NUCLEOTIDE SEQUENCES OF A MALARIA DNA PROBE AND A MOSQUITO LARVICIDAL ENDOTOXIN GENE AS DETERMINED BY THE SANGER 'S DIDEOXY METHOD

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ABSTRACT

The Sanger's dideoxy sequencing method was set up to determine the nucleotide sequence of two different cloned DNAs. One was a cloned DNA from the human malaria parasite, Plasmodium falciparum, pBR K1-14, that had been used as a probe to distinguish various clones and isolates of P. falciparum in Southern hybridization with restriction digested DNA. The other was a cloned DNA from Bacillus thuringiensis var. israelensis, pMU 388, containing the gene that produces a 130 kD mosquito larvicidal δ-endotoxin.

The inserted DNA from the recombinant plasmid, pBR K1-14 and pMU 388, were subcloned into bacteriophage M13 to obtain the single stranded DNA template for the sequencing reaction.

The complete sequence of 753 nucleotides of a K1-14 DNA obtained had a G+C content of 18%, 21 repeating units of 10 base pairs (consensus sequence TAAT$\overset{T}{\underset{C}{A}}A\overset{A}{\underset{T}{A}}AA\overset{A}{T}$), a running sequence of 16 adenine residues and a run of 11 thymine residues. The partial sequence of 577 nucleotides of δ-endotoxin gene was obtained containing a potential ribosome binding site of ten nucleotides, with the Shine-Delgarno sequence (GGAGG) located seven nucleotides upstream from the translation initiation codon ATG. The sequence for the first 142 amino acids of the crystal protein was also deduced.