Curriculum vitae

1. Name: Anurag Singh
2. Father's Name: Shri Rishi Deo Singh
3. Mother's Name: Smt Indra Wati Singh
4. Date of Birth: 21 - 09- 1981
5. Address: Department of Microbiology
Dr. Rammanohar Lohia Avadh University, Faizabad
Email: anurag_singh57@yahoo.com
Mobile no. 8960700844

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- **<u>ACADEMIC QUALIFICATION</u>:**

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- CSIR-UGC NET JUNE-2014(Life Sciences) QUALIFIED (A.I.R.-23 rank)
- ICAR-ASRB NET 2013(Agricultural Biotechnology) QUALIFIED
- ICAR-ASRB NET 2010 (Basic Plant Science) QUALIFIED
- GATE 2007 & 2009 (Life Sciences) QUALIFIED
- CET-Ph.D.-2012 QUALIFIED Conducted by Dr.R.M.L.Avadh University, Faizabad,U.P.
- Ph.D. admission on June 2015, after successful completion of Course work Ph.D. registered on topic *"Continuous decolorization of distillery effluents(Molasses Melanoidin) by indigenous microbe consortia"* and continue under supervision of guide Professor (Dr.) Rajeeva Gaur, Department of Microbiology(Centre of Excellence), Dr. R. L. Avadh University, Ayodhya(Faizabad) U.P.(224001), India.
- M.Sc. biotechnology from JIWAJI University , Gwalior, M.P. in 2007
- B.Sc. from Dr.RML Avadh University, in 2002
- 12^{th} from U.P Board in 1998
- 10^{th} from U.P Board in 1996

➢ RESEARCH AND TEACHING EXPERIENCE:

- 6th month project work on-*" Phylogenetic analysis of Anopheles culicifacies sibling species B & C"*, in Malaria Research Centre New Delhi, India (ICMR institute) based on Molecular biology & Bioinformatics(2007) for partial fulfillment of M.Sc degree.

- Got a short term training from January to December 2010 on I.P.R.(Intellectual property right), under supervision of Mr. Ajitabha Pandey(consultant I.P.R.), New Delhi.

- Joined as **S.R.F**. on project entitle – "*Harnessing of arbuscular mycorrhizae for biofertilization in horticulture*" in G. B. Pant University of Agriculture and Technology ,Pantnagar (from March 2011 to June 2011)

- Joined Dept. of Microbiology, Dr. R.M.L. Avadh university Faizabad as Reseach Scholar in June 2013 under supervision of Dr. Rajeeva Gaur

- Joined as a **Guest lecturer** (P.G.Diploma in Biotechnology) at Government Polytechnic College Faizabad,U.P.(from 1st July 2014 to 30 June 2015).

- Joined as a **Project Fellow** in U.G.C. Major Research Project on 28 August 2015 entitled *"Continuous decolorization of distillery effluent(Molasses Melanoidin) by consortium of Bacterial and Yeasts strains"* and successfully completed on 28 August 2018 under supervision of Professor(Dr.) Rajeeva Gaur, Department of Microbiology, Dr. R. L. Avadh University, Ayodhya(Faizabad), U.P., India.

- Joined Dept. of Microbiology, Dr. R.M.L. Avadh University as a **Guest lecturer**(from 6th September 2019 to Till date)

➢ PUBLICATION::

- R.K.Mishra, R.K.Jaisawal, D.Kumar,P.R.Saable, A.Singh (2014) "Management of major disease and insect pests of onion and garlic" Journal of Plant Breeding and Crop Science. 6(11):160-170 I.F.-0.59, NAAS Rating -6

- Tiwari S, Gaur R and Singh A (2014). "Distillery Spentwash Decolorization by a noval Consortium of *Pediococcus acidilactici* and *Candida tropicalis* under Static Condition" Pakistan Journal of Biological Sciences. 17(6):780-791. I.F-0.73, Citation-01, H-index-26

- Gaur R., Singh A., Tripathi A., Singh R. (2016) Bioreactors. In: Principles and Applications of Environmental Biotechnology for a Sustainable Future, editors Ram Lakhan Singh. Springer Singapore. pp 233-272

- Rajeeva Gaur, Anurag Singh, Soni Tiwari, A. Tripathi (2017) "A noval strain of Bacterium, *Arthrobactor* sp. use for decolorization Melanoidin of distillery effluent*"* Indian Journal of Agriculture and allied science. pp 2455-9709 Volume: 3, No.: 4.

- Gaur R., Singh A., (2019) Microbial Enviornment of Food. In: Food Safety and Human Health, editors Ram Lakhan Singh and Sukanta Mondal. Elsevier. pp 198-218

➢ CONFERENCE:

- Oral presentation of a paper entitled "Naturally isolated *Saccharomyces cerevisiae* strain for suppression of bacterial contamination in alcoholic industries" in National Conference on Biodiversity, Conservation Strategies & Sustainability of Medicinal Plants for their Pharmaceutical use in Human Health and prosperity (from august 24-25,2013), M.G.P.G. College Biotechnology and Molecular Biology Centre Gorakhpur (U.P.), India

- Poster presentation of a paper entitled *"* Decolorization of distillery effluent by a thermotolerant *Candida utilis*" in International Conference on Health, Environment & Industrial Biotechnology (from November 21-23,2013) Department of Biotechnology , Motilal Nehru National Institute of Technology Allahabad(U.P.), India

- Participated in seminar on "Role of CST, U.P. in Promotion of Science & Technology & Facilitation of IPR Protection" from 8 to 9 December 2016 organized by V.B.S. Purvanchal University, sponsored by CST, U.P.

- Poster presentation of paper entitled "A noval strain of Bacterium, *Arthrobactor sp*. use for decolorization Melanoidin of distillery effluent*"* in international conference on "Agricultural, Allied Sciences & Biotechnology for sustainability of agriculture, nutrition and food Security" from 25-26 November, 2017 organized by Mahima Research Foundation and Social Welfare(194, Karaundi, B.H.U. Varanasi, U.P., India)

- Oral Presentation of paper entitled "Evaluation of fermentation kinetics for decolorization potential of *Bacillus* and *Aureobasidium* sp." in 22nd international conference of international Academy of Physical Science on Emerging trends in physical Sciences from April 13-15, 2018 organized by Faculty of Science, Dr. R.M.L. Avadh University, Ayodhya (Faizabad), U.P.,India

- Oral Presentation of paper entitled ".Isolation and Evaluation of Melanoidin Degradation of Distillery Effluent by Bacterial Strains" In National conference of Department of Higher Education, Uttar Pradesh on Interdisciplinary Advancement in Biochemistry from March 28-29, 2019 organized by Department of Biochemistry, Dr. R.M.L. Avadh University, Ayodhya (Faizabad), U.P., India

➢ WORKSHOP:

- Successfully completed National Training Programme on "Molecular Technique to Assess Microbial Community Structure and Identification" from 2nd -9th January, 2017 organized by ICAR-NBAIM, Kushmaur, Maunath Bhanjan(U.P.), India

- Successfully completed Workshop on "Gene cloning & its expression to produce genetically Modified organism" from 23rd-25th October, 2017 organized at Department of Microbiology, Dr. R.M.L. Avadh University in association with Cytogen Research & Development, Lucknow(U.P.), India

- Member of organizing committee in 7 days workshop organized at Department of Microbiology Dr. R. L. Avadh University Ayodhya in Collaboration with Cytogene Research & Development, Lucknow from 03-09 September 2019.

➢ INVITED LECTURES:

- Invited lecture on "*Food & Food productsand preservation of different food materials*" in Govt. Fruit Preservation Centre, Faizabad on 25th October 2018.

- Invited lecture on "*Biochemical properties of food materials*" in Govt. Fruit Preservation Centre, Faizabad on 6th October 2019.

- Invited lecture on "*Microbial Environment of food*" in Govt. Fruit Preservation Centre, Faizabad on 28th February 2019.

➢ AREAS OF INTEREST:

- Plant Biotechnology, Microbiology

Mr. ANURAG SINGH
ASSISTANT PROFESSOR(GUEST)
DEPT. OF MICROBIOLOGY

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Date: 04.08.2020

To,

Head of Department/Coordinators/Director I.E.T.
Dr. Rammanohar Lohia Avadh University,
Ayodhya

Subject: Regarding information of the faculties on format attached.

Dear All,

As per the discussion with Hon'ble Vice Chancellor regarding display of complete profile of all the faculty members working on Regular/Contract/Guest faculty posts in various regular and self finance courses running in your department is to be displayed on university website.

You are therefore, requested to send detail bio-data and information of all faculties working in your department on the format attached herewith along with related documents. The work is urgent and time bound. Please ensure that hard copy of bio data and the information on the format with annexure should reach the office of Director, IQAC and soft copy of the same in pdf format on email-**iqac@rmlau.ac.in** latest by **10-08-2020.**

You are also requested to assign a coordinator in respective department for this work and send the details of the coordinator on following format.

Name of the Department	Name of Coordinator	Email Address of Coordinator	Mobile No. of Coordinator

Director
IQAC

Proforma for Faculty Profile

S.No.	Department	Name of the Teacher	Designation	D.O.B	Post Type (Regular / Contract / Guest)	Date of Joining in this University	Total Teaching Experience	Professional Experience	Research Experience	Total Publication till Date	Publication During Last 05 Years	Project/Patent/ Awards During Last 05 Years	E-Contents Developed During Last 02 Years
1.	Microbiology	Mr. Anurag Singh	Guest faculty	21/09/1981	Guest	06/09/2019	1 years	-	3 years	5 (Annexure-I)	3 (Annexure-II)	-	M.Sc 3rd Sem. Paper I, E- Content (ppt), MSc 3rd Sem. Microbiology Lab (links). https://s.docworkspace.com/d/AJPj7G-9k7ZE4ajygdydFA
2.													
3.													
4.													
5.													
6.													
7.													
8.													
9.													
1													

Note:

1. Please attach evidence of each of the field mentionabove.
2. Please provide complete Bio-Data in hard copy in the office of the IQAC by 10-08-2020 and soft copy of Bio-Data and evidences inpdf format on email-**iqac@rmlau.ac.in** . Please write your name and department in subject field of email.

Director
IQAC

Annexure-1

1) R.K.Mishra, R.K.Jaisawal, D.Kumar,P.R.Saable, A.Singh (2014) "Management of major disease and insect pests of onion and garlic" Journal of Plant Breeding and Crop Science. 6(11):160-170 NAAS Rating -6
2) Tiwari S, Gaur R and Singh A (2014). "Distillery Spentwash Decolorization by a noval Consortium of *Pediococcus acidilactici* and *Candida tropicalis* under Static Condition" Pakistan Journal of Biological Sciences. 17(6):780-791. I.F-0.73, Citation-01, H-index-26
3) Gaur R., Singh A., Tripathi A., Singh R. (2016) Bioreactors. In: Principles and Applications of Environmental Biotechnology for a Sustainable Future, editors Ram Lakhan Singh. Springer Singapore. pp 233-272
4) Rajeeva Gaur, Anurag Singh, Soni Tiwari, A. Tripathi (2017) "A noval strain of Bacterium, *Arthrobactor* sp. use for decolorization Melanoidin of distillery effluent*"* Indian Journal of Agriculture and allied science. pp 2455-9709 Volume: 3, No.: 4.
5) Gaur R., Singh A., (2019) Microbial Enviornment of Food. In: Food Safety and Human Health, editors Ram Lakhan Singh and Sukanta Mondal. Elsevier. pp 198-218

Annexure-2

1) Gaur R., Singh A., Tripathi A., Singh R. (2016) Bioreactors. In: Principles and Applications of Environmental Biotechnology for a Sustainable Future, editors Ram Lakhan Singh. Springer Singapore. pp 233-272
2) Rajeeva Gaur, Anurag Singh, Soni Tiwari, A. Tripathi (2017) "A noval strain of Bacterium, *Arthrobactor* sp. use for decolorization Melanoidin of distillery effluent*"* Indian Journal of Agriculture and allied science. pp 2455-9709 Volume: 3, No.: 4.
3) Gaur R., Singh A., (2019) Microbial Enviornment of Food. In: Food Safety and Human Health, editors Ram Lakhan Singh and Sukanta Mondal. Elsevier. pp 198-218

Paper MB301(Semester III)

Evolution & Microbial Taxonomy

UNIT-II

Presented by

Mr. Anurag Singh

(Assistant Professor-Guest)

Depatment of Microbiology

Dr. R M L Avadh University,Ayodhya

U.P.,India(224001)

Biochemical Methods in Microbial Taxonomy(Chemotaxonomy) :

➢Chemotaxonomy; is the whole of the characterizations made by using the similarities and differences of the biochemical properties of bacteria. In bacterial systematics, chemotaxonomy examines biochemical markers such as: amino acids and peptides (peptidoglycan), lipids (fatty acid, lipopolysaccharides, micolic acid and polar lipids), polysaccharides and related polymers (teicoic acid, whole sugar) and other complex polymeric compounds to find the distribution of members of different taxa and all of this information is used for classification and identification.

➢In general, the characters used in bacterial chemotaxonomy are; peptidoglycan, diamino acids, polysaccharides, teichoic acids, fatty acids, polar acids, isoprenoid quinones, polyamines, prokaryotic pigments and LPS, nucleic acids, proteins, amino acids

Peptidoglycan

➢The cell walls of Gram-positive bacteria are very diverse and the main reason behind this diversity is poly peptidoglycan. Peptidoglycan is a heteropolymerof glycine threads formed from cross-linked peptide bonds. The backbone of glycan is linked by a β-1,4 glycosidic bonds, consisting of alternating N-acetylglucose amine and N-acetyl muramic acid units.

➢Peptidoglycan has been of great interest in the antibiotic and mechanisms of resistance researches, specifically in the behaviour of mobile phages, immuneresponses, identification and classification of bacteria. The discriminatory strength of peptidoglycan structure is limited by Gram-positive bacteria. Some differences found within the structure of peptidoglycan distinguish the two groups of bacteria as Gram-positive and Gram-negative. According to the differences in the cross-linking of the amino-sugar backbone, peptidoglycans can be divided into two groups; A-type peptidoglycan and B-type peptidoglycan

Analysis of diaminopimelic acid

➢ Diaminopimelic acid is found on the cell wall at the 3rd position. Structurally there are three forms; LL-, meso-, and OH-.They are named as; α-and ε-according to their different chemical structures. The presence or absence of these forms in Gram positive bacteria, particularly in Actinobacteria, separate them into different groups according to the type of diaminopimelic acid .The diamino acids in Gram (-) bacteria; including Spirochetes, with the exception of few groups of the Gram (-) bacteria, have a remarkably homogenous peptidoglycan structure. Here, the taxonomically significant diversity can be found in the peptide side chains, especially at the 3rd position. The most commonly distributed diamino acid in this position is, m-diaminopimelic acid, nonetheless, L-lysine is also quite common. The least common are; L-diaminobutric acid, LL-diaminopimelic acid and L-ornithine. Thin Layer Chromatography (TLC) can be used in the analysis of diamino acids.

Sugar analysis

➢In the presence of DAP, Actinomycetes are groups according to the type of sugar they contain and are expressed in some chemotypes. In a study of a 16 Actinomycetes; four groups were identified according to the sugar content present in Actinomycetes. In group A, galactose and arabinose are present, xylose is absent. Madurose present in B group, but xylose or arabinose are absent. In group C, no identifiable sugar exists, however, xylose and arabinose present in group D. Thin Layer Chromatography (TLC) is used for the comparative analysis of sugars.

Teichoic acid

- In gram positive bacteria cell wall, the polymer backbone contains various sugars and glycopolymers such as; phosphate, alanine, succinate, pyruvate, chlorine or mycolic acid binds together via diester bonds forming, teichoic acid.
- Teichoic acids are divided into five different groups according to the difference in the chemical composition of the polyol groups;

I-Polyolteichoic acid type I: (alditol-phosphate) glycerol, ribitol, erythrol, mannitol, arabinitol and their phosphodiester bonds may vary among species.

I-Polyteichoic acid type II: (glucose-alditol-phosphate); there is a glycosyl group in the main chain of the teichoic acid.

III-Polyteichoic acid type III: (sugar-1-phosphate-aldethol-phosphate); in this type of teichoic acid, unsaturated bonds are formed between glyceric acid and acyl components. The dominant sugar in this type is N-acetyl hexosamine. Bacillus subtilis.

IV-Polyteichoic acid type IV: (sugar-1-phosphate-polyol phosphate); glycerol phosphate and sugar-1 phosphate are linked to the teichoic acid chain; it is used in the detection of Staphylococcusstrains. The N-acetyl hexosamine is the dominant sugar

V-Poly teichoic acid type V: (polyol phosphate); this type of teichoic acid contains; glycerol phosphate and glucose-glycerol-phosphate and it is used in the identification of Nocardiopsis strains.

Polar lipids

➢Polar lipids are only associated with cell membranes; they are lipid derivatives and are not limited to phospholipids. The most common ones are phospholipids which are phosphatidic acid derivatives (phosphoglycerides). Although the general pattern of polar lipids is homogeneous nevertheless, they are an important chemical characteristic used in the differentiation between families and species.

➢The simplest form are the acylglycosides, in which a mono- or oligosaccharide is esterified by a long chain fatty acid. 1,2 diol are a rare form of polar lipids that can be observed in both *Thermomicrobium roseum* and *Chloroflexus aurantiacus* .

Isoprenoid Quinones

➢Isoprenoid quinones are vitamin-like structures found in the prokaryotic cell membranes. They are formed from isoprene subunits. It is found in both aerobic and anaerobic organisms. Structurally there are two main classes; benzoquinones(Ubiquinone) and naphthoquinones (Menaquinones), act as an electron carrier or antioxidants. The structural variation of the quinones, the differences in hydrogenation length as well as the differences in the polyprenyl side chains carry an important value in classification. Quinones are used as an important chemotaxonomic marker in the classification of the genus and family.

➢The natural composition mixture of bacterial quinones can be easily identified using various chromatographic techniques. It is sensitive to photooxidation in the presence of oxygen and light. Gram positive bacteria only synthesize menaquinones or dimethylquinones, but they never synthesize ubiquinones. Facultative anaerobic Proteobacteria groups such as *E. coli* have both MQ and UQ. The majority of aerobic gram-negative bacteria produce only ubiquinones.

Polyamines

➢Polyamines are chemical structures that occur following the formation of many amine groups from different conformations. The general types used in chemotaxonomy are 1,3-diamino propane, putrescine, 2-hydroxy putrescine, cadaverine, sym-norspermidine, spermidine, sym-homospermidin, and spermin. They can be found in prokaryotes, however; their concentration is significantly low. As a result, the prognosis when chemotaxonomy is used can be relatively low. Nevertheless, when classification is made, the dominant polyamine pattern in the group can be observed.

Prokaryotic Pigments

➢ In many prokaryotic organisms, a wide range variety of colours can be observed in their cultures and colonies. These colours may be; yellow, red, purple, green, and many other different colours. Some of the pigments used in chemotaxonomy includes; lutein, cryptoxanthin, phytocyanin, zeaxantin (betacarotene), chlorophyll A and chlorophyll B.

Lipopolysacchrides

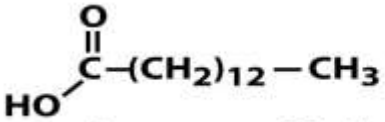
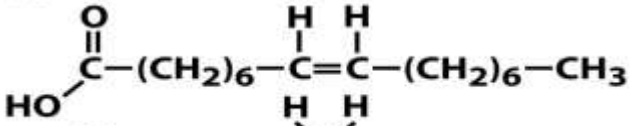
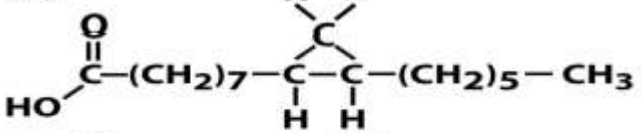
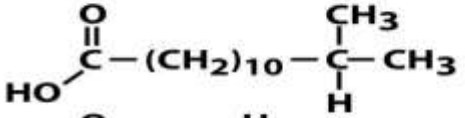
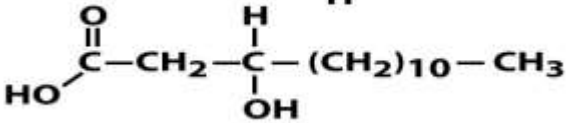
➢The chemical complex of lipopolysaccharides (LPS) found on the outer membrane of Gram (-) bacteria are composed of different polysaccharide (PS) and lipids (Lipid A) parts. Most of their biological effects are caused by a lipid and the polysaccharide moiety that acts as an antigen for the actual cell. LPS is the most important component of the outer membrane. It is a major contributor to the structural integrity of gram (-) bacteria, and serves in the protection against chemical attacks on the cell. The lipopolysaccharide structure is generally comprised of three parts; O-antigen or chain, oligosaccharide nucleus and lipid A. Some properties of the inner nucleus are preserved among the taxa. Many bacterial LPS nucleus contain; carbohydrates, phosphates components, amino acids, and ethanolamine. The polysaccharide of major surface antigens is called O-antigen. O-antigen or O-chain varies among different bacteria.

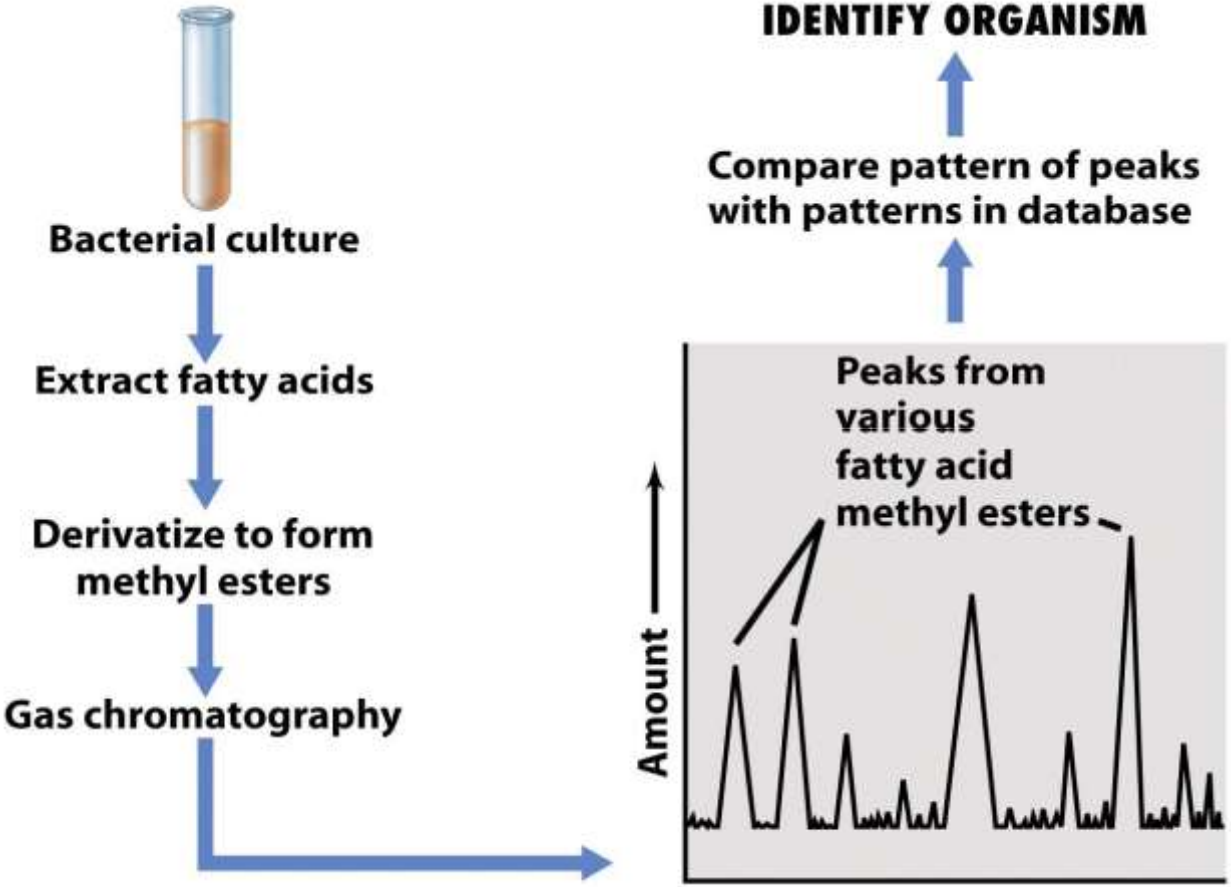
Fatty Acid Analysis(FAME)

➢The type and proportions of fatty acids present in cytoplasmic membrane lipids and the outer membrane lipids of gram negative bacteria. The technique for identifying these fatty acids has been nicknamed FAME, for fatty acid methyl ester.

➢The fatty acid composition of Bacteria varies from species to species in chain length and in the presence or absence of double bonds, rings, branch chains, or hydroxyl groups.

Classes of Fatty Acids in *Bacteria*

Class / Example	Structure of example
I. ***Saturated:*** tetradecanoic acid	
II. ***Unsaturated:*** *omega-7-cis* hexadecanoic acid	
III. ***Cyclopropane:*** *cis 7, 8* methylene hexadecanoic acid	
IV. ***Branched:*** 13-methyltetradecanoic acid	
V. ***Hydroxy:*** 3-hydroxytetradecanoic acid	


Bacterial culture
Extract fatty acids
Derivatize to form methyl esters
Gas chromatography
Amount
Peaks from various fatty acid methyl esters
Compare pattern of peaks with patterns in database
IDENTIFY ORGANISM

DNA-DNA Hybridization

➢When two organisms share many identical or highly similar genes, their DNAs are expected to hybridize in approximate proportion to the similarities in their DNA sequence. For this reason, measurement of DNA-DNA hybridization between the genome of two organisms provides a rough index of their similarity to each other.

➢70% or greater; considered same species

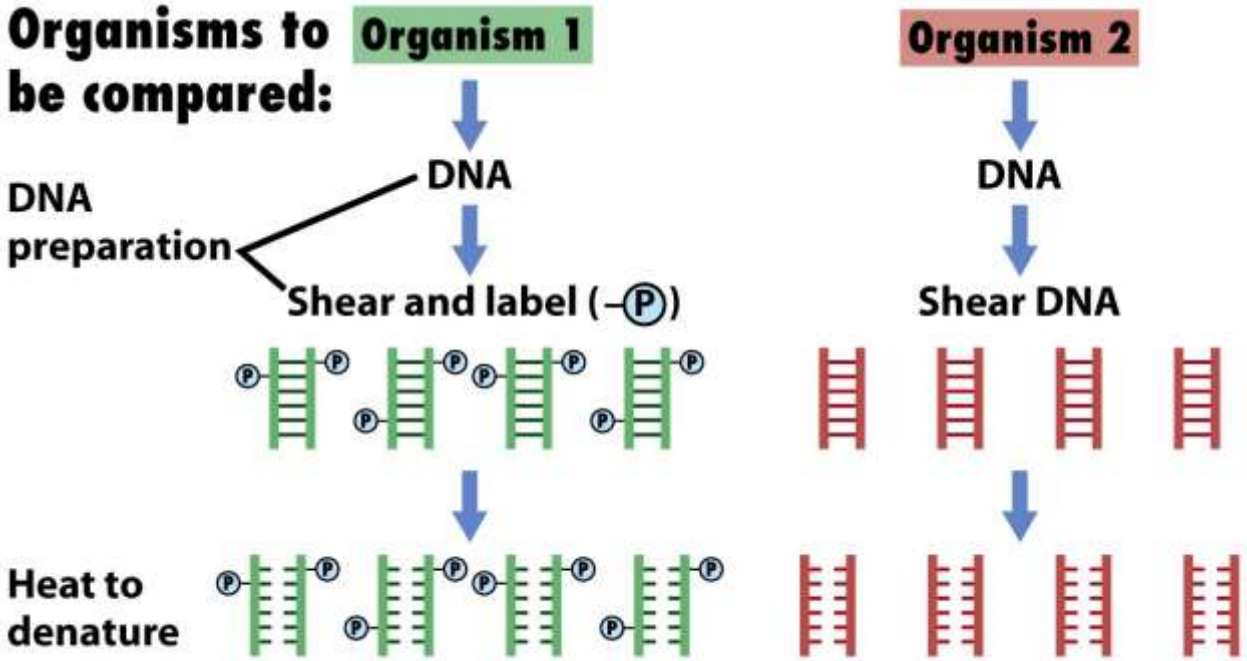
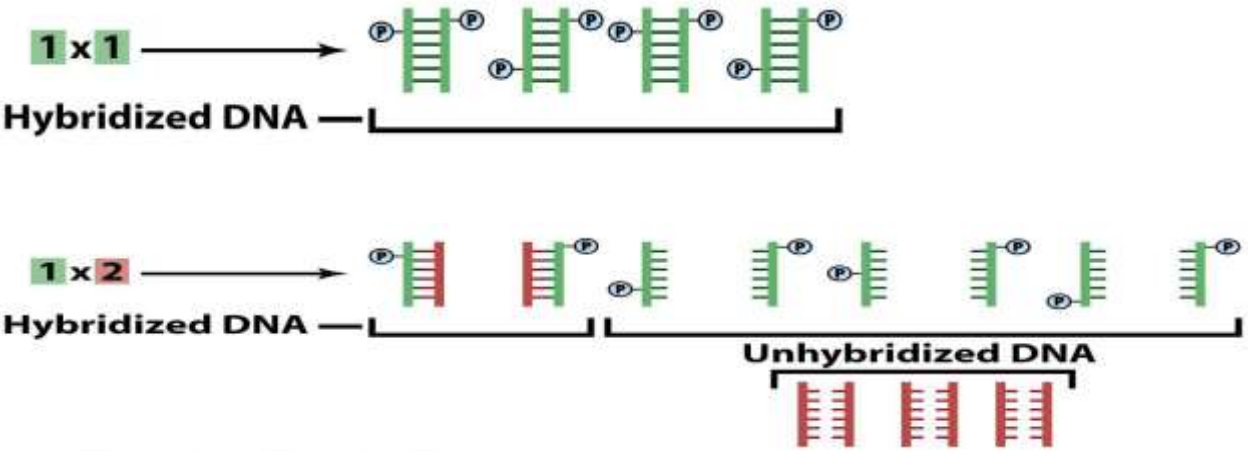


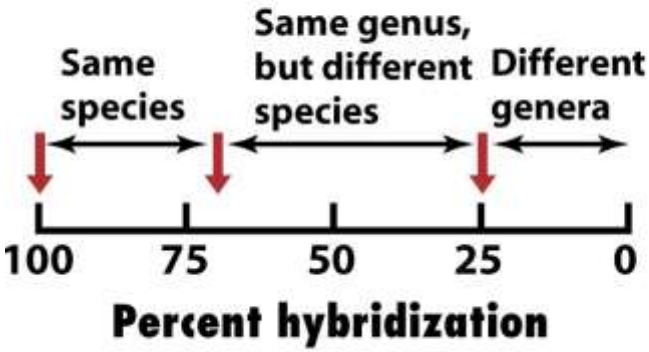
Figure 11-22a Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

Hybridization experiment:

Mix DNA from two organisms—unlabeled DNA is added in excess:



Results and interpretation:



1 x 1
100%
Same strain (control)

1 x 2
25%
1 and 2 are likely different genera

Figure 11-22c Brock Biology of Microorganisms 11/e

GC Ratio

➢GC ratio is percentage of guanine-cytosine base pairs in the genome. If the GC ratio of two organisms differs by more than about 5%, they cannot be closely related, but organisms with similar or even identical GC ratio may be unrelated. Not much used now in taxonomy because of poor resolution.

Molecular Methods in Microbial taxonomy:

There are mainly three types of methods

- By Molecular Markers
- Multiple Sequence Typing
- Multigene and Whole Genome analysis

(A) By Molecular Marker

A molecular marker is a DNA sequence in the genome which can be located and identified. As a result of genetic alterations (mutations, insertions, deletions), the base composition at a particular location of the genome may be different in different organisms.

Molecular markers are of two types:

1. Based on nucleic acid (DNA) hybridization (non-PCR based approaches).
2. Based on PCR amplification (PCR-based approaches).

Markers Based On DNA Hybridization:

➢The DNA piece can be cloned, and allowed to hybridize with the genomic DNA which can be detected. Marker-based DNA hybridization is widely used. The major limitation of this approach is that it requires large quantities of DNA and the use of radioactivity (labeled probes). Example-RFLP

Restriction fragment length polymorphism (RFLP):

➢RFLP was the very first technology employed for the detection of polymorphism, based on the DNA sequence differences. RFLP is mainly based on the altered restriction enzyme sites, as a result of mutations and re-combinations of genomic DNA.The procedure basically involves the isolation of genomic DNA, its digestion by restriction enzymes, separation by electrophoresis, and finally hybridization by incubating with cloned and labeled probes

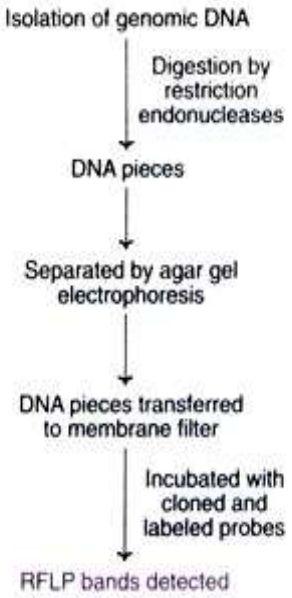


Fig. 53.2 : An outline of restriction fragment length polymorphism (RFLP) analysis as a molecular marker in plant breeding.

- Ribotyping and ARDRA are derivatives of RFLP

Ribotyping:

- DNA fingerprint called a ribotype, when DNA from an organism is digested by a restriction enzyme and the fragments are separated by gel electrophoresis, DNA fragments are transferred from the gel onto nylon membrane and hybridized with an rRNA gene probe.
- Due to multiple copy number of 16 SrRNA gene(1-14 depending upon the group of Bacteria), a complex profile is obtained

Presently, riboprinters are used to automate the method and read the profile

ARDRA:

- ARDRA employs digestion of amplified ribosomal DNA with different restriction enzymes and a profile is obtained using the combination of these patterns.
- This can be applied to screen large number of isolates simultaneously

Markers Based on PCR Amplification:

➢Polymerase chain reaction (PCR) is a novel technique for the amplification of selected regions of DNA .The advantage with PCR is that even a minute quantity of DNA can be amplified. Thus, PCR-based molecular markers require only a small quantity of DNA to start with.

PCR-based markers may be divided into two types:

1. Locus non-specific markers e.g. random amplified polymorphic DNA (RAPD); amplified fragment length polymorphism (AFLP).
2. Locus specific markers e.g. simple sequence repeats (SSR)

Random amplified polymorphic DNA (RAPD) markers:

➢RAPD is a molecular marker based on PCR amplification. The DNA isolated from the genome is denatured the template molecules are annealed with primers, and amplified by PCR.

➢Single short oligonucleotide primers (usually a 10-base primer) can be arbitrarily selected and used for the amplification DNA segments of the genome (which may be in distributed throughout the genome). The amplified products are separated on electrophoresis and identified.

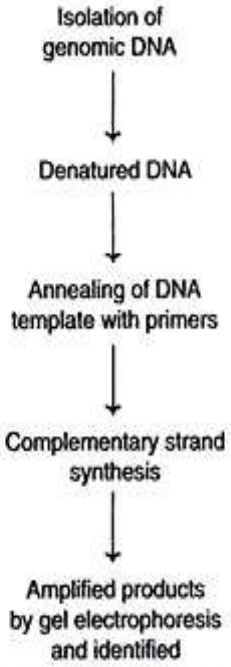


Fig. 53.4 : An outline of random amplified polymorphic DNA (RAPD) analysis as a molecular marker in plant breeding.

➢Based on the nucleotide alterations in the genome, the polymorphisms of amplified DNA sequences differ which can be identified as bends on gel electrophoresis. Genomic DNA from two different organisms often results in different amplification patterns i.e. RAPDs. This is based on the fact that a particular fragment of DNA may be generated from one individual, and not from others. This represents polymorphism and can be used as a molecular marker of a particular species.

➢SCARs(Sequence characterized amplified region) are the modified forms of STS markers. They are developed by PCR primers that are made for the ends of RAPD fragment. The STS-converted RAPD markers are sometimes referred to as SCARs. SCARs are useful for the rapid development of STS markers.

➢RAPD-PCR assay have been used extensively to define fungal populations specific, intraspecific, race and strain levels.

Amplified fragment length polymorphism (AFLP):

➢AFLP is a novel technique involving a combination of RFLP and RAPD. AFLP is based on the principle of generation of DNA fragments using restriction enzymes and oligonucleotide adaptors (or linkers), and their amplification by PCR. Thus, this technique combines the usefulness of restriction digestion and PCR.

➢The DNA of the genome is extracted. It is subjected to restriction digestion by two enzymes (a rare cutter e.g. Msel; a frequent cutter e.g. EcoRI). The cut ends on both sides are then ligated to known sequences of oligonucleotides.

➢PCR is now performed for the pre-selection of a fragment of DNA which has a single specific nucleotide. By this approach of pre-selective amplification, the pool of fragments can be reduced from the original mixture. In the second round of amplification by PCR, three nucleotide sequences are amplified.

➢This further reduces the pool of DNA fragments to a manageable level (< 100). Autoradiography can be performed for the detection of DNA fragments. Use of radiolabeled primers and fluorescently labeled fragments quickens AFLP.

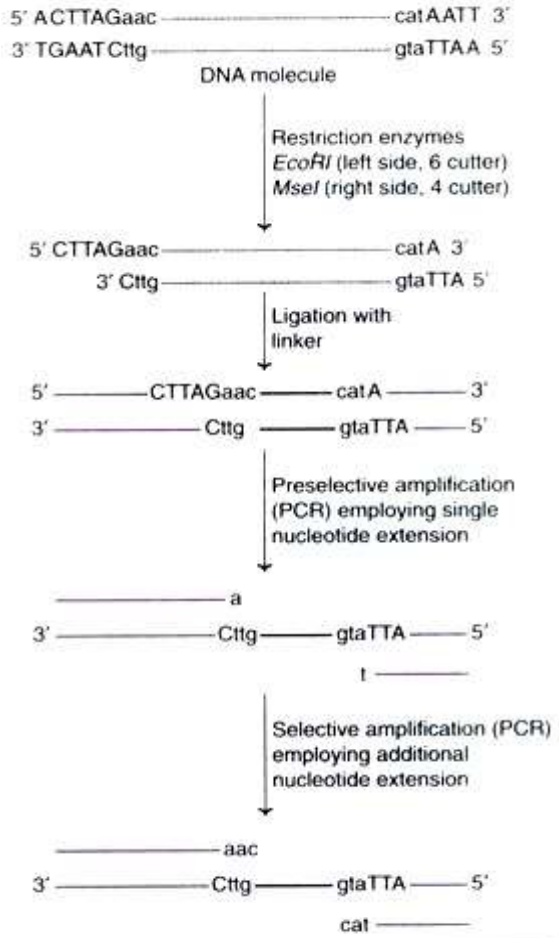


Fig. 53.5** : A diagrammatic representation of the amplified fragment length polymorphism (AFLP)* ***(Note : The lower case letters represent the sequences found within the amplified region; the coloured lines indicate linkers).

➢AFLP analysis is tedious and requires the involvement of skilled technical personnel. Hence some people are not in favour of this technique. In recent years, commercial kits are made available for AFLP analysis. AFLP is very sensitive and reproducible. It does not require prior knowledge of sequence information. By AFLP, a large number of polymorphic bands can be produced and detected.

➢A technique similar to AFLP called T-RFLP (terminal restriction fragment length polymorphism) is widely used in phylogenetic analysis of natural microbial communities.

Microsatellites:

➢Microsatellites are the tandemly repeated multi-copies of mono-, di-, tri- and tetra nucleotide motifs. In some instances, the flanking sequence of the repeat sequences may be unique. Primers can be designed for such flanking sequences to detect the sequence tagged microsatellites (STMS). This can be done by PCR.

(B)Multiple Sequence Typing

- Some analyses such as ribotyping focus on only a single gene, which may not provide sufficient information for unequivocal discrimination of bacterial strains.
- Multilocus sequence typing(MLST) circumvents this problem and is a powerful technique for characterizing strains within a species.
- For MLST, first of all different housekeeping genes are isolated. For each gene, an approximately 450-bp sequence is amplified using PCR and then sequenced. Each nucleotide along the sequence is compared and differences are noted. Each difference, or sequence variant, is called an allele and is assigned a number.
- The relatedness between each allelic profile is expressed in a dendrogram of linkage distances that vary from 0(strains are identical) to 1(strains are only distantly related, if at all).

(C)Multigene and Whole Genome analysis

- The use of multiple genes for the identification and description of bacteria can avoid problems associated with reliance on individual genes.
- Multigene sequence analysis is similar to MLST, except that complete or nearly complete gene sequences are obtained and comparisons are made using cladistic methods
- Analyses of whole genome sequences provide comparative analysis of gene content(presence or absence of genes) and the order of genes in the genome.

Thank you