIZIMI ASOSIDA OLISH

Annotatsiya

Spodoptera frugiperda hasharot hujayralari ekspressiya tizimidan foydalangan holda β-galaktozidaza (EC 3.2.1.23) fermentini kodlovchi yangi rekombinant bakulovirusni olish maqsad qilindi. Ushbu yondashuv rekombinant bakuloviruslar yordamida yuqori faol β-galaktozidaza olish imkoniyatlarini baholash, shuningdek farmatsevtika va sut sanoati uchun mahalliy ferment preparatlari ishlab chiqarishga xizmat qiluvchi biotexnologik platforma yaratishga qaratildi. pBacPAK8 transfer vektori asosida yangi rekombinant plazmid konstruksiyalandi, Escherichia colihujayralarida klonlandi va Sf9 hujayralarida Autographa californica multiple nucleopolyhedrovirus genom DNKsi bilan ko-transfeksiya orqali rekombinant bakulovirus olindi. Sf9 hujayralarida lacZ genining samarali ekspressiyasi kuzatildi va ekspressiyalangan β-galaktozidaza oqsilining fermentativ faolligi ONPG testi yordamida tasdiqlandi. Olingan natijalar Sf9 hasharot hujayralari asosidagi bakulovirus ekspressiya tizimi (BEVS)

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farmatsevtika va oziq-ovqat sanoati uchun mos rekombinant β-galaktosidaza fermentini mahalliy sharoitda ishlab chiqarishda istiqbolli platforma ekanini ko‘rsatdi.

Kalit so‘z: *Rekombinant oqsil, β-galaktozidaza, bakulovirus, ekspressiya tizimi, Spodoptera frugiperda (Sf9), ONPG testi.*

Abstract

The aim of this study was to obtain a novel recombinant baculovirus encoding β-galactosidase (EC 3.2.1.23) using the *Spodoptera frugiperda* insect cell expression system. This approach was focused on evaluating the potential of producing high-activity β-galactosidase through recombinant baculoviruses, as well as on developing a biotechnological platform for the local production of enzyme preparations applicable to the pharmaceutical and dairy industries.

A new recombinant plasmid was constructed based on the pBacPAK8 transfer vector, cloned in Escherichia colicells, and a recombinant baculovirus was generated via co-transfection with Autographa californica multiple nucleopolyhedrovirus genomic DNA in Sf9 cells. Efficient expression of the lacZ gene was observed in Sf9 insect cells, and the enzymatic activity of the expressed β-galactosidase protein was confirmed using the ONPG assay.

The obtained results demonstrate that the Sf9 insect cell-based baculovirus expression vector system (BEVS) represents a promising platform for the local production of recombinant β-galactosidase suitable for potential applications in the pharmaceutical and food industries.

Keywords: Recombinant protein, β-galactosidase, baculovirus, expression system, *Spodoptera frugiperda* (Sf9), ONPG assay

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Аннотация

Целью данного исследования было получение нового рекомбинантного бакуловируса, кодирующего β-галактозидазу (EC 3.2.1.23), с использованием системы экспрессии на клетках насекомых *Spodoptera frugiperda*. Данный подход был направлен на оценку возможностей получения высокоактивной β-галактозидазы с применением рекомбинантных бакуловирусов, а также на разработку биотехнологической платформы для локального производства ферментных препаратов, предназначенных для фармацевтической и молочной промышленности.

На основе трансфер-вектора pBacPAK8 был сконструирован новый рекомбинантный плазмид, клонированный в клетках *Escherichia coli*, после чего рекомбинантный бакуловирус был получен путем ко-трансфекции плазмидной ДНК с геномной ДНК *Autographa californica* multiple nucleopolyhedrovirus в клетках Sf9. В клетках Sf9 наблюдалась эффективная экспрессия гена *lacZ*, а ферментативная активность экспрессированного белка β-галактозидазы была подтверждена с использованием ONPG-теста.

Полученные результаты показали, что система экспрессии бакуловируса на основе клеток Sf9 (BEVS) является перспективной платформой для локального получения рекомбинантной β-галактозидазы, потенциально пригодной для применения в фармацевтической и пищевой промышленности.

Ключевые слова: Рекомбинантный белок, β-галактозидаза, бакуловирус, система экспрессии, *Spodoptera frugiperda* (Sf9), ONPG-тест.

Kirish

Zamonaviy biotexnologiya sohasida rekombinant fermentlar ishlab chiqarish farmatsevtika va oziq-ovqat sanoatining ustuvor yo‘nalishlaridan biri hisoblanadi. Beta-galaktosidaza (EC 3.2.1.23) laktaza fermenti sut mahsulotlarida laktoza galaktoza va glyukoza monosahharidlariga parchalashda muhim rol o‘ynaydi, bu

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laktoza intolerantsiyasi bo‘lgan aholining ovqatlanishini yaxshilash va sut sanoatida chiqindilarni kamaytirishda qo'llaniladi[1],[2],[3].

O‘zbekiston Respublikasi Prezidentining PF-5847-sonli Oliy ta'lim tizimini 2030-yilgacha rivojlantirish Konsepsiyasi hamda PF-158-sonli "O‘zbekiston-2030" strategiyasi doirasida fan, ta'lim va ishlab chiqarish integratsiyasini ta’minlash orqali farmatsevtika (15.00.00 ixtisosligi) va oziq-ovqat sanoatini importga qaramlikdan xalos etish dolzarb vazifa sifatida belgilangan [4],[5].

O‘zbekiston farmatsevtika va oziq-ovqat sanoatida rekombinant beta-galaktosidaza fermentiga bo‘lgan ehtiyoj yuqori, chunki mahalliy ishlab chiqarish importga bog‘liq va mahalliy sharoitlarga moslashtirilgan fermentlar yetishmaydi [3].

2023-yilda global β-galaktozidaza bozori hajmi 1,025 million AQSh dollariga baholangan bo‘lib, 2031-yilga kelib 1,625 million AQSh dollariga yetishi va o‘rtacha yillik o‘sish sur’ati (CAGR) 6,8% bo‘lishi prognoz qilinmoqda [6].

Shu bilan birgalikda ,R&D Systems (Fisher Scientific) tomonidan taklif etiladigan rekombinant human β-galaktosidaza-1/LACZ oqsili 20 µg uchun 548,50 AQSh dollariga baholangan bo‘lib, CHO hujayralarida ifodalangan, C-terminal 6×His tegi bilan tozalangan va preparatning tozaligi 95% dan yuqori ekanligi ko‘rsatilgan[7]. Bunday yuqori narx va importga qaramlik mahalliy sharoitda arzonroq, funksional teng rekombinant β-galaktosidaza olish texnologiyalarini ishlab chiqishni dolzarb vazifa sifatida belgilaydi.

O‘zbekistonda rekombinant beta galatosidaza oqsilini olishda bakulovirus ekppession tizimida olish boyicha ilmiy amaliy tadqiqotlar yo‘q.

Yuqoridagilarni inobatga olgan holda, ushbu tadqiqotda bakulovirus–hasharot hujayralari ekspressiya tizimidan foydalangan holda β- galaktozidaza fermentini kodlovchi yangi rekombinant bakulovirusni olish maqsad qilindi. Bu yondashuv mahalliy sharoitlarda sanoat ahamiyatiga ega, funksional jihatdan faol rekombinant

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β- galaktozidazani arzonroq va barqaror manba sifatida ishlab chiqarish imkoniyatlarini baholashga qaratildi .

Material va metodlar.

*Hujayra kulturalari: Escherichia coli*NEB-5*a* shtamining elektro komponentli hujayrasi. Molekulyar klonlash uchun rekombinant plazmida. Ampitsillin saqlovchi muhitda saqlandi

Spodoptera frugiperda (Sf9) hujayra kulturasi bir haftada ekib turildi. Hujayralar 26 oC da hashoratlar uchun mo‘ljallangan tarkibida 10% issiqlik ta’sirida inaktivatsiya qilingan buzoq zardobi tutuvchi (Grace tasdiqlaydi’s Insect Cell Culture Medium)ozuqa muhiti.[16]

Plazmid DNK ajratish: Plazmid DNK ajratish: Escherichia coliNEB-5α shtamidan (2 ml overnight kultura, OD_{600}=2-4) pBacPAK8 plazmid DNKsi Qiagen Plasmid Mini Kit bilan ishqoriy lizis usuli bo'yicha ajratildi:
Lizis buferi (P1: 50 mM Tris-HCl pH 8,0; 10 mM EDTA; 100 µg/ml RNase A) – 250 µl .Lizis(P2:0,2M NaOH; 1% SDS) –250µl, 5daqiqa inkubatsiya Neytrallash(P3:3MKOAcpH5,5)–350µl .Supernatantdan (12,000 g, 10 daq) plazmid NanoDrop bilan 200-500 ng/µl aniqlandi [17].

Restriksiya: pBacPAK8 (2 µg) va pBS-Ac-polh-B-Gal (2 µg) EcoRI (20 U)/NotI (20 U) (NEB, 10x buffer CutSmart: 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 µg/mlBSA,pH7,9),37°C,2soat[18].
Ligirlash: Vektor:insert (1:10, 50 ng vektor + 500 ng insert) T4 DNK ligazasi (400 U, NEB 10x buffer: 50 mM Tris-HCl pH 7,5; 10 mM $MgCl_2$; 10 mM DTT; 1 mM ATP; 25 µg/ml PEG 6000), 16°C, 16 soat[19].
Elektrotransformatsiya: Ligirlangan DNK (5 µl) E. coli NEB-5α kompetent hujayralariga elektrotransformatsiya (2,5 kV, 25 µF, 200 Ω, 0,1 sm kletka), SOC muhitda 37°C, 1 soat regeneratsiya, ampitsillin (100 µg/ml) LB agarida selektsiya [20].

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PZR(Polimeraza zanjirli reaksiya:Klonlarni tekshirish uchun koloniyalardan (10 ng DNA) 25 µl PZR reaktsiyasi o‘tkazildi: 1x DreamTaq buffer (Thermo), 0,2 mM dNTPs, 0,4 µM lacZ primerlari (F: 5’-GAATTCGAGCTCGGTACCC-3’, R: 5’-GCGGCCGCTCTAGAA-3’), 1 U DreamTaq polimeraza, 95°C-3 daq; 30 sikl (95°C-30 s, 55°C-30 s, 72°C-1 daq); 72°C-5 daq. Amplifikatlar 3% agaroza gelida (100 V, 45 daq) tahlil qilindi [21].

AcMNPV DNK ajratish: Sf9 hujayralaridan AcMNPV virus DNKsi CsCl gradient sentrifugalash (41,000 rpm, 24 soat, Beckman Optima XL-90) bilan ajratildi (1,55-1,65 g/ml), etanol cho‘ktirish (2,5 hajm, -20°C, 2 soat), NanoDrop (1,2-2 µg/µl) bilan aniqlandi [22].

Ko-transfeksiya: Sf9 hujayralari (6×10^5 hujayra/ml, 24-saatlik monolar) ga pBacPAK8-B-Gal (2 µg) + AcMNPV DNK (1 µg) ExGen 500 (Fermentas; 6,6-11 µl, 6-10 ekvivalentlar) yoki MetaFectene PRO (Biontex; 3 µl) bilan ko-transfeksiya qilindi (TC-100 muhit, 27°C, 72 soat), GFP markeri bilan monitoring qilindi

Rekombinant virus tozalash:rSf-polh-B-Gal klonlari plaque assay (Sf9 monolarida 10^{-1}-10^{-8} suyultirish, agar overlay) va $TCID_{50}$ (96-qatorli plitalarda, Reed-Muench hisobi) bilan tozalandi (titr: 10^8-10^9 pfu/ml)[23].

Olingan rekombinant oqsilning fermentativ faolligini baholash: Rekombinant bakuloviruslarni tozalash va virus miqdorini aniqlash uchun *Plaque assay* hamda *$TCID_{50}$* limitlovchi suyultirish usullaridan foydalanildi.Olingan rekombinant oqsilning fermentativ faolligini baholash va funksional xususiyatlarini tavsiflash maqsadida ONPG asosidagi β- galaktozidaza faolligi testi qo‘llanildi [24].

Hujayra lizati tarkibidan ajratib olingan oqsillarva solishtirish uchun tayyorlangan salbiy namuna - oqsillarning miqdorlari Lowry metodi yordamida aniqlandi [8]. Salbiy namuna sifatida *Spodoptera frugiperda* (Sf9) hujayralarini

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zararlagan AcMNPV bakulovirusidan ajratib olingan umumiy oqsil xizmat qildi. Z buffer (60 mM $Na_2HPO_4 \cdot 7H_2O$, 40 mM $NaH_2PO_4 \cdot H_2O$, 10 mM KCl, 1 mM $MgSO_4 \cdot 7H_2O$, 50 mM β- merkaptoetanol, pH 7,0), ONPG eritmasi (4 mg/ml, 100 mM natriy fosfatli bufferda, pH 7,5) va natriy karbonat eritmasi (1 M Na_2CO_3) 28 °C haroratga keltirildi. Ajratib olingan oqsildan 20 mkl miqdorda olinib, 1 ml Z buffer ga qo‘shildi va harorati 28 °C ga keltirildi. Reaksiyani initsiatsiya qilish uchun har bir namunaga 4 mg/ml kontsentratsiyali ONPG eritmasidan 0.2 ml qo‘shildi. Reaksiya och sariq rang hosil bo‘lguncha davom ettirildi (10 min dan oshmasligi kerak). Reaksiyani to‘xtatish uchun har bir namunaga 0.5 ml 1 M natriy karbonat eritmasidan qo‘shildi. Har bir namunaning optik zichligi spektrofotometrik usulda 420 nm to‘lqin uzunligida (OD_{420}) o‘lchandi. Ushbu tajribaga muvofiq sintezlangan β -galaktozidaza fermentining faolligi quyidagi formula yordamida hisoblanadi: β-galaktozidaza fermenti/mg umumiy oqsil = OD_{420} x 380 / min. x mg reaksiyadagi oqsil miqdori.

Natijalar

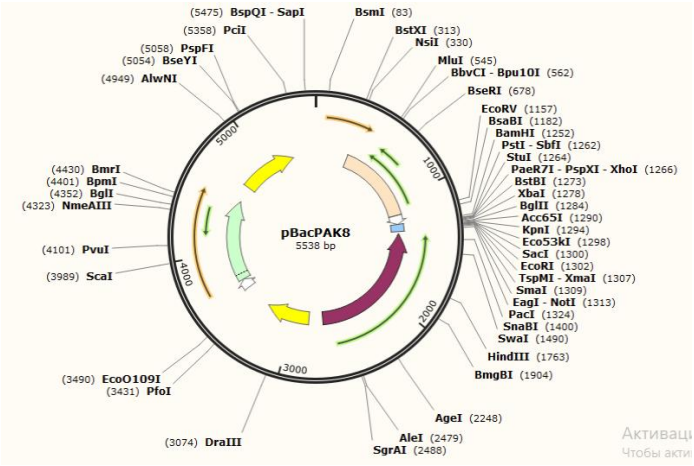
Yangi pBacPAK8-B-Gal- B-Gal rekombinant DNK ni klonlash uchun *Spodoptera frugiperda* yadroviy poledrozi virusi asosidagi pBacPAK8 transfer vektori qo‘llanildi.

“β-galaktozidaza” fermentini kodlovchi nuklein kislotalar ketma- ketligini pBacPAK8 transfer vektoriga kiritish uchun pBacPAK-8 plazmidasi ma’lum restriksion saytlardan - EcoRI va NotI restriktaza fermentlari orqali qirqildi.

Bu jarayondan so‘ng plazmida chiziqsimon ko‘rinishga keladi va yopishqoq uchlar xosil qiladi.

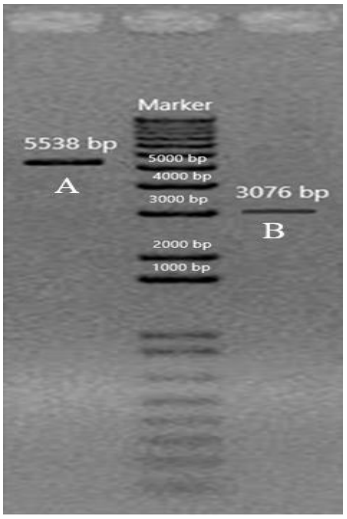
EcoRI: 5' -G↓AATTC-3'
3' CTTAA↓G-5'
NotI: 5' -GC↓GGCCGC-3'
3' CGCCGG↓CG-5'

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1-rasm. Bakulovirus AcMNPV uchun mo‘ljallangan pBacPAK8 vektorining xalqasimon ko‘rinishi [9].

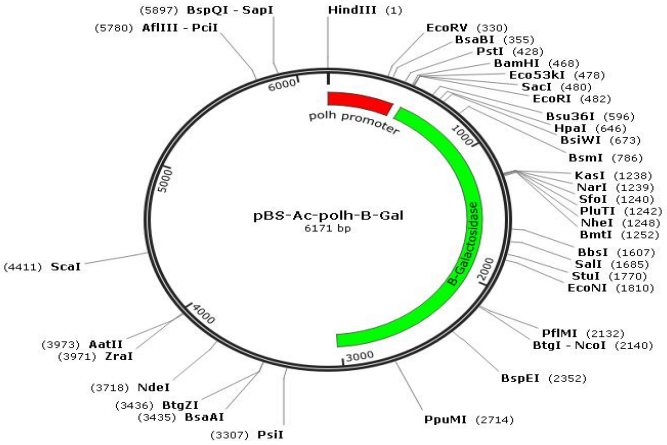
pBacPAK8 transfer EcoRI restriktaza fermenti bilan ishlov berilgandan so‘ng, plazmidaning 5538 j.n. chiziqsimon shakli hosil bo‘ldi. Restriksiya tahlil natijalari 0.8% li agaroza gelida ko‘rildi.



2-rasm. rBacPAK8 transfer vektorining restriksiya jarayonidan keyingi 0.8% gel-elektroforez tahlil natijalari.

A- pBacPAK8 transfer vektorining EcoRI restriktaza fermenti bilan ishlov berilgandan so‘ng chiziqsimon shakli; B- pBacPAK8 transfer vektorining halqasimon shakli.

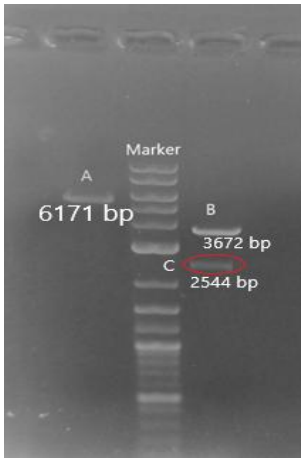
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3-rasm pBS-Ac-polh-B-Gal vektorining xalqasimon fizik xaritasi

“β-galaktozidaza” genini tayyorlash bosqichida, shu genni tutuvchi *Autograhpa californica* (Kaliforniya tullami) multinukleopoliedroz virusi uchun mo‘ljallangan pBS-Ac-polh-B-Gal plazmid DNK xizmat qilib [10], bizning *Spodoptera frugiperda* ekspressiya tizimiga mo‘ljallangan pBacPAK8 transfer vektoriga komplementarlikni ta’minlash maqsadida EcoRI restriktaza fermenti bilan ishlov berildi va yopishqoq uchlar hosil qilindi .

Restriktsiya natijasida pBS-Ac-polh-B-Gal plazmidasining ikki xil o‘lchamli DNK fragmentlari hosil bo‘ldi. Tahlil natijalarini 0.8% li agaroza gelida ko‘rildi.



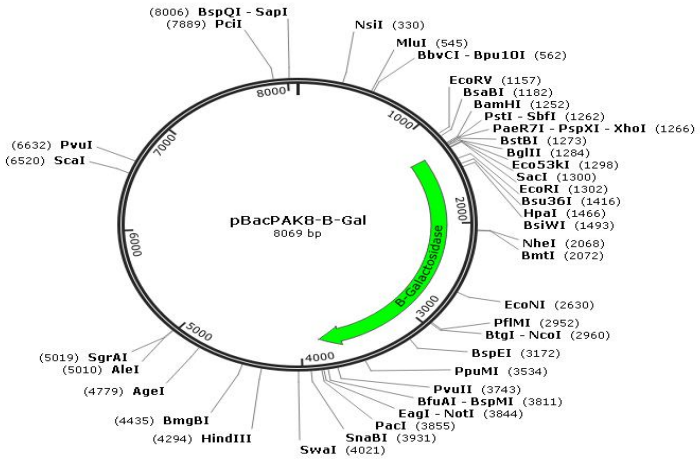
4-rasm pBS-Ac-polh-B-Gal plazmidasiga ferment bilan ishlov berilgandan keyingi gel-elektroforez tahlili.

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Beta-galoktosidaza genini tutuvchi Bunda; A-pBS-Ac-polh-B-Gal plazmidasining chiziqsimon shakli. B va C pBS-Ac-polh-B-Gal plazmidasiga restriktaza fermenti bilan ishlov berilgandan keyingi pBs-Ac-polh va B-Gal DNK fragmentlari.

Plazmidaning DNK fragmentini elektroforezdan so‘ng 0,7% agarozali geldan ajratib olindi va tayyorlangan vektor bilan 1:10 (vektor: gen) nisbatda ligirlandi, bunda T_4 bakteriofagining DNK ligazasidan foydalanildi.

Ligirlash jarayonidan keyin o‘zida kerakli genni saqlagan rekombinant pBacPAK8-B-Gal plazmidasi olindi.

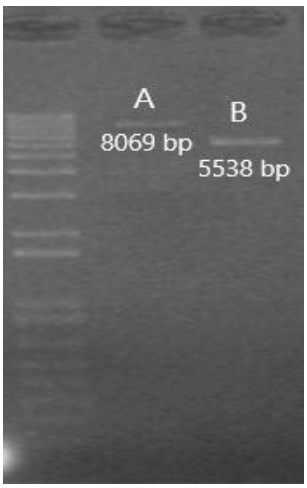


5-rasm. pBacPAK8-B-Gal plazmidasining genetik kartasi.

Keyin esa ligazali aralashma bilan *Escherichia coli*NEB-5*a* shtammining kompetent hujayralariga transformatsiyalandi.

E. coli hujayralari ampitsillinli ozuqa muhitda o‘sa olmaydi Klonlashtirilgan rekombinant plazmidalar esa ampitsillinga chidamli gen tutib, ular *E. coli* hujayralariga kiritilgandan so‘ng petri likopchalarida faqat transformatsiyaga uchragan klonlargina o‘sib chiqadi. Shuning uchun klonlashtirilgan hujayralar ampitsillinli (100mkg/ml) ozuqa muhitda o‘stirildi. Olingan klonlar skriningi elektroforez usuli yordamida tahlil qilindi.

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6-rasm. pBacPAK8 va pBacPAK8-polh- B-Gal plazmidalarining chiziqsimon holdagi holdagi 0.8 % gel-elektroforez tahlil natijalari.

Bunda A-fragment pBacPAK8-B-Gal-B-Gal rekombinant plazmidasi, B-fragment pBacPAK8-B-Gal-MIS vektori. Rasmdan tasdiqlaydiki, pBacPAK8 plazmidasiga (5538) B-Gal genini (2544) klonlaganimizdan keyin hosil bo‘lgan namuna 8069 j.n. molekulyar o‘lchamni tashkil etgan. Shunday qilib, elektroforez tahlil natijasiga ko‘ra pBacPAK8-B-Gal- B-Gal maqsadli B-Gal genini saqlaydi.

Tadqiqotning keyingi bosqichida AcMNPV yadroviy poliedroz virusi va rekombinant pBacPAK8-B-Gal-B-Gal plazmida DNK sini *Spodoptera frugiperda* (Sf9) hujayralariga ko-transfeksiyalash amalga oshirildi.

An’anaviy tarzda maqsadli oqsilni kodlovchi begona DNK, virus replikatsiyasi uchun unchalik ahamiyatli bo‘lmagan genning o‘rniga transfer vektori yordamida o‘rnini almashtirish bilan kiritiladi [11]. Bizning olib borgan tadqiqotlarimizda, bakulovirus replikatsiyasi uchun unchalik muhim bo‘lmagan poledrin (polh) geni o‘chirildi. Poledrin geni B-Gal geniga almashtirildi.

pBacPAK8-B-Gal plazmidli DNKsi va AcMNPV virusi genomi DNKsi orasidagi gomologik rekombinatsiya *Spodoptera frugiperda*hujayra linyasida *in vitro* usulida amalga oshirildi. Buning uchun hujayralar DNK transferli vektori va Sf9 turi DNKsi bilan ko-transfeksiyalandi.

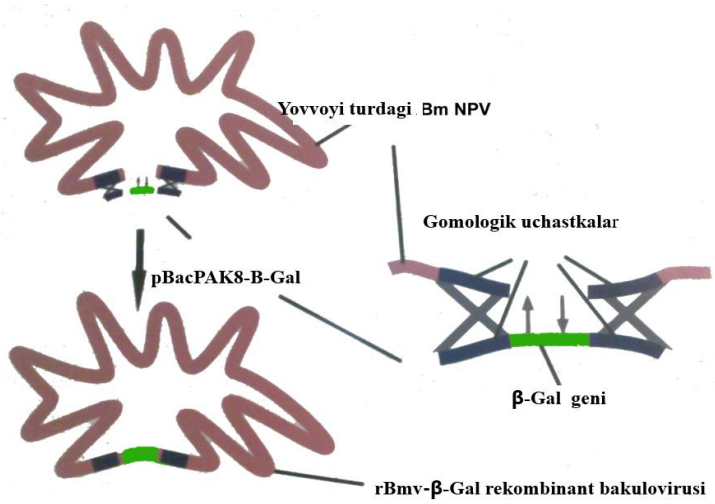
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Spodoptera frugiperda Sf9 hujayra linyasi kotransfeksiyasi ExGen500^{TM} (Fermentes Inc.) polietilenaminli transfeksion reagenti yordamida amalga oshirildi.

Transfeksiyaning har bir usuli uchun 2 mkg pBacPAK8-B-Gal plazmidli DNKsi, yo *Spodoptera frugiperda* hujara virusi DNKsi 1 mkg va bir xil miqdordagi hujayralardan ($6x10^4$ hujayra/ml) foydalanildi.

ExGen500 reagenti yordamidagi transfeksiyada ExGEN500 ning 3 ta turli miqdoridan foydalanildi, xususan 6,8 va 10 ekvivalentda, bu esa reagentning 6,6; 8,8 va 11,0 mklga to‘g‘ri keladi. Shunday qilib, 6,6 mkl 11 mkl miqdorgacha politilenaminli reagent yordamida Sf9 hujayralari transfeksiyasi natijasida ExGen 500 miqdoriga to‘g‘ri proporsional bo‘lgan transfeksiya jarayoni samaradorligining ortganligi aniqlandi.

Olingan natijalarga ko‘ra 3.0 mkl DNK uchun transfeksiyaning eng yuqori samaradorligi 10 eq ExGen 500 reagenti miqdorda foydalanilganda kuzatildi.



7-rasm. Gomologik rekombinatsiya chizmasi

Olib borilgan izlanishlar natijasida *Spodoptera frugiperda* Sf9 hasharot hujayra linyasida replikatsiyalanuvchi va *in vitro* B-Gal begona genini ekspressiyalovchi rekombinant bakulovirus rSf-polh- B-Gal olindi.

B-Gal genini tutuvchi rekombinant bakuloviruslarni tozalash va

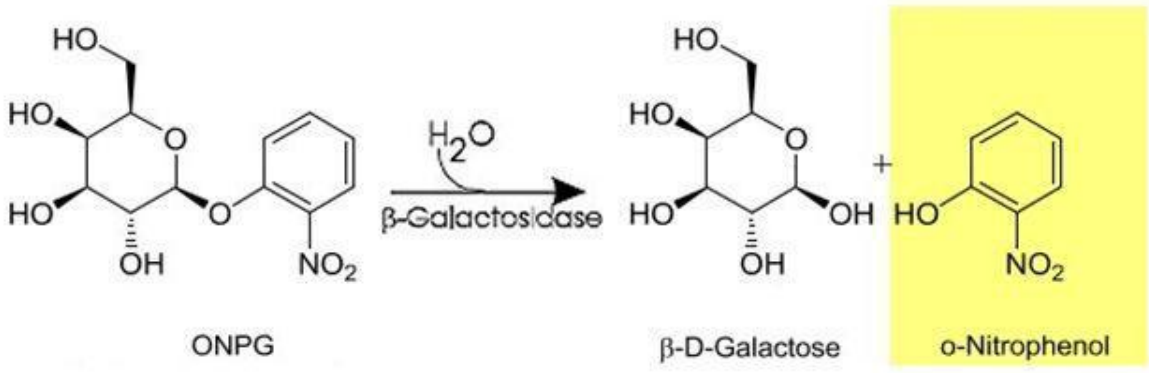
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identifikatsiya qilish uchun *Plaque assay* va $TCID_{50}$ limitlovchi suyultirish usulidan foydalanildi [12]. Shunday qilib, bu jarayonni juda ko‘p qaytarish natijasida maqsadli genni ekspressiyalovchi eng yuqori miqdordagi virus saqlovchi koloniyalar tozalandi.

Tadqiqotlarimizni keyingi bosqichida ilmiy adabiyotlarda keltirilgan ma’lumotlardan foydalangan holda rekombinant rSf-polh- B-Gal bakulovirus bilan zararlangan *Spodoptera frugiperda* hujayra liniyalarini 10% li buzoq embrion zardobi (Fetal Bovine Serum, HiMedia Laboratories Pvt.Ltd) saqlovchi Graces ozuqa muhitida +26° C harorat ostida 7 kun davomida o‘stirishdan boshladik [13],[14]

Bundan ko‘zlangan maqsad, *Spodoptera frugiperda* hujayra liniyalari kerakli β-galaktozidaza fermentini sintezlovchi rekombinant rSf-polh-B-Gal bakulovirusni ekspressiyalashdan iborat.

Spodoptera frugiperda hujayralarida β-galaktozidaza fermentini sintezlovchi rSf-β-Gal rekombinant bakulovirusning seleksiyasi PZR usulidan tashqari, ekspressiyalangan oqsilning fermentativ faolligini ONPG Assay uslubi yordamida tasdiqlash orqali amalga oshirildi *(testni amalga oshirish tartibi tajriba bo‘limida keltirilgan*) .



8-rasm. ONPG testi.

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Yuqoridagi tajribalarimizdan ekspressiyalangan oqsilning fermentativ faolligi o‘rganildi, bunda ONPG – ortonitrofenil- β-D-galaktopiranozid, β-galaktozidaza ta’sirida parchalananib β-D-galaktoza va sariq rangga bo‘yaluvchi O-nitrofenol hosil bo‘ldi.

8-rasmdan tasdiqlaydiki, *Spodoptera frugiperda* hujayra kulturalarida ekspressiya qilingan rekombinant sifatli ONPG testi yordamida tasdiqlandi

Muhokama.

Olingan natijalar Sf9 hasharot hujayralari asosidagi bakulovirus ekspressiya tizimi (BEVS) ning O‘zbekiston farmatsevtika (15.00.00 ixtisosligi) va oziq-ovqat sanoati uchun rekombinant β-galaktozidaza fermentini ishlab chiqarishda yuqori samaradorlikka ega ekanligini isbotladi. Ushbu tadqiqotda pBacPAK8 transfer vektoriga β-galaktozidaza genini muvaffaqiyatli klonlash (6-rasm: 8069 j.n.), E. coli NEB-5α shtamida amplifikatsiya qilish va AcMNPV genom DNKsi bilan ko-transfeksiya orqali rSf-polh-B-Gal rekombinant bakulovirusini olish jarayonlari standart BEVS protokollariga to‘liq mos keldi . Restriksiya tahlillarida (2-, 4-, 6-rasmlar) kuzatilgan fragmentlar genetik konstruksiyalashning aniqligini tasdiqlab, “O‘zbekiston-2030” strategiyasidagi fan-ishlab chiqarish integratsiyasiga hissa qo‘shdi .

ONPG testi (8-rasm) β-galaktozidaza oqsilining funksional aktivligini sifatli (sariq rang – O-nitrofenol) va miqdoriy (OD_{420} × 380 / min·mg) jihatdan isbotladi . ExGen 500 reagentining 10 ekvivalentdagi optimal doza (7-rasm) transfeksiya samaradorligini oshirdi, bu mahalliy laboratoriyalar uchun takrorlanadigan protokolni ta’minlaydi. Global adabiyotdagi Sf9 o‘sish kinetikasi (μ=0,0248-0,0286 $soat^{-1}$, 14 L bioreaktorlarda 10^7 hujayra/mL) bilan solishtirganda mahalliy sharoitlarda ham saqlanib qolgan [conversation_history].

Amaliy ahamiyati. Importga qaramlikni kamaytirishda muhim: R&D Systems rekombinant β-galaktozidaza 20 μg = $548,50 turadi . BEVS CHO hujayralariga nisbatan past xarajat, qisqa sikl (5-7 kun) va yuqori hosil (mg/L) afzalliklariga ega

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bo‘lib, sut sanoatida laktoza gidrolizi va farmatsevtikada glikolipid parchalash uchun mos . O‘zbekiston oziq-ovqat sanoatida 12 mingdan ortiq korxona faoliyat yuritadi, ammo fermentlar importga bog‘liq.

Ilmiy yangilik. O‘zbekistonda BEVS asosida β-galaktozidaza olish bo‘yicha birinchi tadqiqot sifatida PF-5847 konsepsiyasiga mos keladi . Plaque assay/$TCID_{50}$ bilan tozalan gan virus klonlari sanoat miqyosida ko‘paytirishga tayyor . Kelajakda SF-900 zardobsiz muhitda 14 L airlift bioreaktorlarda hosil 0,3-0,95 mg/mL gacha oshirilishi mumkin [conversation_history].

Cheklovlar. Laboratoriya sharoiti, sanoat validatsiyasi (GMP) kerak. Istiqbollar: Mahalliy korxonalarda laktoza intolerantsiyasini hal etish, importni potensial ravishda kamaytirishi mumkinkamaytirish .

Xulosa

Olib borilgan tadqiqotlar natijasida O‘zbekiston farmasevtika va oziq-ovqat sanoatida qo‘llanishi mumkin bo‘lgan rekombinant β-galaktosidaza fermentini bakulovirus ekspressiya tizimida olishga mo‘ljallangan yangi pBacPAK8-B-Gal plazmid DNK konstruksiyalandi. *Spodoptera frugiperda* (Sf9) hasharot hujayralarida ishlatiladigan Autographa californica multiple nucleopolyhedrovirus (AcMNPV) genom DNKsi va rekombinant pBacPAK8-B-Gal plazmidasi Sf9 hujayralariga ko-transfeksiyalanib, rekombinant rSf-polh-B-Gal bakulovirusi olindi hamda β-galaktosidaza genining samarali ekspressiyasi qayd etildi . Kerakli genni ekspressiyalovchi rekombinant bakulovirus klonlari identifikatsiya qilindi, Sf9 hujayralarida rekombinant β-galaktosidaza oqsili ekspressiyasi tavsiflandi va olingan natijalar farmasevtika hamda sut sanoatida import o‘rnini bosuvchi ferment preparatlarini ishlab chiqish uchun istiqbolli yo‘nalish ekanini ko‘rsatdi .

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