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OPTIMIZATION OF CULTURE MEDIA TO PRODUCE BACILLUS THURINGIENSIS STRAINS

Balabek Ainaz (0000-0002-0092-4129)
Adilkhankyzy Ainura (0000-0001-8048-7987)
Shissenbaeva Nurgul (0009-0005-8930-4274)

Kazakh Research Institute of Plant Protection and Quarantine named after Zh. Zhiembaev
Almaty, Kazakhstan

Annotation*: currently, biological preparations occupy a special place in the protection of agricultural crops from pests. Microorganisms of various origins are used in the production of pathogenic biological preparations against agricultural pests. In this work, experiments were conducted to select nutrient media for growing entomopathogenic bacteria. Nitrogen and carbon sources were used as optimization factors. The control growing medium was medium "A", traditionally used for cultivating bacteria of the genus Bacillus. As a result, nutrient media SG and KR showed significantly better results than nutrient media in the control variant.*

Key words: *Bacillus thuringiensis, entomopathogen, nutrient medium, optimization, biopreparation, strain.*

ОПТИМИЗАЦИЯ КУЛЬТУРАЛЬНЫХ СРЕД ДЛЯ ПОЛУЧЕНИЯ ШТАММОВ BACILLUS THURINGIENSIS

Балабек Айназ (0000-0002-0092-4129)
Адилханкызы Айнура (0000-0001-8048-7987)
Шиссенбаева Нургуль (0009-0005-8930-4274)

Казахский научно-исследовательский институт защиты и карантина растений им. Жиембаева
Алматы, Казахстан

Аннотация*: в настоящее время биологические препараты занимают особое место в защите сельскохозяйственных культур от вредителей. Для производства патогенных биологических препаратов против сельскохозяйственных вредителей используются микроорганизмы различного происхождения. В данной работе проведены эксперименты по подбору питательных сред для выращивания энтомопатогенных бактерий. В качестве факторов оптимизации использовали источники азота и углерода. Контрольной средой для выращивания была среда «А», традиционно используемая для культивирования бактерий рода Bacillus. В результате питательные среды SG и KR показали значительно лучшие результаты, чем питательная среда в контрольном варианте.*

Ключевые слова: *Bacillus thuringiensis, энтомопатоген, питательная среда, оптимизация, биопрепарат, штамм.*

БАЦИЛЛУС ТЮРИНГЕНСИС ШТАМДАРЫН АЛУУ ҮЧҮН КУЛЬТУРАЛЫК КАРАЖАТТАРЫН ОПТИМАЛАШТЫРУУ

Балабек Айназ (0000-0002-0092-4129)
Адилханкызы Айнура (0000-0001-8048-7987)
Шиссенбаева Нургуль (0009-0005-8930-4274)

Ж. Жиембаев атындагы өсүмдүктөрдү коргоо жана карантини боюнча Казак илимий-изилдөө институту, Алматы, Казахстран

***Аннотация**: азыркы учурда айыл чарба өсүмдүктөрүн зыянкечтерден коргоодо биологиялык препараттар өзгөчө орунду ээлейт. Айыл чарба зыянкечтерине каршы патогендик биологиялык препараттарды өндүрүүдө ар кандай тектүү микроорганизмдер колдонулат. Бул иште энтомопатогендик бактерияларды өстүрүү үчүн азыктык чөйрөлөрдү тандоо боюнча эксперименттер жүргүзүлдү. Оптималдаштыруу фактору катары азот жана көмүртек булактары колдонулган. Контролдоочу азык чөйрөсү "А" орточо болгон, адатта Bacillus тукумундагы бактерияларды өстүрүү үчүн колдонулган. Натыйжада SG жана KR азыктандыруучу чөйрөлөрү контролдук вариантта азыктандыруучу чөйрөлөргө караганда бир кыйла жакшы натыйжаларды көрсөтүштү.*

***Өзөктүү сөздөр**: Bacillus thuringiensis, энтомопатоген, азык чөйрөсү, оптималдаштыруу, биологиялык продукт, штамм.*

1. Introduction

Obtaining environmentally friendly products is one of the most important tasks of agriculture. Microorganisms of various origins are used in the production of pathogenic biological preparations against crop pests. One of them is biological products made from entomopathogenic bacteria Bacillus thuringiensis (Grishechkina, S. D., 2015).

Bacillus thuringiensis are bacteria that constantly circulate in biocenoses and are the basis for many biological insecticides. To date, more than 80 serovars are known, which are contained in the collection of the International Center for Entomopathogenic Bacteria at the Pasteur Institute (Paris) (Nurzhanov, A. A., 2019).

Bacillus thuringiensis are found in the soil, isolated from various insects, leaf litter, from a wide variety of biological objects of the external environment (Burceva L. I., 2001.).

The entomopathogenic potential of Bacillus thuringiensis has been actively used for more than 50 years to protect agricultural crops. The importance of the toxin produced by this bacterium is that it is effective not only against insects, but also against other organisms, such as nematodes and protozoa. Preparations with the active substance Bacillus thuringiensis in terms of toxicity to humans belong to hazard class 4 and are characterized as low-hazard (Lacey L.A., 2000.)-(Jain D, 2016). Unfortunately, biological preparations are not currently used as widely in agricultural production as they were a couple of decades ago. One of the reasons for this is the underestimation of their positive qualities and the enthusiasm of specialists for the high initial efficiency of chemical pesticides. The desire to quickly achieve maximum effect is still a priority in choosing protective agents (Grabova, A.Yu. 2015). However, this does not take into account the negative consequences of using chemical pesticides: the emergence of resistant forms of phytophages and phytopathogens and, as a consequence, increased pesticide pressure; disruption of biological balance in agrocenoses, which leads to outbreaks of mass

reproduction of not only dominant harmful species, but sometimes secondary ones; general deterioration of the environment (Smirnov O.V., 2011).

2. Materials and methods

The research was carried out in the biotechnological laboratory of the Kazakh Research Institute of Plant Protection and Quarantine named after Zhazken Zhiembayev. The strain Bacillus thuringiensis F5/1 (hereinafter strain F5/1) was obtained from the collection of entomopathogenic cultures of the Kazakh Research Institute of Plant Protection and Quarantine named after Zhazken Zhiembayev as a material for inoculation into various nutrient media. To select the optimal nutrient medium, nutrient media with different compositions of nitrogen and carbohydrate sources were prepared [2] (Table 1).

The composition of the nutrient media was measured and prepared. 100 ml were poured into 500 ml flasks and sterilized in an autoclave under a pressure of 1 atm for 30 minutes. The pH before sterilization was 7.0-7.6, after sterilization - 6.8-7.2. Strain F5/1, grown on nutrient medium "A", was inoculated at the rate of 0.5 ml of inoculum per 50 ml of culture medium cooled to 30-35 ° C. Flasks were grown at 200 rpm and 29°C for 56-70 hours until complete maturation, sporulation, and formation of crystalline endotoxin. Each culture medium was prepared in triplicate, and culture medium “A” was used as a control. A Goryaev chamber was used to calculate the spore titer (Burceva L. I., 2001.). Formation

Table 1. Composition and amount of different media used in the cultivation of strain F5/1, 100 ml/g

Material	NM 1	NM 2	KPDS	NM 4	NM 5	MPS	KR	KP	SG	NCC
Pea flour	0,5	2,0		0,5						
Corn flour			1,5		0,5					
Glucose	0,5									
K_2HPO_4	0,3									
$MgSO_4$	0,02									
$CaCl_2$	0,1		0,15							
Potato starch		1,0		1,0	1,0					
$(NH_4)_2HPO_4$		0,15								
$MnSO_4$		0,01	0,02	0,01	0,01					
K_2SO_4		0,1								
NaCl						1	0,5	0,5	0,5	
Peptone						1		1		
Yeast extract			3,0	3,0	3,0					
Meat						50				
Bone meal							1	1		
Fish meal							1			
Grasshopper flour									1	
Sucrose									1	1.4
Potassium										0,1
$(NH_4)_2SO_4$										0.6
$Ca(H_2PO_4)_2$										0.05

of crystalline endotoxin in nutrient media was determined by the Gram method (Smirnov O.V., 2011).

3. Results

As a result of the laboratory experiments, samples were taken from the control nutrient medium "A" and 10 different nutrient media after 3 days of incubation to determine the formation of crystalline endotoxin and stained using the Gram method. According to the results of microscopy of the stained samples, the process of crystal formation was observed in the control and culture media NM 1, NM 2, MPS, KR, KP, SG. In culture media NM 4, NM 5 and KPDS, although the cells were fully developed, crystals and spores were not formed.

Table 2. Spore titer of strain F5/1 and the formation of crystalline endotoxin on nutrient media

Culture media	Spore titer	Formation of crystalline endotoxin
«A»	$1{,}85\times10^{9}\pm0{,}11$	+
NM 1	$0{,}90\times10^{8}\pm0{,}30$	+
NM 2	$0{,}96\times10^{8}\pm0{,}13$	+
KPDS	-	-
NM 4	-	-
NM 5	-	-
MPS	$2{,}00\times10^{9}\pm0{,}20$	+
KR	$2{,}80\times10^{9}\pm0{,}15$	+
KP	$1{,}60\times10^{8}\pm0{,}14$	+
SG	$2{,}15\times10^{9}\pm0{,}14$	+
NCC	-	-

The highest spore titer on nutrient media was observed on the KR nutrient medium. The second successful result was found in the SG culture medium. The amount of spore titer in the remaining nutrient media will significantly contribute to the selection of new formulations in the future (Table 2).

4. Discussion

The study investigated the formation of crystalline endotoxins and spore titer production by strain F5/1 across different nutrient media. The findings revealed variations in the ability of the strain to produce spores and crystalline endotoxins depending on the composition of the nutrient medium, indicating that media composition plays a critical role in the physiological response of microorganisms.

The highest spore titer was observed in the KR nutrient medium, reaching 2.80×10^{9} spores/mL, followed closely by the SG medium (2.15×10^{9} spores/mL) and the MPS medium (2.00×10^{9} spores/mL). These media demonstrated not only robust spore production but also successful crystalline endotoxin formation, highlighting their potential suitability for optimizing the production of biocontrol agents or other microbial metabolites.

5. Conclusion

According to the results of the laboratory experiment on the selection of the composition of nutrient media for cultivation of the Bacillus thuringiensis F5/1 strain, the KR nutrient medium showed the highest indicator. In the future, research work will be continued on replacing nitrogen and carbohydrate sources in nutrients, as well as optimization work. It is planned to achieve higher results than the KR nutrient medium indicator.

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