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COMPARATIVE GENOMICS OF *STAPHYLOCOCCUS AUREUS* COAGULASE GENE

Kelmani Chandrakanth[1], Virupakshaiah DBM*, Gavimath C.C[2], Udaykumar Morabad[3], Kangralkar V.A[4].

1 Department of Biotechnology, Gulbarga University, Gulbarga. Karnataka, India.

* 2 &3. Department of Biotechnology, Basaveshwar engineering college, Bagalkot, Karnataka, India.

4. Department of Pharmacology, MM Pharmacy College, Belgaum. Karnataka, India.

ABSTRACT

Comparative Genomics is an attempt to take advantage of the information provided by the signatures of selection to understand the function and evolutionary processes that act on genomes. The sheer amount of information contained in the modern genomes necessitates that the methods of comparative genomics are automated. The comparative genomics is an up coming concept in the in molecular studies of pathogenic organisms. In our study we have selected *Staphylococcus aureus*, highly pathogenic bacteria for human beings. By its infection millions of people die world wide. Keeping this concept in our mind, we have selected *staphylococcus aureus* organism with respect to Coagulase gene which codes for enzyme coagulase, plays a vital role in blood coagulation in infected person. In our study we have selected Coagulase gene from two different strains of *Staphylococcus aureus* namely NCTC 8325 and Newman. By the use of bioinformatics resources and genomics-proteomics tools we have carried out comparative studies of coagulase genes from these two strains and also carried out restriction mapping of these two coagulase genes.

Keywords: GenBank, ExPASy, *staphylococcus aureus*, comparative genomics, Coagulase gene, serial cloner.

INTRODUCTION

In the early eighties, the word bioinformatics was not widely used and it was academics interest. The term bioinformatics first came in the use in 1990's because of human genome project. Today it is an emerging field and one of the leading segments in biotechnology and pharmacy industries. The bioinformatics is not only a subject of database but it has crossed beyond to become part and parcel of biological scientist.

Comparative genomics is the study of relationship between the genomes of different species or strains. Comparative studies of whole genome helps researchers to understand what part of the genome in one organism are similar to those in another, how the overall structure of genes and genomes have evolved, and what this findings tell us about the gene expression, gene regulation and how to interfere with this events in model organisms. Comparative genomics is also a critical enabling field for functional genomics because it gives researchers an indication of which model organism is most appropriate for particular study.

Staphylococcus aureus is a spherical or ovoid, non motile, gram-positive bacteria arranged in grape-like clusters on solid media. On agar the growth is opaque and of a golden or white color. It shows catalase positive, oxidase negative, aerobic and facultative anaerobic organism that require complex media for growth [1]. It causes the infection like skin, soft tissue, respiratory, bone, joint, and endovascular disorders. *Staphylococcus aureus* can grow on many selective media and presence of this organism can be confirmed by coagulase positive test. *Staphylococcus* produces various enzymes such as protease, lipase, beta lactamase and coagulase, a prothrombin activator, converts fibrinogen into fibrin. Coagulation is brought about by extra cellular substances often called free coagulase to distinguish it from bound coagulase or clumping factor. Coagulase is formed by Staphylococcus aureus. When coagulase positive *Staphylococcus aureus* are grown on plasma agar a zone of turbidity due to the deposition of insoluble fibrin appears around the colonies [2]. Coagulase enzyme gene approximately contains between 1800bp to 2100bp. In our comparative studies we have chosen two strains two knock out the differences in their restriction maps.

MATERIALS AND METHODS

For the present study bioinformatics online databases and software like serial cloner were used. The databases and the software used are as follows.

GenBank was developed by the national center for biotechnology information (NCBI) at the national library of medicine. The National Center for Biotechnology Information provides a comprehensive website for biologists that includes biology-related databases, and tools for viewing and analyzing the data inherent in the databases [4]. The protein sequences of Coagulase gene from two different strains namely NCTC 8325 and Newman of *Staphylococcus aureus* are retrieved. The ExPASy (Expert Protein Analysis System) is a proteomic server of the SWISS institute of bioinformatics (SIB) which analyses protein sequences and structures and two-dimensional gel electrophoresis [5]. The server functions in collaboration with the European Bioinformatics Institute (EBI). ExPASY also produces the protein sequence knowledgebase, UniProt KB /SWISS Prot, and its computer annotated supplement, UniProt KB/Trembl. Reverse translate tool is used to translate the protein sequence to nucleotide sequence.

Serial Cloner is the molecular biology software. It provides tools with an intuitive interface that assists you in DNA cloning, sequence analysis and visualization. Serial Cloner is available for MacOSX, MacOS9 and Windows. Serial Cloner has been developed to provide light molecular biology software to both Macintosh and Windows users. Serial Cloner reads and write DNA Strider-compatible files and import and export files in the universal FASTA format (as well as in pDRAW32 format). Powerful graphical display tools and simple interfaces help the analysis and construction steps in a very intuitive way. All the tools you need to analyze and manipulate your sequences are available in an all-in-one-window concept. Numerically select fragments, find restriction sites, ORF or any motif,

calculate Tm of selected fragments, %GC or dynamically determine the translation your selection into peptide and calculate the MW using a compact interface. Serial Cloner also lets you build restriction map and quickly format it to add multi-frame translation or only show single cutters for example. The graphic map of Serial Cloner is really graphic as you can easily select and extract a fragment or show single, double or multiple cutters all in the same window. Serial Cloner will assist you in setting-up new sub-cloning projects and in preparing the electronic versions of your constructs. The main window of serial cloner is shown in figure 1

RESULT

Result was obtained by retrieving protein sequences of Coagulase gene of two different strains of *Staphylococcus aureus* namely NCTC 8325 and Newman. The nucleotide length of each gene is 1908nt and 1905nt respectively. This protein sequences were converted into nucleotide sequences by using online ExPASy (Reverse transcript tool). This converted nucleotide sequences of Coagulase gene (FASTA format) was then pasted in the main sequence field of the tool 'serial cloner' and the results (comparison of Coagulase gene) were obtained in the form of images as shown in the figure.

Figure 2 and figure 3 shows the graphical restriction maps of NCTC 8325 and Newman strains of *Staphylococcus aureus* respectively which clearly shows that the restriction enzyme BcgI and NspI are present in the strain NCTC 8325 but they are absent in Newman strain of *Staphylococcus aureus*. Thus the position of restriction sites for NspI and BcgI was found to be located at 627^{th} to 632^{nd} and 632^{nd} to 644^{th} ntds respectively as highlighted in figure 2 and figure 3.

Figure 4 indicates the sequence field of serial cloner pasted with the nucleotide sequence of Coagulase gene of NCTC 8325 strain of *Staphylococcus aureus* and figure 5 indicates the sequence field of serial cloner pasted with the nucleotide sequence of Coagulase gene of Newman strain of *Staphylococcus aureus.* By using 'Find' option we found out restriction sites for BcgI and NspI which are 'cgaaagaactgc' and 'acatgc' respectively (figure 4, figure5,figure 6 and figure 7)

By close observation of fig 4,fig 5,fig 6, and fig 7 (results of the tool serial cloner) we can find out that there is a presence of an extra 'gcg' sequence in NCTC 8325 strain in between 631st to 633rd nucleotides which is absent in Newman strain of Staphylococcus aureus. Hence we can predict that due to the lack of this extra 'gcg' sequence in Newman strain there is an absence of restriction sites for NspI and BcgI restriction enzymes in between 627^{th} to 644^{th} ntds but its present in NCTC 8325 strain of *Staphylococcus aureus.* This lack of extra 'gcg' sequence could have been occurred due to any sort of mutation.

DISCUSSION

By preparing the graphical map in the tool serial cloner we have found out the absence of particular restriction sites namely NspI and BcgI in Newman strain of *staphylococcus aureus* and by using the option 'Find' of the tool serial cloner we've found out the exact location of the differences in between the Coagulase

gene sequences of two strains of *Staphylococcus aureus. The difference found was the lack of an extra 'gcg' sequence in Newman strain resulting in the absence of two particular restriction sites of restriction enzymes NspI and BcgI.*

serial cloner, we can come out with exact location of mutation in different strains of same species and we can also carryout comparative genomic studies in between two different species [3].

CONCLUSION

Thus by using the concept of comparative genomics and bioinformatics tools like

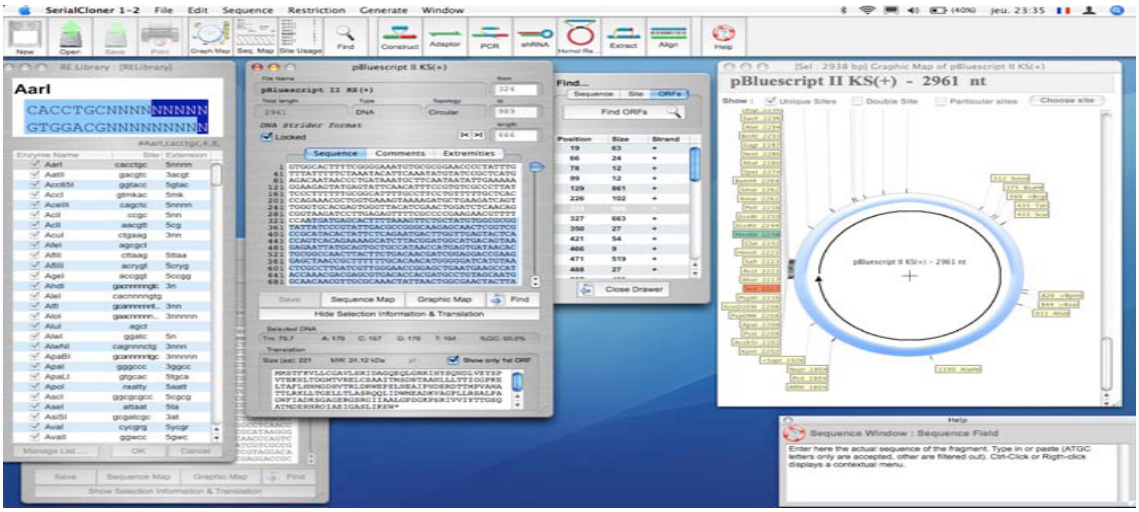

Figure1

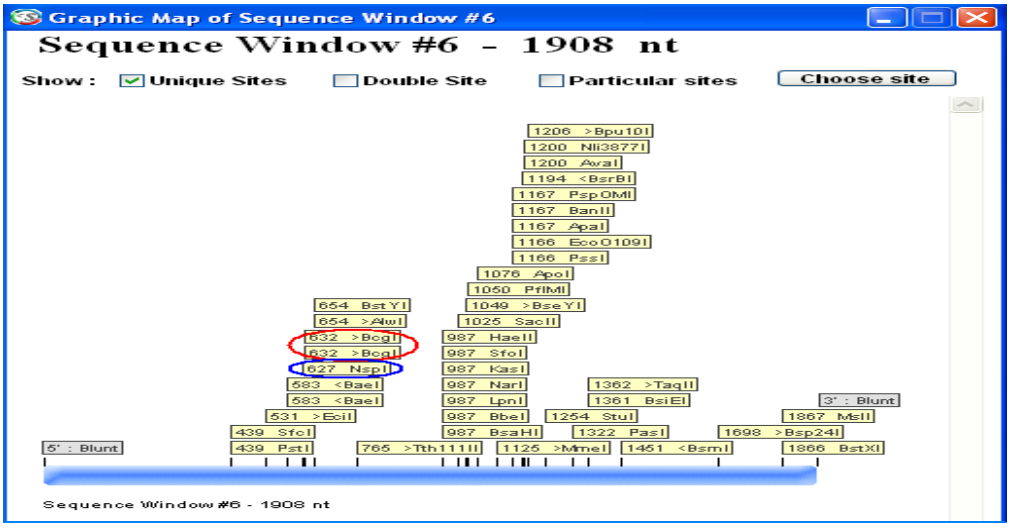

Figure2

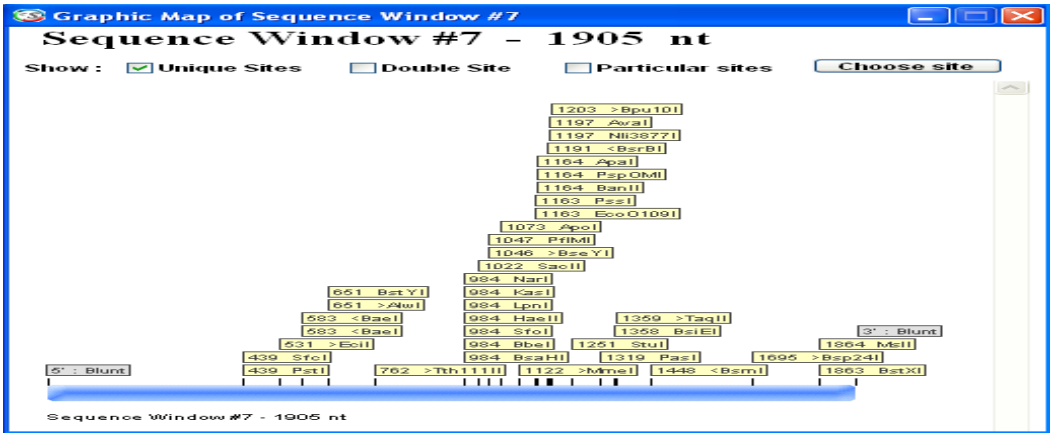

Figure 3

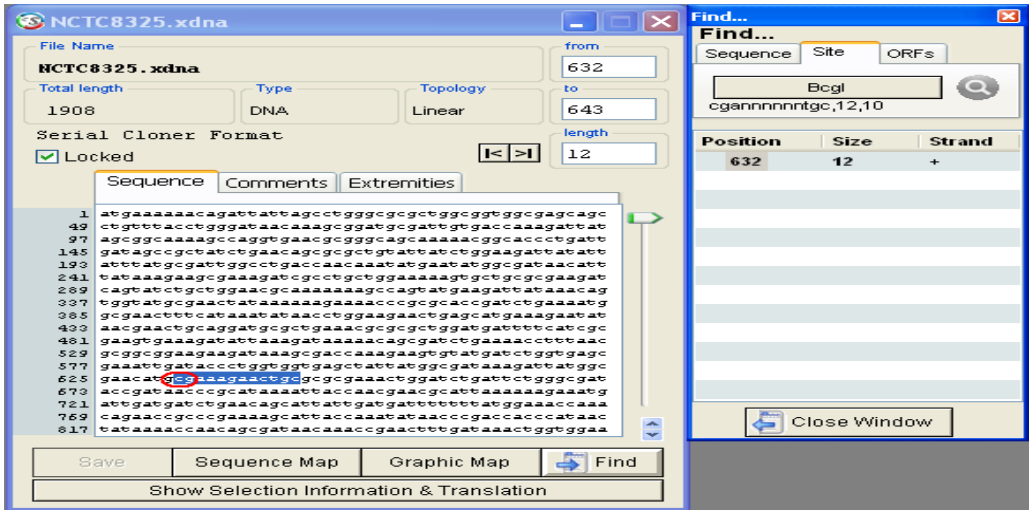

Figure4

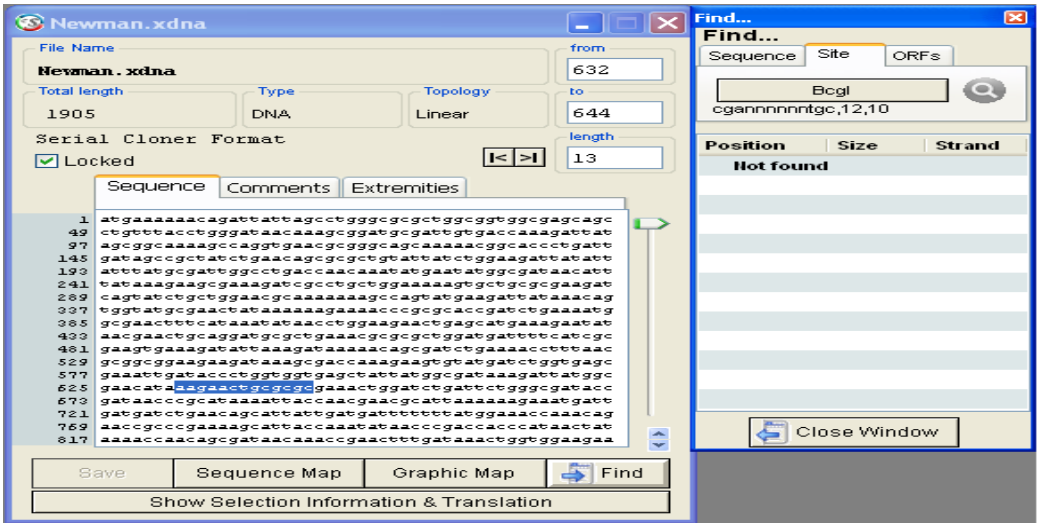

Figure5

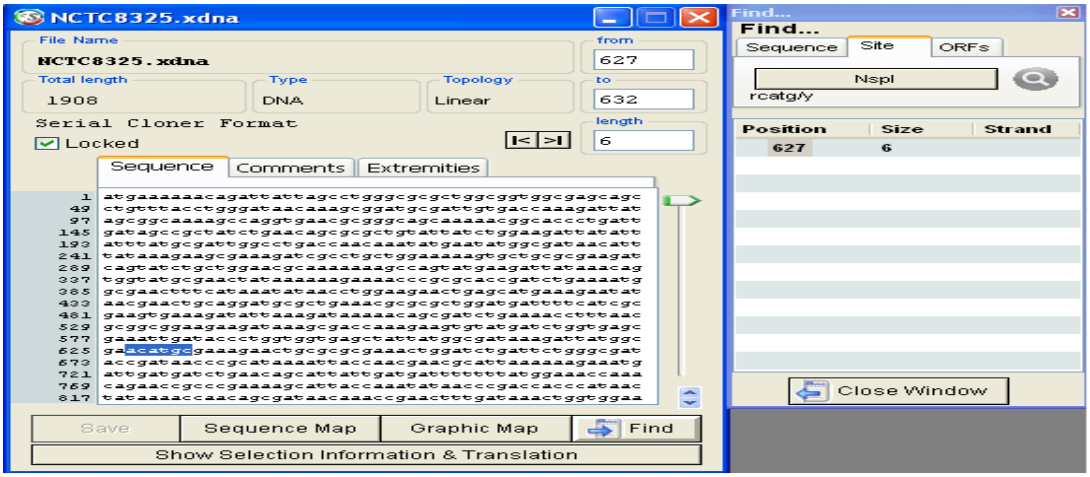

Figure6

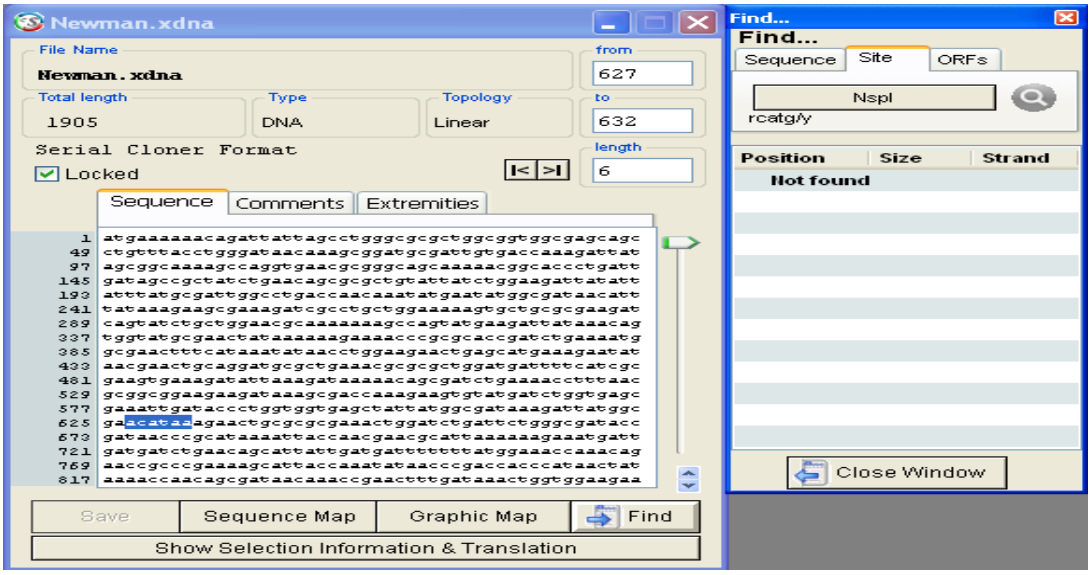

Figure7

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