"GENETIC MANIPULATION OF 'COIRRET' FOR THE APPLICATION ON COIR FOR QUALITY IMPROVEMENT"

AIM

To improve the performance of 'COIRRET' and to formulate a novel eco-friendly microbial consortium for field use on coir fibre/yarn.

INTRODUCTION

Genetic Manipulation is changing the hereditary characteristics of plants and animals by modifying the genetic material. Genetic engineering also called genetic modification, is the direct human manipulation of an organism genome (organism hereditary information) using modern DNA Technology.

Artifical manipulation, modification and recombination of DNA or other nucleic acid molecules inorder to modify an organism or population of organism. The term initially meant any of a wide range of techniques for modifying or manipulating organisms through heredity and reproduction. Now the term denotes the narrower field of recombinant DNA technology, or gene cloning, in which DNA molecules from two or more sources are combined, either within cells or in test tubes, and then inserted into host organisms in which they are able to reproduce.

This technique is used to produce new genetic recombinations that are of value to science, medicine, agriculture or industry. Genetic manipulation has a lot of advantages, but it also comes with a lot of risks.

COIRRET

The 'COIRRET' is a consortium of microbes: ***Mycoplana bullata, Mycoplana dimorpha and Pseudomonas desmolyticum*** belongs to the family actinomycetes.. Coirret is a formulation of phenolic bacteria. They posses the ability of degrading phenolic compounds. Central Coir Research Institute (CCRI) has developed this bacterial consortium 'COIRRET'. (Das A.R,2001)

By applying coirret on mechanically treated fibers we can reduce the retting period from 11 month to 3 month and improve the quality of green husk fiber within 72 hrs. 'COIRRET' is a bacterial cocktail which biosoftens in just 3 days. This process ensure a good quality fiber with less effluent problems.

In the present work, coirret bacterial strains: *Mycoplana bullata (NCIM 2382), Mycoplana dimorpha (NCIM 2383) and Pseudomonas desmolyticum (NCIM 2028)* obtained from National collection of Industrial Micro organism (NCIM), Pune. These bacterial strains were isolated and used for phenol degradation and retting.

OBJECTIVES

Development of an eco friendly biological package for retting coconut husk/coir fibre tanks by the application of biotechnology.

To produce superior quality fibre for the coir industry.

The project target improving the performance of 'coirret' on coconut husk and coir fibre by genetic manipulation.

APPLICATION OF BIOTECHNOLOGY IN COIR INDUSTRY

- It may be well adopted for coir softening and brightening.
- New eco-friendly methods for fibre production having the potential to produce more consistent quality of fibre are of interest.
- Microbial treatment will produce soft, white fibres having better tensile strength and elongation properties. Expected outcome will ensure that the coir fibre achieve the desired softness required for faultless spinning without end breaks and hairiness.
- In coir sector, process efficiency can be achieved by means of using biotechnological tools i.e. by altering the genetic make up of a particular strain for a desired purpose using genetic engineering techniques. All the information process by biotechnology is stored and analysed using bioinformatics softwares.
- Sequence analysis is the use of various bioinformatics methods and tools to determine the biological function and structure of genes and the proteins they code for. Thus we can produce an improved strain having better characteristics. Therefore special techniques have to be formulated to explore the possibilities of enzymatic quality improvement of coir fibre. There by we can minimize the environmental issues related to the coir industry.

EXPERIMENTAL DESIGN

1. To isolate pure cultures of the stains in COIRRET
2. Morphological and biochemical analysis of the bacterial strains.
3. To isolate DNA from 24 hour old broth culture of each of the bacterial strain
4. PCR amplification of DNA with 16s rRNA primer followed by sequencing of the DNA.
5. Sequence analysis by using bioinformatics tools.
6. To perform sequence similarity search by using bioinformatics software to compare the sequence of a particular gene with sequence from other related organisms.
7. Determination of quality and quantity of enzyme production by the bacterial strains.
9. To identify whether the DNA is plasmid or genomic DNA.
10. Physical mutagenesis of the 3 bacterial strains.
11. Determination of quality and quantity of enzyme production by the mutated bacterial strains.
12. Isolation of DNA from the 3 mutated bacterial strains.
13. Designing primers using primer3 bioinformatics software for PCR amplification of the isolated DNA.
14. Sequencing the mutated organism and with the help of Refseq bioinformatics software, check whether the mutation has occurred in the genes coding for enzymes tannase, cellulose, xylanase, pectinase, tannase and laccase etc.
15. Testing of improvement in quality of COIRRET by application on coir fibre (In accordance with the Biosafety rules and regulation guidelines).
16. Testing the quality improvement of coir fibre in brightness, softness and flexural rigidity, tenacity, elongation and breakage force etc and the scanning electron microscope (SEM) analysis and Fourier transform infrared spectroscopy (FTIR) studies.
17. To develop a bio-package for husk retting in the tanks using microbes isolated from the stressed environment.

Flow Chart-

Schematic Representation of "Genetic Manipulation Of 'Coirret' For The Application On Coir For Quality Improvement"

- *COIRRET*

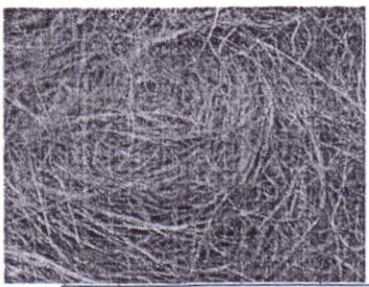
- *Modifying 'COIRRET' by incorporation of new phenol degrading strains.*

- *Study of DNA in all strains*

- *Genetic Manipulation of the DNA in all strains.*

- *Laboratory scale trials on treatment of coir fibre using the modified 'COIRRET'*

- *Assessment of bio brightening / biosoftening of coirfiber by new consortium through tests.*



- *Scale up studies on coir fibre*

- *Standardisation of production of modified 'COIRRET' for large scale treatment.*

- *Standardisation of dosage of 'COIRRET' for treatment on coir in large scale.*

- *Demonstrations of treatment of coir entrepreneur through field trials.*