RIBOTYPING

SUBMITTED BY-

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SYNOPSIS

- Introduction
- History
- Microbial Systematics

- Technique used in Microbial Systematics Analysis
  - Phenotypinc Analysis
  - Genotypic Analysis
  - Phylogenatic Analysis

- Application of Phylogenetic Method
  - Signature Sequences
  - Phylogenetic probes and FISH
  - Microbial Community Analysis
  - Ribotyping.

- Application of Ribotyping
- Disadvantages of Ribotyping
- Summary
- Conclusion
- References

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INTRODUCTION

- Variations are found in different organisms are called as diversity of life.
- Systematics is the study of the diversity of organisms and their relationship. It links together phylogeny, just discussed, with **taxonomy.**
- **Taxonomy** uses three kinds of methods .Phenotypic, Genotypic, and Phylogenetic for the identification of bacteria.
- Information from rRNA-based phylogenetic analysis also finds application in a technique for bacterial identification called **Ribotyping.**

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HISTORY

- **CAROLUS LINNEUS** is a Swedish biologist originated the present method of classification.
- He is regarded as father of **Taxonomy**.

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MICROBIAL SYSTEMATIC

- **Systematics** is the study of the diversity of organisms and their relationships.
- Organisms are characterize, named, and placed into group according to their natural relationship
- Basic elements of bacterial taxonomy- the identification of bacterial classification and nomenclature.
- Taxonomy uses three kinds of methods phenotypic, genotypic, and phylogenetic, for the identification and description of bacteria.

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TECHNIQUE USED

Technique used in microbial systematic Analysis

1. Phenotypic Analysis:

Phenotypic analysis examines the morphological, metabolic and chemical characteristics of the cell. the observable characteristics of a bacterium provide many traits that can be used to differentiate between species.

Example :

Fatty Acid Analysis: FAME

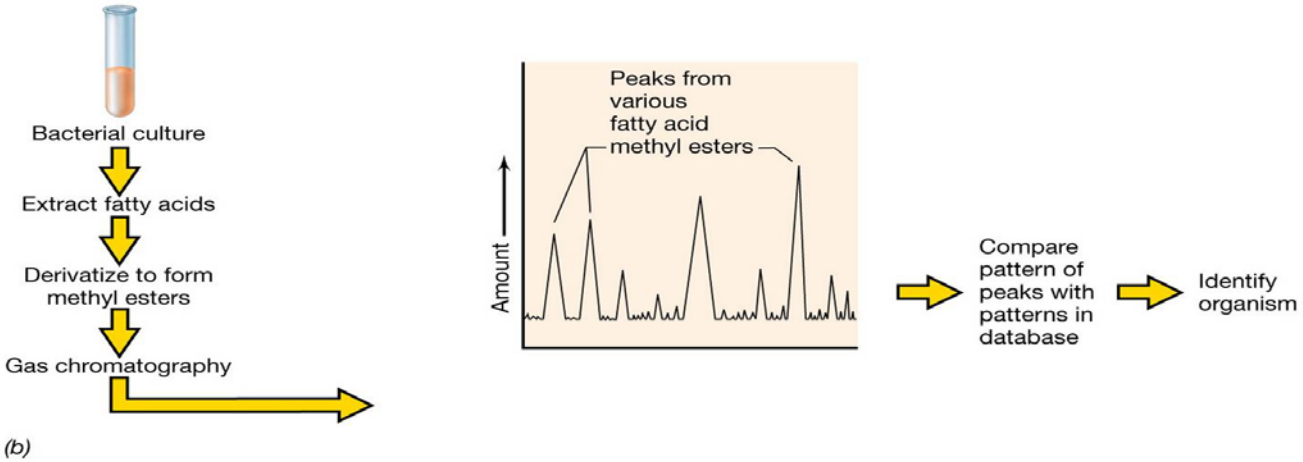

Fig : 1. FAME analysis in bacterial identification

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TECHNIQUE USED

2. Genotypic Analysis

- Genotypic Analysis consider comparative aspects of cells at the level of the genome.
- Genotypic analysis has particular appeal in microbial taxonomy because of the insights it provides at the DNA level.
- Several Methods of genotypic analysis are used.

1. DNA-DNA hybridization
2. DNA profiling methods
3. Multilocus sequence typing

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TECHNIQUE USED

3. Phylogenetic Analysis :

- Phylogenetic Data are being used increasingly in microbial taxonomy to complement phenotypic and genotypic information.

- Phylogenetic analysis is beginning to use multiple gene sequences to describe and identify organisms

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APPLICATION

Application of Phylogenetic Methods:

- Signature sequences
- Phylogenetic Probes and Fish
- Microbial Community Analysis
- **Ribotyping**

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APPLICATION

a. Signature Sequence :

- Signatures defining a specific group within a domain or, in some cases, a particular genus or even a single species, are also known or can be determined by computer inspection of aligned sequence.
- Signature sequence can help place newly isolated or previously misclassified organisms into their correct phylogenetic group.
- The most common use of signature sequences is for the design of specific nucleic acid probes.

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b. Phylogenetic probes and FISH :

- Probe is a strand of nucleic acid that can be labelled and used to hybridize to a complementary nucleic acid from a mixture.
- Probes can be general or specific.
- This technique is called FISH for fluorescent in situ hybridization.
- It is used in microbial ecology and clinical diagnostics.

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APPLICATION

c. Microbial community Analysis :

- PCR is used to amplify genes encoding SSUrRNA from membrane to that community.

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APPLICATION

d. Ribotyping :

Principle of Ribotyping :

- Ribotyping is a molecular technique that takes advantage of unique DNA sequences to differentiate strains of bacteria.

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What is Ribotyping :

- Ribotyping is a method that can identify and classify bacteria based upon differences in rRNA.
- It generates a highly reproducible and precise fingerprint that can be used to classify bacteria .
- DNA is extracted from a colony of bacteria and then restricted into discrete-sized fragments.

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- The DNA is then transferred to a membrane.
- The pattern is recorded, digitized and stored in a database.
- It is variations that exist among bacteria in both the position and intensity of rRNA bands that can be used for their classification and identification

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Why Ribotype:

- Ribotyping allows the establishment of unequivocal relationships between bacterial isolates recovered from any of these sources and the finished product.
- Molecular epidemiology A number of diseases in animals that are caused by bacteria can be traced to the consumption of contaminated feed.
- Ribotyping can help to identify the contaminated feed source for elimination.
- Identification RiboPrint patterns can be used to identify a bacterium if that pattern is in the database.

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PROCEDURE

- Extract the genomic DNA from your bacterial isolate.
- Cut it with a specific restriction enzyme.
- Run the DNA in an agarose gel by electrophoresis.
- DNA fragments are separated by size as they move through the gel
- Transfer the DNA pieces to a nylon membrane.

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PROCEDURE

- Incubate the nylon membrane with a specific enzyme-linked DNA probe (a DNA fragment that hybridizes to the genes coding for 16S and 23s rRNA).
- Wash the nylon membrane and add the enzyme substrate to produce a chromogenic or fluorescent signal and visualize the pieces of DNA of interest.
- In the case of chromomeric signal the reaction will be seen in the nylon membrane. In the case of fluorescent reaction the nylon membrane is coupled with a photographic film and the results are seen on film.

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PROCEDURE

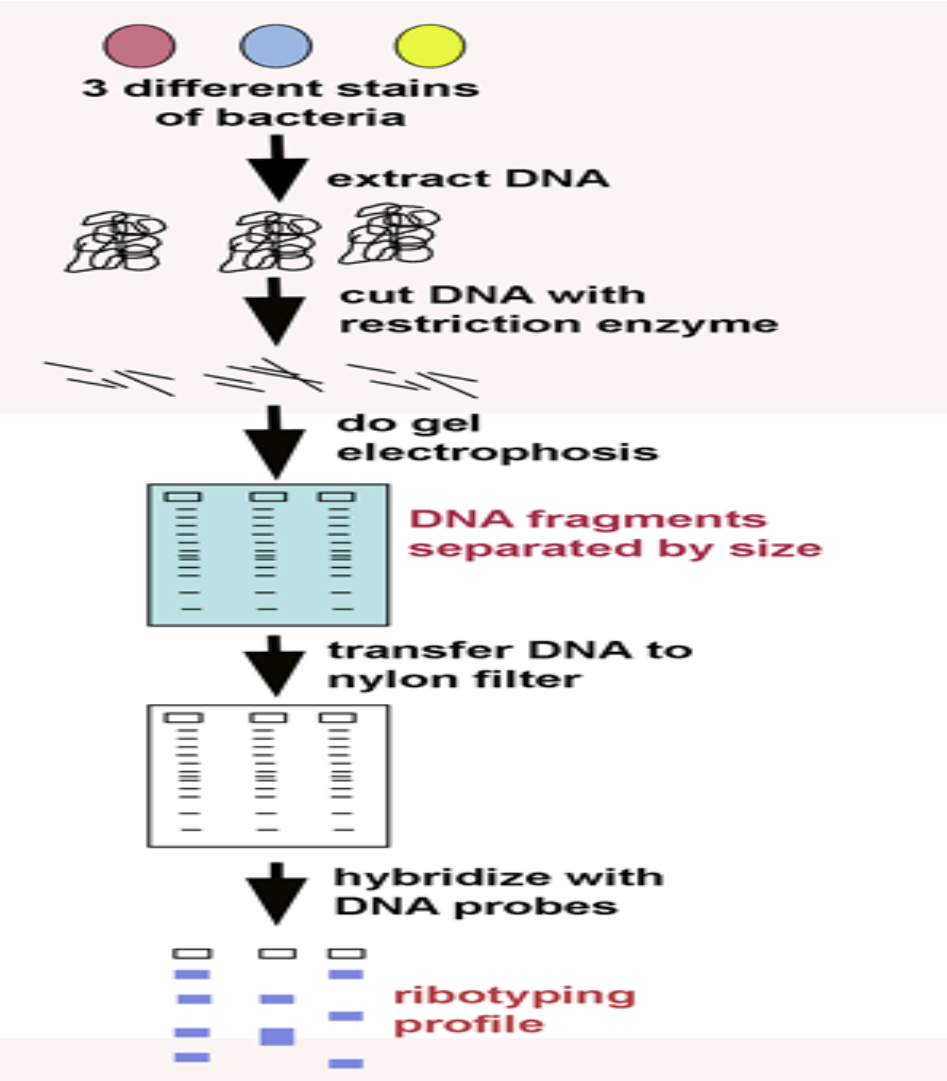

Fig: 2 Procedure of Ribotyping

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APPLICATION

- For bacterial identification in clinical diagnostics.
- Microbial analysis of food water and beverages.
- Allows you to differentiate different strains of bacteria in a very sensitive manner.

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DISDVANTAGES

- Must carefully choose probe so there is no cross reactivity.
- Must carefully choose probes so that they successfully bind to sequences.

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SUMMARY

Taxonomy uses three kinds of methods phenotypic, genotypic, and phylogenetic, for the identification and description of bacteria

Phylogenetic Data are being used increasingly in microbial taxonomy to complement phenotypic and genotypic information.

Information from rRNA-based phylogenetic analysis also finds application in a technique for bacterial identification called Ribotyping.

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CONCLUSION

- Ribotyping is a method that can identify and classify bacteria based upon differences in rRNA.

- Ribotyping is a molecular technique that takes advantage of unique DNA sequences to differentiate strains of bacteria.

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REFERENCES

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THANK YOU !