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(RESEARCH ARTICLE)

Design of primers in detection of Chronic Bee Paralysis Virus (CBPV) using a nested Polymerase Chain Reaction

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Abstract

Diseases in bees are quite common and when the bees are no longer on the face of the earth, a great catastrophe will begin. The above has been attributed to a Nobel Prize winner: Albert Einstein, who predicted that Man would only have 4 years left on our planet. The detection of one of the pathogens that descend to bees constitutes a small contribution to the knowledge of this pathogen and its control. The chosen method is currently the most effective tool in this regard and we must thank the great North American biochemist Kary Mullis, who died in 2019.

Keywords: CBPV; Bee disease; In silico;Design primers; Detection

1. Introduction

The honey bee is a recognized producer of honey and plays an important role in the agriculture by aiding in the pollination of a wide variety of crops. Currently, bees have been disappearing from their hives and colonies have weakened or died [1].

Viral infections are the least known of the bee diseases, due to a lack of information on the mechanisms underlying possible diseases and/or available experimental data on different modes of spread, transmission and persistence [2, 3].

Bee colony losses have been reported in Europe and America that cannot be attributed to the Varroa mite (*Varroa destructor*), so a combination of stressors, including other pathogens, is suspected to be the cause [1, 2, 4]. Therefore, knowledge of the mechanism of spread and synergy of pathogens within hives is crucial to understand the dynamics of bee diseases [4–6].

Chronic bee paralysis virus (CBPV) is the cause of a contagious disease among adult honey bees that leads to death and is known as chronic paralysis syndrome [7, 8]. CBPV virion morphology and organization of the RNA genome are unique among other viruses that descend to bees. CBPV is classified as a positive-sense single-stranded RNA virus that was first isolated in 1963 and infection can affect any colony in the apiary, resulting in thousands of deaths.

One way to detect this pathogen is via the Polymerase Chain Reaction (PCR) with *In Silico* primer design, knowing its genome from the Genbank® database [9].

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2. Material and methods

The choice of method to attempt the detection of CBPV involves the reaction and the Polymerase Chain Reaction [10] associated with a previous reverse transcription (RT-PCR).

In this sense, the appropriate temperatures for the nested-PCR protocol and the necessary functions to use must be known. For this, a free *online*biotool will be used: *Invitrogen's Oligoperfet Design*®.[11].

As an input for the above, the genome of Genbank® should be consulted, which consists of a positive single-stranded RNA virus that exhibits a worldwide distribution and subsequently align at least 20 of the known sequences and recognize conserved sequences among them that will serve as target sequences for the design of specific primers. These 20 sequences correspond to one of the segments of the virus and in this case it is the fragment known as RNA2, with a size close to 2300 nucleotides. (https://www.ncbi.nlm.nih.gov/nuccore/KU950354.1) and they were aligned using the Clustal Omega program in order to find 100% identical zones to apply the program that designs the primers. The access numbers used correspond to: MZ821998.1; MZ821996.1; MZ821994.1; MZ821992.1; MZ821990.1; MZ821988.1; MZ821986.1; MZ821984.1 ; MZ821982.1; MZ821980.1; MZ821978.1; MZ821976.1; MZ821974.1; MZ821970.1; MZ821968.1; MZ821966.1; MK637523.1; KX168413.1 ; OK491521.1; KU950354.1

3. Results

After the use of the Clustal Omega program [12]. The common nucleotide sequence chosen for the design of primers for RT-PCR corresponds to

>seq1
CAAGGCCTACGTTATCTCAGGTCCGGCAACCAGTTTGCTGTTCTTTCTAGGGCCGCCCAACGAACCTTCATCTGTCCATCTCAAT
ACATCGAATACACAACCATACCATTAGACTGTGACGAACTCAATGTGCGATGGCCCGTTCACGCTCAAGCTCCCGCAGACGCTC
TCGATCACTGAGCGCCAATCGTCGTCCGAGCCGACCACGCCGGTCCATTCTGGATCGCATCGGTCGTGCATTCCAGCGAGTCCTT
GCCAATCCAACTGCTGTGGTCGCCTTCGTCGTATGCGCGAT

Indicating in red letters the common nucleotides in the 20 sequences considered (considering the first 600 nucleotides of the RNA2 fragment). The design of indicates that the optimal matches correspond to P1: GCCTACGTTATCTCAGGTCCG and P2: ATCGCGCATACGACGAAGG, which generates a 291 bp DNA fragment (Figure 1)

Available Primers

Sequence	Length	% GC	Tm (°C)	Start	Stop
GCCTACGTTATCTCAGGTCCG	21	57.14	60.00	5	25
ATCGCGCATACGACGAAGG	19	57.89	60.66	277	295

Figure 1 Characteristics of the pair of primers designed for the generation of a fragment of 291 base pairs

For the nested PCR, the primers P3: GAACTCAATGTGCGATGGCC and P4: ATCGCGATACGACGAAGG are proposed (Figure 2)

Available Primers

Sequence	Length	% GC	Tm (°C)	Start	Stop
GAACTCAATGTGCGATGGCC	20	55.00	59.90	10	29
ATCGCGCATACGACGAAGG	19	57.89	60.66	166	184

Figure 2 Characteristics of the pair of primers designed for the generation of a fragment of 184 base pairs

4. Discussion

The detection of CBPV is a permanent challenge, especially for countries that do not have a diagnostic technique such as Chile. Currently, suspect samples must be sent to Montevideo, Uruguay. Designing a molecular diagnostic method using PCR and incorporating the nested variant would undoubtedly provide greater specificity by using two pairs of primers.

This type of molecular detection has been used in several experiences related to the detection of pathogens of veterinary interest together with obtaining the title of veterinary medical degree [13, 14, 15].

5. Conclusion

The design of this pair of primers constitutes a promising use of the PCR protocol, considering the Tm delivered by the program used. Without a doubt, the fabulous method invented by Kary Mullis constitutes a turning point in the molecular detection of any pathogen, be it viral, bacterial or parasitic, in conjunction with some molecular bio-tools.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors not declared conflict of interest.

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