LAPORAN

Call For Paper
International Conference on Biology, Sciences and Education

Padang, 17-18 Oktober 2019
Universitas Negeri Padang

Oleh

Febriana Dwi Wahyuni, M.Si

UNIVERSITAS ESA UNGGUL

2019

PENDAHULUAN

Setiap dosen berkewajiban untuk melaksanakan Tridarma Perguruan Tinggi, yaitu pengajaran, penelitian, dan melakukan pengabdian kepada masyarakat. Kinerja dosen masih banyak berfokus pada pengajaran sehingga mengesampingkan dua kewajiban lainnya yaitu penelitian dan pengabdian kepada masyarakat. Saat ini, dosen mulai dituntut untuk banyak melakukan penelitian dan mempublikasikan hasil penelitiannya. Salah satu media untuk mempublikasikan hasil penelitian adalah melalui kegiatan Call For Paper. Melalui kegiatan ini, para dosen maupun peneliti bisa menyebarluaskan hasil penelitiannya.

TUJUAN

Konferensi ini bertujuan untuk *sharing knowledge* dari hasil penelitian yang telah dilakukan.

PELAKSANAAN

Waktu dan tempat

Konferensi dilaksanakan pada:

- Hari dan tanggal : Kamis-Jumat, 17-18 Oktober 2019
- Waktu : 08.00 WIB – selesai
- Lokasi : Universitas Negeri Padang

Peserta Workshop

Mahasiswa, dosen maupun peneliti dari berbagai Perguruan Tinggi di Indonesia dan Luar negeri

Pendaftaran

Peserta yang bisa mendaftar untuk kegiatan Call For Paper International Conference on Biology, Sciences and Education adalah mahasiswa, dosen dan peneliti dari semua Perguruan Tinggi maupun lembaga penelitian di Indonesia dan Luar Negeri.
Panitia pelaksana adalah dari Departemen Biologi, Universitas Negeri Padang.

Output dan Outcome

Setelah mengikuti kegiatan ini, diharapkan peserta dapat mentransfer ilmu dari hasil penelitian yang telah dilakukan

Penutup

Demikian Term of Reference ini dibuat sebagai panduan kegiatan. Semoga dapat memberikan informasi bagi pihak-pihak yang terkait.

Rundown Acara

CONFERENCE SCHEDULE

DAY 1	***17 October 2019***
07.30 – 08.30	Registration
08.31 – 09.30	Opening Ceremony (*ICoBioSE* 2019)
09.30 – 10.00	Coffee Break
10.01 – 12.00	Keynote Speaker I Prof. Datin Dr Mariani Binti Md Nor University of Malaya Keynote Speaker II Dr. Katsuyuki Eguchi Tokyo Metropolitan University, Tokyo, Japan
12.00 – 13.15	Lunch
13.16 – 14.00	Poster Session
14.01 – 15.00	Parallel Session I
15.01 – 15.15	Coffee Break
15.16 – 17.00	Parallel Session II
17.01 – 19.00	Break
19.01 – 22.00	Sarasehan di Gubernuran Sumbar* (masih dikonfirmasi)
DAY 2	***18 October 2019***
08.00 – 10.00	Keynote Speaker III Prof. Madya Dr. Haniza Hanim Mohd Zain Universitas Pendidikan Sultan Idris, Tanjung Malim, Malaysia Keynote Speaker IV Prof. Si-Min Lin, Ph.D National Taiwan Normal University, Taipei, Taiwan Keynote Speaker V Dr. Wendy Wang Yanling Lee Kong Chian Natural History Museum,Singapore
10.00 – 10.15	Parallel Session III
10.15 – 10.30	Coffee Break
10.30 – 12.15	Parallel Session IV
12.15 – 13.30	Praying break + Lunch
13.30 – 15.45	Parallel Session V
15.45 – Selesai	Penutupan

In Silico Analysis for *Cry*I Gene Amplification from Local Isolate of *Bacillus thuringiensis*

F D Wahyuni[1*], C Fransisca[1], S E Pratiwi[1], H Saraswati[1], K S Dewi[2]

[1]Biotechnology Department, Faculty of Health, Universitas Esa Unggul, Jakarta, Indonesia
[2]Research Center For Biotechnology, Indonesian Institute of Sciences, Cibinong

[*]febriana@esaunggul.ac.id

Abstract. *Bacillus thuringiensis* is one of bacterial type that was used as a microbiological control agent for pests and vectors of plant disease. It was because in *B. thuringiensis* there is Cry protein which is specific insect repellent. This Cry Protein is only toxic to certain insects. One of them is CryI protein which is toxic to Lepidoptera insects. Lepidoptera can attack various types of plants. CryI Proteins was encoded by *cry*I gene. The method can be used for *cry*I gene amplification is Polymerase Chain Reaction (PCR). Primer is one important component in PCR. The aim of this research was get a good primer candidate for *cry*I gene amplification from *B. thuringiensis*. In silico analysis for designing *cry*I primer was carried out using several software, such as BLAST for searching sequence *cry*I gene, Bioedit for alignment, and DINAmelt for analyze dimer structure of primer. Ten primer candidates were successfully obtained based on the result of the primer3. One primer was selected that could represent *cry*I gene, with forward primer 5'-CGGTGAATGCCCTGTTTACT -3' and reverse primer 5'- CGGTCTGGTTGCCTATTGAT -3'. Amplification of the *cry*I gene by PCR *in silico* produces a product with a length about 200 bp.

Keywords: bioinformatics, cry gene, PCR in silico, primer

1. Introduction

One of barrier in the production of a commodity in tropical and humid countries is the attack of plant-disturbing organisms such as insect pests and plant pathogens [1]. Even in certain plants such as rice, pest insects are still a major obstacle and become a serious problem, for example brown plant hopper and stem borer [1]. The control effort that is usually done by farmers is spraying insecticides. The use of insecticides was allegedly caused a negative impact on the environment and caused resistance to target insects. Concerns about the negative impact of the use of insecticides have increased public attention to bioinsecticides as an alternative to reducing pest populations [2].

Bacillus thuringiensis (Bt) is a bacterium that produces crystalline proteins that are toxic to insects and nematodes during sporulation [3]. *B. thuringiensis* bioinsecticides constitute 90-95% of the bioinsecticides commercialized for use by farmers in various countries [2]. The advantages of using this bacterium as a biopesticide include a protein produced by *B. thuringiensis* which is a specific buffer and is called a Cry protein (from the word crystal) or also called as δ-endotoxin. Cry protein is only toxic to certain types of insects and is not toxic to useful insects or to other organisms [3]. One type of Cry protein, CryI, is known to be able kill insects from the Lepidoptera group [4] . Insects of this type Lepidoptera are pests that attack various plants. The development of recombinant DNA technology is now possible to carry out the transformation of cry genes into plant cells. The introduction of cry genes into plant genomes is expected to produce plants capable of expressing endotoxins that can cause pest

insect death. In this way it is also expected to reduce the use of insecticides that can pollute the environment.

Advances in technology in the field of molecular biology have encouraged scientists to isolate chromosomes or plasmids that contain cry genes. The cry gene is a DNA sequence that codes for the formation of Cry proteins [3]. Several studies have been carried out for cry gene amplification [5,6]. One method that can be used for cry gene amplification is the Polymerase Chain Reaction (PCR) which will be carried out in this study by applying a discipline of bioinformatics.

Bioinformatics is an interdisciplinary field which is broadly defined as a combination of biology (molecular biology) and computing using computer and software assistance [7]. One of the most significant roles of bioinformatics is to design and produce primary sequences. Primary is one component that plays an important role in the PCR process [8]. A good primer is a specific primer. Unspecified primers can cause amplification of other regions in the genome that are not targeted or otherwise there are no amplified genome regions. To get a primer that meets the good primary criteria for amplification, in silico design is carried out [7]. The aim of this study was to analyze the combination of primary pairs used in the PCR process to amplify the *cry*I gene from *B. thuringiensis*. The *cry*I gene that will be amplified is a domain of the total *cry*I gene sequences that already exist in *genebank*.

Materials and Methods

B. thuringiensis isolate

Bacteria culture of *B. thuringiensis* was obtained from the InaCC culture collection with code number B432 and B327

Bacterial DNA extraction

B. thuringiensis DNA extraction method was used the gSYNC DNA Extraction kit from *Geneaid*.

Target Gene Sequences

Target gene used in this research is *cry*I gene from *Bacillus thuringiensis* (accession number X56144). These sequence were obtained from the NCBI website (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov).

Blast

The gene then was analyzed with Basic Local Alignment Search Tools (BLAST) software with other *B. thuringiensis* strains so that identity values from the highest to the lowest were obtained. Gene sequences that have high identity values are used in the multiple alignment method to obtain conserved sequences.

Multiple Alignments

The determination of sustainable areas is determined using the Bioedit program. The sequence of bases obtained is aligned with the ClustalW Program and then the one which has the most similarity in its constituent bases is similar to 90-100%. As a basis for tracking primary attachments [9].

Primary Design

Primary DNA determination analysis was performed with Primer3 software, and manually selected the most sustainable areas by considering the area is the cipher sequence, with general requirements, including the number of nucleotides, GC content and there is no possibility of complementarity between bases in one primary chain or between one primer with another.

Analysis of Primary Dimer

Sometimes the primers that are designed can recognize the sequence of themselves, bind to one another to form a structure called a dimer. This can be a problem because primers will tend to stick together, not with the target gene and this can reduce DNA concentration. For this reason, it is necessary to do a dimer prediction analysis using available software to predict the presence of dimers in primary candidates, namely DINAmelt (http://unafold.rna.albany.edu/?q=DINAMelt) made by NM Markham and Michael Zucker of Rensselaer Polytechnic Institute [10,11]. From the results of this analysis using DINAmelt, it can be seen whether the dimers are formed in the primer, how much the formation of the G-C bonds, and the presence of the 3 'end of the complement.

Analysis of Restriction Sites in the cryI Gene

To find out the restriction sites contained in the *cry*I gene, an analysis was performed using *Snapgene* software. The purpose of knowing the restriction site is so that genes can be cut with one of the desired endonuclease restriction enzymes.

Amplification of the *cry*I gene by PCR

Amplification of the *cry*I gene using specific primer cry1 forward 5'-CGGTGAATGCCCTGTTTACT-3' and reverse 5'-CGGTCTGGTTGCCTATTGAT-3' with a total volume of 25 µl containing 1 µl DNA genome, 12.5 µl PCR HS redmix master mix (Bioline), 1 µl for each primer and 9.5 ddH_2O. PCR amplification was carried out as many as 35 cycles using Sensoquest. For one cycle consists of 3 stages, namely denaturation, attachment, and elongation. The predenaturation stage is carried out for 3 minutes at a temperature of 95^0C once, the denaturation stage is 1 minute, the primary attachment stage is at 50-60^0C for 1 minute, the extension of the DNA chain at a temperature of 72^0C for 1 minute. In the last cycle, the chain was extended longer at 72^0C for 5 minutes.

PCR Electrophoresis

PCR results were migrated into 1% agarose gel under 100 volt 30 minute conditions. 1 kb DNA marker is used as a marker. Gel staining used fluorosafe DNA stain which is directly added to the agarose gel. Gels containing DNA fragments were visualized using UV Trans Illuminators and documented using the Digibox Camera Documentation System gel.

Result and Discussion

a. Result

***cry*I gene Sequences**

Based on the search results of the *cry*I gene sequences in genebank with accession number X56144, *cry*I gene sequences were obtained from *Bacillus thuringiensis* consisting of 3516 bp (figure 1.)

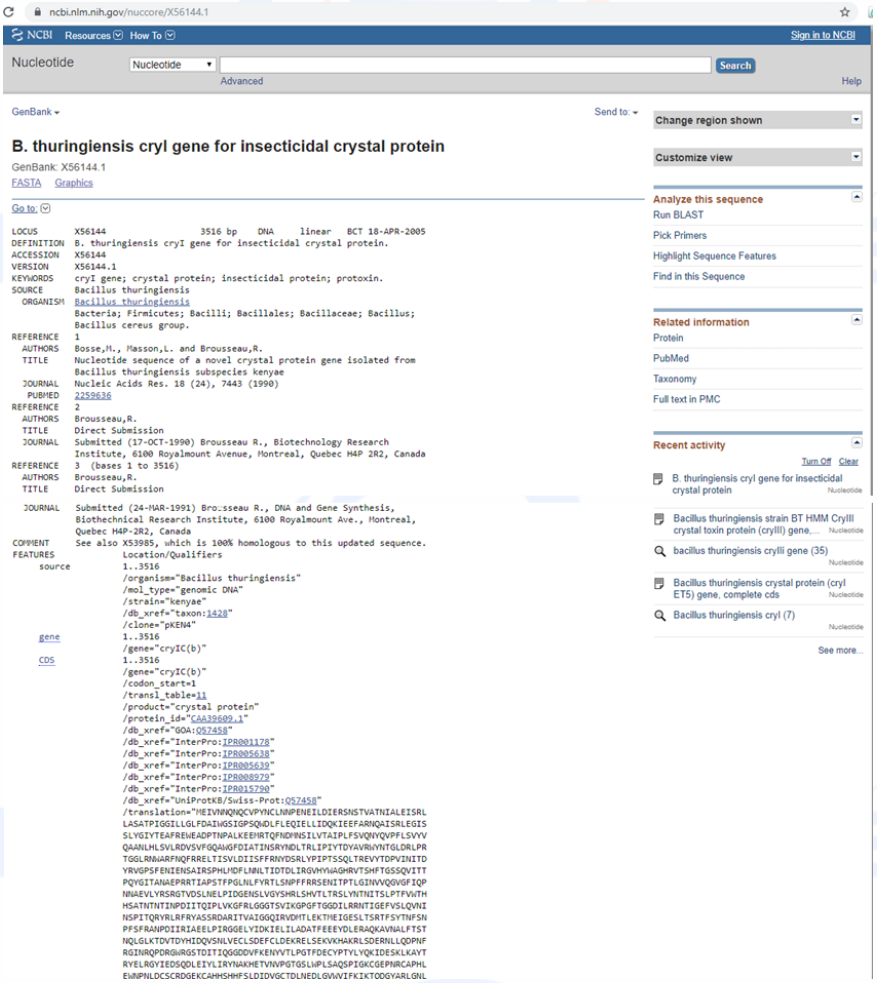

Figure 1. The *cry*I gene *Bacillus thuringiensis* source from NCBI

Basic Local Alignment Search Tools (BLAST)

The BLAST process was carried out in *Bacillus thuringiensis cry*I gene as a determinant strain with other *B. thuringiensis* strains. *B. thuringiensis cry*I gene for insecticidal crystal protein as a determinant because it has a long DNA sequence. BLAST results obtained an identity value of 100%-96% *cry*I gene against several strains of *B. thuringiensis*.

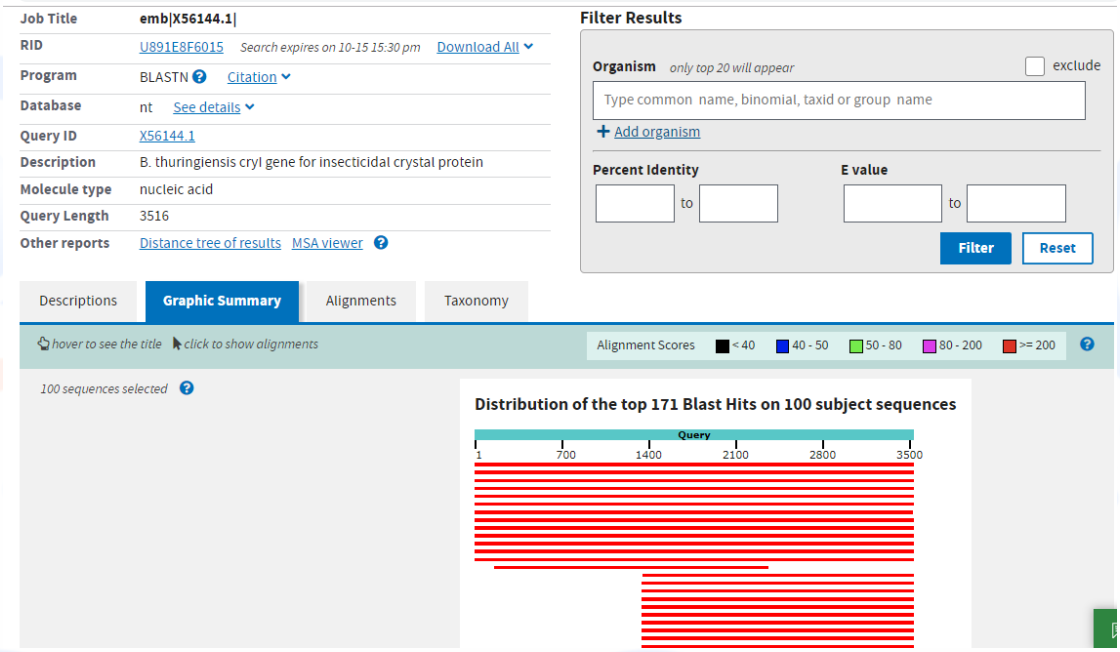

Figure 2. BLAST *cry*I gene with several strains of *Bacillus thuringiensis*

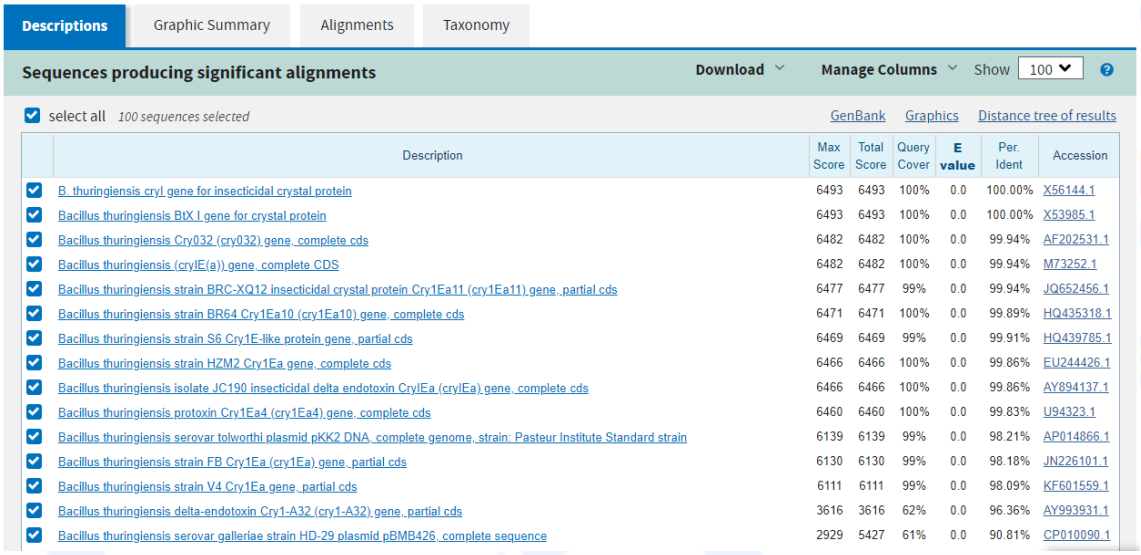

Figure 3. Analysis of the *cry*I gene identity with the *cry*I gene from another *Bacillus thuringiensis* strain

Multiple Alignment

From several strains of BLAST results that have 100-96% DNA sequence identity values, multiple alignment is performed, so that several species with DNA sequences of *cry*I genes that have parallel DNA sequences are obtained (Figure 4). Sequences selected parallel to the CDS region that corresponds to the primary DNA sequence that has been designed with Primer3 software.

Figure 4. The multiple alignment process of *cry*I gene from *B. thuringiensis* with other strain that have 100-96% DNA sequence identity using Bioedit software

DNA primer *cry*I gene

The design of the primary DNA of the *cry*I gene from Bacillus thuringiensis uses primer3 software that has passed the BLAST process and multiple alignment (Table 1).

Table 1. cry1 Primer design from *B. thuringiensis*

Primer (*cry*I)	Sequence (5'-3')	Tm (^{0}C)	GC%	Self 3' complementarity
Forward	CGGTGAATGCCCTGTTTACT	60	50	1
Reverse	CGGTCTGGTTGCCTATTGAT	60	50	2

Dimer Structure in Primer Candidate

The results of the analysis with DINAmelt software, it can be seen that the primers that have been designed only have 2 G-C bond. This is advantageous because it can increase the concentration of PCR products and the possibility for the primer bind to each other is very small.

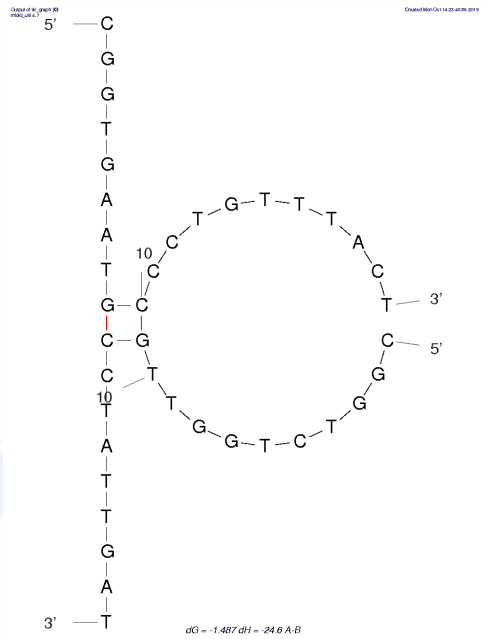

Figure 5. This figure shows DINAmelt analysis results to predict dimer structure on primer pair previously designed

Map of *cry*I gene

The results of the snapgene analysis showed that the amplified *cry*I gene had 8 restriction sites. This information can be used as a basis for cloning.

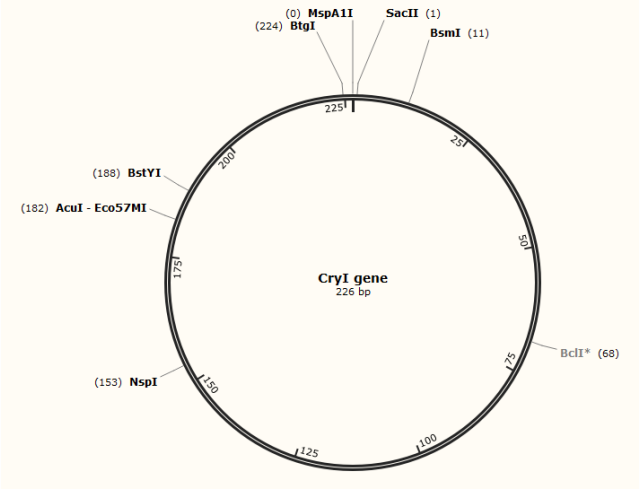

Figure 6. Mapping Restriction Enzymes in *cry*I Genes with Snapgene software

PCR Electrophoresis

DNA visualization results that have been amplified with cry1 forward and cry1 reverse primers show DNA bands with a size of about 200 bp.

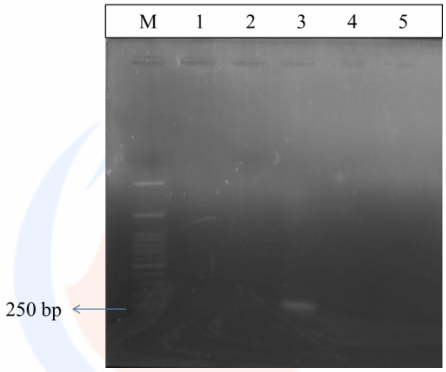

Figure 7. Amplification of *cry*I gene

b. Discussion

Primary design is the first step that determines the success of DNA amplification by PCR method [12]. Criteria that need to be considered in the selection of primers include the length of primer, temperature melting (Tm), GC content and bonds at the end of 3 '. Good primers range from 18-30 base pairs. Primers which have a length more than 30 base pairs will cause the primary attachment to be unspecified. The second characteristic to consider in primary selection is Tm. A good primer has a Tm difference of around 5^0C. This is intended to prevent a decrease in the amplification process. The percentage between bases G and C also needs to be considered because the content of the number of bases G and C can affect the Tm of a primary [13]. A good primary has a percentage of G and C around 40-60%. Another criteria for good primers is having a low self 3'complementarity so that there is no attachment between primer pairs and forms a structure called a hairpin [14].

Dimers are structures formed between primer pairs, where they are united because they have a complementary basis. This process occurs at the appropriate attachment temperature, usually at low temperatures. By looking at the stages of the PCR process, it can be seen that the primary attachment to the printed DNA occurs at optimal annealing temperatures. This process can occur simultaneously with the formation of dimers. The problem that may arise is that primers have a tendency to stick to one another, and not stick to printed DNA. If this dimer bond is too strong, it will interfere with the DNA extension process and will result in low DNA concentration. From the analysis using DINAmelt, it can be seen that the primer forms a dimer with two GC bonds. The bond between bases G and C is a strong bond because it consists of 3 hydrogen bonds. That will make the primer easier to put together.

In silico analysis is an important computational prediction in primary design. Primers must also be tested through a series of optimizations in the laboratory. Primary candidate optimization involves optimization in annealing temperature (Ta) using PCR gradient and optimization of primary concentration. In addition to primers, optimization of the PCR reaction is also carried out to check the minimum detection and quantification of nucleic acids in the reaction, and this requires work in the laboratory to produce a good PCR test.

Conclusion

The primary designed can amplified the cryI gene with a size of about 200 base pairs. Annealing temperature optimization is needed to get a more specific amplicon.

REFERENCES

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INTERNATIONAL CONFERENCE ON BIOLOGY, SCIENCES AND EDUCATION
BIOLOGY DEPARTMENT
FACULTY OF MATHEMATICS AND NATURAL SCIENCES
UNIVERSITAS NEGERI PADANG
Jl. Prof. Dr. Hamka Kampus FMIPA – UNP Air Tawar Padang, Email: icobiose@fmipa.unp.ac.id

No. : 10 /ICoBioSE/2019
Attachment : -
Subject : **Letter of Acceptance**

Dear Febriana Dwi Wahyuni, M.Si,
We are pleased to inform you that this following abstract :

Title : **IN SILICO ANALYSIS FOR AMPLIFICATION CRYI GENE FROM BACILLUS THURINGIENSIS LOCAL ISOLATE**
Author(s) : **Febriana Dwi Wahyuni, Cindy Fransisca, Selvi Erna Pratiwi, Henny Saraswati**
Reg. ID : **Bio00126**

Has been reviewed and accepted to be presented in International Conference on Biology, Sciences and Education (ICoBioSE) to be held on October 17th – 18th,2019 at Universitas Negeri Padang, Padang City, West Sumatera, Indonesia.

1. You are kindly requested to make payment amount IDR 1.500.000 for local presenter or USD 105 for international presenter, and IDR 900.000 for local student presenter or USD 63 for international student presenter no later than October 11th 2019. The payment should be transferred to Bank Negara Indonesia 1946 (BNI 46) with account number 0668110902 and account name RPL 010 BLU UNP UTK DKE. Please confirm your payment through your account by sending the scan of receipt. For student conference, please attach the scan of student ID'card.
2. The paper should be submitted no longer than October 2nd ,2019. All full papers will be reviewed and selected to be submitted to IOP Publisher indexed by Scopus or Jurnal Biodjati (Sinta 2). Please kindly follow the guidelines for paper's structure available on our website.
3. The selected papers to be published will be charged additional fee of IDR 900.000 or USD 93 for each paper and should be transferred to Bank Negara Indonesia 1946 (BNI 46) with account number 0534437975 and accound name Dezi Handayani, S.Si. It can be also paid to committee directly on the day of the conference.

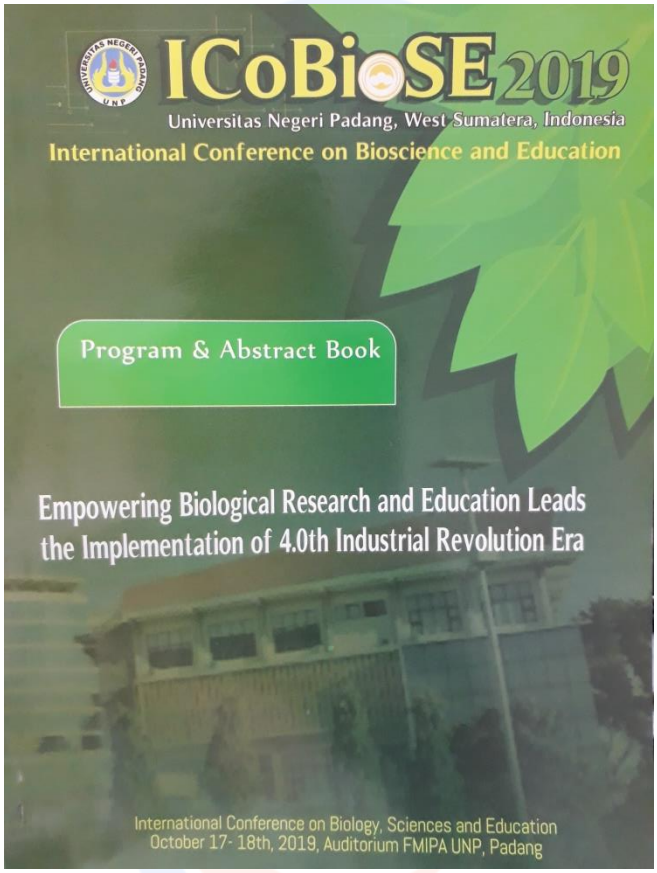
We look forward to meet you in International Conference on Biology, Science and Education (ICoBioSE).
Your faithfully,

ICoBioSE

Dr. Yuni Ahda, M.Si
General Chair

Bio00126

PROSIDING



PARALLEL SESSION SCHEDULE

Thursday, October 17, 2019

BIODIVERSITY Mod : Fitra Arya Dwi Nugraha, M.Si Ruang : BIO 1			BIOFUNCTION Mod : Dr. Rijal Satria, Ph.D Ruang : BIO 2	
No	Reg. ID	Nama	Reg. ID	Nama
		Sesi I		
1		Syazwan Saidin		Syafruddin Ilyas
2	BIO00145	Dr. Suwarno, M.SI	Bio00234	Dr. Dewi Imelda Roesma, M.Si
3	BIO00046	Mareta Widiya, M.Pd.Si	BIO00172	Dr. Haliatur Rahma, S.Si., MP
4	BIO00133	Fatimah, S.Si	BIO00199	Dr. Hasmiwati, M.Kes
5	BIO00054	Dharma Ferry, M.Pd	BIO00010	Dr. Syamsurizal
6	BIO00164	Retno Prihatini	Bio00003	Dr. Dwi Hilda Putri, M. Biomed.
		Sesi II		
1	Bio00115	Dra. Zuchrotus Salamah, M.Si		Dr. Djong Hon Tjong, M.Si
2	Bio00160	Prof. Zainus Salimin	Bio00126	Febriana Dwi Wahyuni, M.Si
3	Bio00059	Khairissa Trisliani Asmara, S.Si	Bio00131	Indri Puspita Sari, S.Si.
4	Bio00060	Wika Mardhiyah	Bio00165	Armaita. S,KM. M.Si
5	Bio00146	Nabela Fikriyya, S.Pd		Dasumiati
6	Bio00117	Drs. Hadi Sasongko, M.si.	Bio00005	Dr. Violita., S.Si., M.Si

Bio00134

IN SILICOANALYSIS FOR AMPLIFICATION CRYI GENE FROM BACILLUS THURINGIENSIS LOCAL ISOLATE

FebrianaDwiWahyuni, HennySaraswati*
Department of Biotechnology, Faculty of Health, UniversitasEsaUnggul, Indonesia
febriana@esaunggul.ac.id

ABSTRACT

Bacillus thuringiensis is one of bacterial type that was used as a microbiological control agent for pests and vectors of plant disease. It was because in B. thuringiensisthere is Cry protein which is specific insect repellent. This Cry Protein is only toxic to certain insects. One of them is CryI protein which is toxic to Lepidoptera insects. Lepidoptera can attack various types of plants. CryI Proteins was encoded by cryI genc. The method can be used for cryI gene amplification is Polymerase Chain Reaction (PCR). Primer is one important component in PCR. The aim of this research was get a good primer candidate for cryI gene amplification from B. thuringiensis. In silico analysis for designing cryI primer was carried out using several software, such as BLAST for searching sequence cryI gene, Bioedit for alignment, and DINAmelt for analyze dimer structure of primer. Ten primer candidates were successfully obtained based on the result of the primer BLAST from NCBI. One primer was selected that could represent cryI gene, with forward primer 5'-GTACCGATTCCCTATGGCCG-3' and reverse primer 5'-GTACGCTGTGACACGAAGGA-3'. Amplification of the cryI gene by PCR in silico produces a product with a length about 770 bp.

Keywords: Bioinformatics, Cry gene, PCR in silico, Primer

Bio00136

UTILAZATION OF C-CINNAMALKALIX [4] RESORSINARENA OF SYNTHESIS AS ABSORBENT MATERIAL FOR DANGEROUS DYES OF RHODAMIN B

Sri Benti Etika, Edi Nasra*
-
-

ABSTRACT

This study aims to determine the optimum conditions of Rhodamin B using C-Cinnamal Calix [4] Resorsinarena (CCCR) absorbent synthesized by UV-VIS Spectrophotometer. CCCR is synthesized by reacting cinnamaldehyde with resorcinol through an electrophilic substitution reaction. Cinnamaldehyde was obtained from isolation using cinnamon oil as much as 7.036 grams with a yield of 14.072% from 50 g cinnamon oil and CSKR produced as much as 5.6775 g brownish red. Cinnamaldehyde and CSKR were characterized using FTIR and UV-VIS. The results of the UV-VIS spectrum and FTIR data by the CSKR from isolated cinnamaldehyde showed the same results as pure cinnamaldehyde. The results of CSKR characterization using UV-VIS have absorption at a wavelength of 336 nm with an absorbance of 3,6685 Au (0,36685A) which indicates that there is a double bond conjugated to cinnamaldehyde and CSKR. The results of CSKR characterization using FTIR showed a peak of 3806.68 cm-1 indicating the presence of an O-H group. Furthermore, there is absorption at wave number 1610.56 cm-1 which indicates the presence of C=C groups. At wave number 1448.90 cm-1 shows the aromatic ring. Furthermore, absorption at the fingerprint wave number 692.31 cm-1 - 836.05 cm-1 indicates the presence of (CH2). The CSKR melting point is 358^0 C.

Keywords: Absorbent, CCCR, Rhodamin, UV-VIS Spectrophotometer

DOKUMENTASI ACARA