

**METILATROFIK ACHITQINI METANOLLI OZUQA MUHITIDA KO'PAYTIRISH**

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<https://doi.org/10.5281/zenodo.7357784>

**Annotatsiya.** Hozirgi kunda rekombinant oqsillarni sintez qilishda eng ko'p ishlatilayotgan tizimlardan biri bu metilatropik *Pichia pastoris* achitqi ekspressiya tizimidir. Mazkur achitqi turi metanolni uglerod manbai va oqsil ekspressiyasida inisiator vazifasini bajaruvchi noyob tizim hisoblanadi. Bizning tadqiqotda rekombinant oqsil ekspressiyasi uchun ishlatiladigan metanolning optimal miqdorini o'rganish maqsad qilingan. Buning uchun ozuqa muhit tarkibiga 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5 % miqdorda methanol solindi va achitqining o'sish darajalari o'rganildi. Natija shuni ko'rsatdiki, ozuqa muhitga 1.5% methanol solinganda eng yuqori o'sish darajasini namayon qildi.

**Kalit so'zla:** metilatropik *Pichia pastosis* achitqisi, ekspressiya, MD, BMG, BMM ozuqa muhitlari, MOX, AOX.

**РАЗМНОЖЕНИЕ МЕТИЛАТРОФНЫХ ДРОЖЖЕЙ В МЕТАНОЛОВОЙ ПИТАТЕЛЬНОЙ СРЕДЕ**

**Аннотация.** Одной из наиболее широко используемых систем для синтеза рекомбинантных белков является система экспрессии метилатрофных дрожжей *Pichia pastoris*. Этот вид дрожжей представляет собой уникальную систему, использующую метанол в качестве источника углерода и инициатора экспрессии белка. Наше исследование направлено на изучение оптимального количества метанола, используемого для экспрессии рекомбинантного белка. Для этого в питательную среду добавляли 0,5, 0,75, 1,0, 1,25, 1,5, 2,0, 2,5% метанола и изучали уровень роста дрожжей. Результат показал, что наибольшая скорость роста наблюдалась при добавлении в среду 1,5% метанола.

**Ключевые слова:** метилатрофные дрожжи *Pichia pastosis*, экспрессия, питательные среды MD, BMG, BMM, MOX, AOX.

**REPRODUCTION OF METHYLATROPHIC YEASTS IN METHANOL NUTRIENT MEDIUM**

**Abstract.** One of the most widely used systems for the synthesis of recombinant proteins is the expression system of the methylatrophic yeast *Pichia pastoris*. This yeast species is a unique system using methanol as a carbon source and protein expression initiator. Our study is aimed at studying the optimal amount of methanol used for the expression of the recombinant protein. To do this, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5% methanol was added to the nutrient medium and the level of yeast growth was studied. The result showed that the highest growth rate was observed when 1.5% methanol was added to the medium.

**Keywords:** Methylatrophic yeast *Pichia pastosis*, expression, nutrient media MD, BMG, BMM, MOX, AOX.

Metilotrofik achitqilar dastlab 1960-yillarning oxiri va 1970-yillarning boshlarida ajratib olingan [1]. Bu achitqilar uglerod va energiyaning yagona manbai sifatida metanoldan samarali foydalanish xususiyatiga ega. Metanni utelizatsiya qilishi metanol yoki alkagol oksidaza (MOX yoki AOX) fermentlarining mustahkam ekspressiyalanishi bilan bog‘liq. Mazkur fermentlarning sinteziga javob beradigan genlarni (MOX yoki AOX) boshqaruvchi promotorlar tomonidan mahalliy va heterologik oqsillar yuqori darajada ekspressiyalanib kelinmoqda. [2,3] Sintezlangan geterologik oqsillar peroksisomalarda to‘planadi va yuqori darajada ekspressiyalanganda peroksisomal hujayra hajmining 80% ni egallab, hujayraning noyob xususiyatini namoyon qiladi. *Pichia pastoris* metilotrofik achitqi xisoblanib rekombinant oqsillar ishlab chiqararishda keng qo‘llanilayotgan ekspressiya tizimlaridan biri xisoblanadi [4]

**Material va metodlar.** *Pichia pastoris* achitqisi (invitrogen), shaker inkubator, metanol, BMG ozuqa muhiti (100 mM kaliy fosfat pH 6.0, 1% glitserin, 1X YNB), Yeast Nitrogen Base (YNB), BMM ozuqa muhiti (100 mM kaliy fosfat pH 6.0, metanol, 1X YNB), MD agar qattiq ozuqa muhitib(1% glyukoza, 1X YNB, 1.5% agar agar), fotokolorimetr KΦK-2.

**Olingan natijalar va ularning muhokamasi.** Mazkur olib borilgan tadqiqot ishidan maqsad *Pichia pastoris* metilatrofik achitqisining metanolda optimal ko‘payish sharoitlarini aniqlashdan iborat. Buning uchun MD agar ozuqa muhitida o‘rib turgan *Pichia pastoris* achitqi koloniyasidan olib 30 ml BMG ozuqa muhitli kolbaga ekdik. Kolbani shaker inkubatorga 30 °C li haroratga qo‘ydik. Bunda kolbaning aylanib turishini ta‘minlash maqsadida uskunani 140 ay/min ga sozladik (1-rasm).



**1-rasm.** *Pichia pastoris* hujayralarining shaker inkubatorida o‘shishi.

Hujayralar ularning optik zichligi ( $OD_{600}$ ) 2-6 bo‘lgunga qadar o‘stirildi (15-20 soat) va ularning optik zichliklarini fotokolorimetrdan o‘lchandi. Shundan so‘ng kolbalar olinib undagi o‘sgan hujayralar probirkalarga ko‘chirildi va 4500 ay/min da sentrifuga qilindi. Cho‘kma metanollin BMM ozuqa muhitida eritilib toza steril kolbalarga solindi va shaker inkubatorga

joylanib yuqorida keltirilgan sharoitlarga sozlandi. Bunda ozuqa muhitdagi metanolning miqdori turlicha (0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5) qilib belgilandi (1-jadval).

1-jadval

Turli konsentratsiyali metanolda *Pichia pastoris* hujaralarining o‘shish darajalari

№	Methanol miqdorlari % da	O‘sgan hujayralarning optik zichliklari (OD <sub>600</sub> )	O‘stirish davomiyligi (metanoli muhitda)
1	0.5	68	72 soat
2	0.75	70	72 soat
3	1.0	75	72 soat
4	1.25	78	72 soat
5	1.5	80	72 soat
6	2.0	77	72 soat
7	2.5	73	72 soat

**Xulosa.** Jadvaldan ko‘rinib turibdiki hujayralar 1.5% metanolda yaxshi o‘shish sur‘atini namoyon qilgan. Metanolning miqdori tushishi yoki ko‘tarilishi bilan achitqi hujayralarining o‘shish darajalari kamayib borgan. Demak *Pichia pastoris* achitqisi 1.5% metanolda optimal o‘shish darajasini namoyon qular ekan.

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