939/E2/Poster

Screening of bacteria for restriction enzymes

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Restriction endonucleases (REs) are enzymes that are capable of cleaving double stranded DNA in a sequence specific manner, irrespective of the source of DNA. The cleavage occurs either within or adjacent to the restriction site (recognition sequence). The majority of recognition sites are four, six or eight bases long and are palindromic. There are four major types of restriction enzymes and are designated type I, II, III and IV. This classification is based on their subunit structure, cofactor requirements, specificity of cleavage and associated methylase activity. Restriction enzymes have a wide variety of applications in genetic engineering including cloning, molecular diagnosis of diseases etc. There is a growing demand for restriction endonucleases exhibiting new specificities. To date no research has been carried out to screen and/or isolate REs from the microbial flora in Sri Lanka. Being a tropical island with a rich microbial diversity it is likely that many bacteria producing novel REs could be identified. The objective of this study was to screen bacteria for isolation of restriction enzymes. Bacteria from different regions and habitats in Sri Lanka including Matale, Matara, Galle, Anuradhapura and Kandy were isolated from soil and /or water samples collected into sterile tubes (50 ml). Five milliliter cultures of the isolated colonies were grown in LB broth medium. The bacteria were then screened for restriction enzymes. Briefly, an extract of bacteria was made by disintegrating the cell mass by sonication. After centrifugation the supernatant was collected and used directly in the screening assay. The assay was carried out by incubating lambda DNA with the extract. To determine the presence of restriction enzyme(s) an aliquot of the reaction mixture was separated by agarose gel electrophoresis. The presence of distinct bands indicates the presence of restriction endonucleases. Initial screening of many isolates revealed the presence of restriction enzymes in two isolates. The putative restriction enzymes cleaved lambda DNA producing several bands. Further characterization of the bacteria is in progress.

Keywords: Restriction enzymes

Acknowledgement: National Science Foundation, Research grant RG/2014/BT/02

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