Oral Biology
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Lecture - 16
Evaluation of Oral microbiome

Welcome to today's session. So, today's session is on topic Evaluation of Oral Microbiome. Oral microbiome includes the microorganisms which are found at the oral cavity. It is also known as Oral Microbiota.

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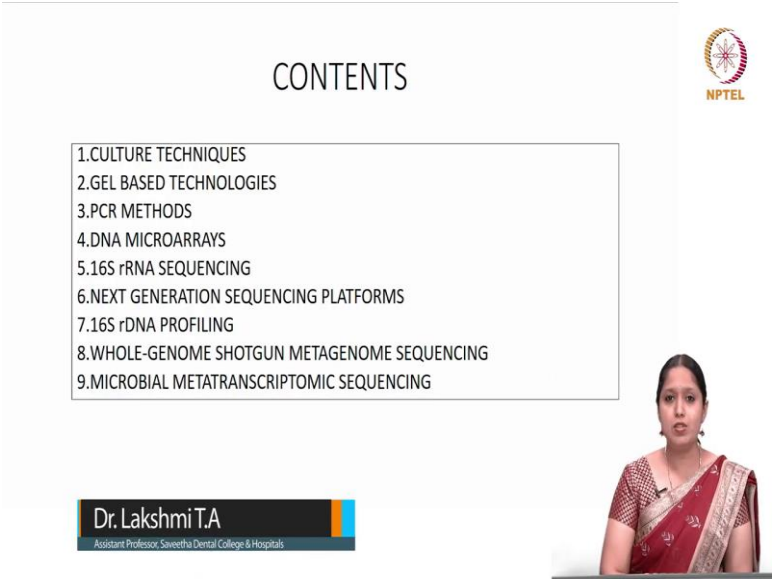

So, there are various methods which are used for evaluating the oral microbiome, which includes the traditional basic Culture techniques, Gel Based Technologies, Polymerase Chain Reaction methods, DNA Microarrays, 16S rRNA Sequencing, Next Generation Sequencing Platforms, 16s rDNA Profiling, Whole Genome Shotgun Metagenome Sequencing and Microbial Meta Transcriptomic sequencing. Now let us see each and everything in detail.

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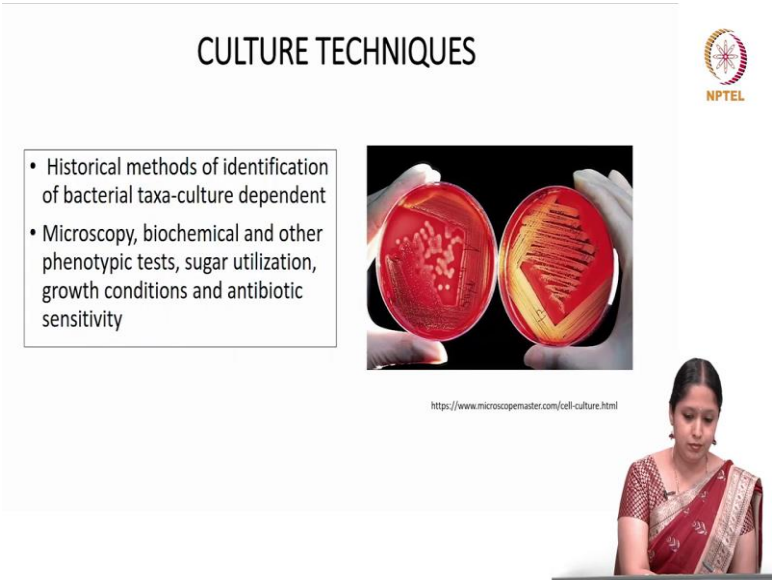

So, the first technique is the Culture Technique. Culture Technique is the basic gold standard method for the identification of bacteria present in the oral cavity. So, it is a historical method of identification of bacterial taxa. So, it is culture dependent. It depends upon microscopy, biochemical and other phenotypic tests, sugar utilization, growth conditions and antibiotic sensitivity.

There are different culture media for different types of bacteria and special culture media is also there and most commonly used culture media include agar, which includes blood agar, chocolate agar and various other culture mediums.

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The actual diversity of oral microbiome cannot be completely revealed by culture-based methods. This is because the main difficulty with the conventional culture and culture based analytical technologies is many of the bacterial species in the biological samples, they cannot be cultured making these approaches unsuitable for research.

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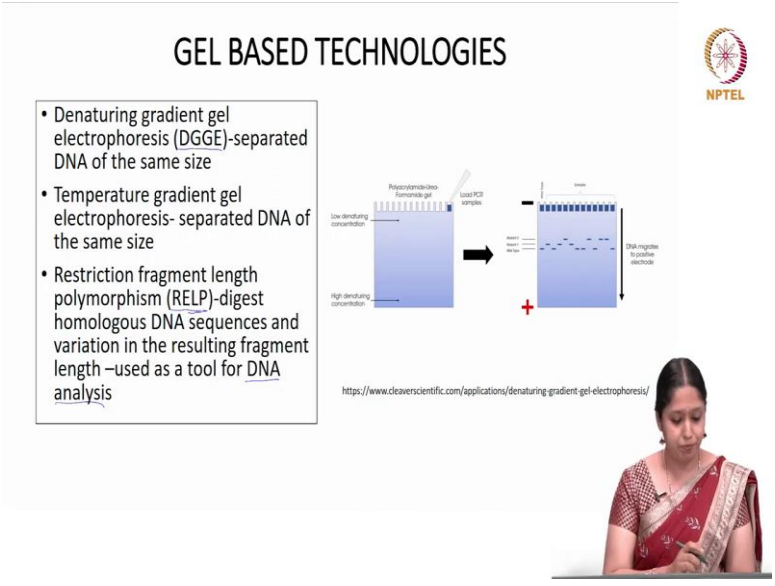

Next coming to the Gel Based Technologies. So, under gel-based technologies comes Denaturing Gradient Gel Electrophoresis or DGGE and temperature gradient gel electrophoresis. So, both these methods use separated DNA of the same size. They will

separate the DNA of the same size and there is something called as restriction fragment length polymorphism.

It digests homologous DNA sequences and variation in the resulting fragment length is used as a tool for DNA analysis. So, under gel-based technologies comes denaturing gradient gel electrophoresis, temperature gradient gel electrophoresis and restriction fragment length polymorphism.

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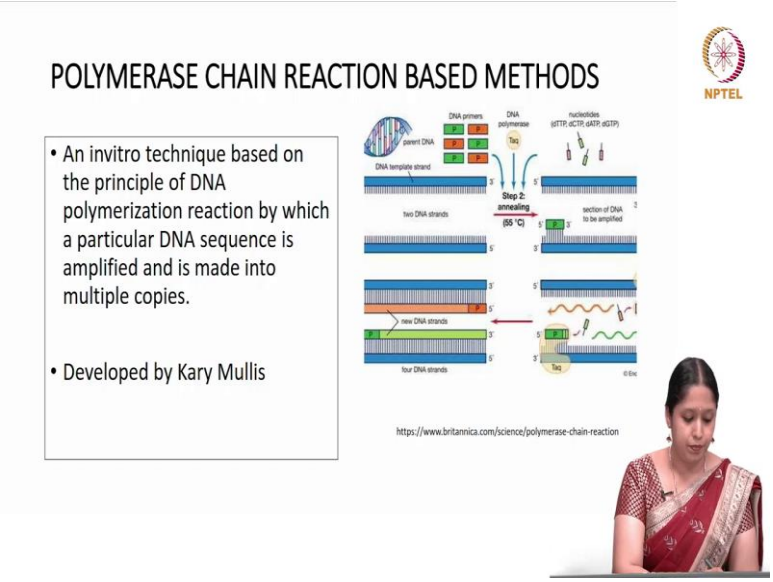

The next important method which is used for the evaluation is the Polymerase Chain Reaction. So, this technique was developed by Kary Mullis, an Invitro technique based on the principle of DNA polymerization reaction by which a particular DNA sequence is amplified and it is made into multiple copies. So, this is a basic principle of polymerase chain reaction.

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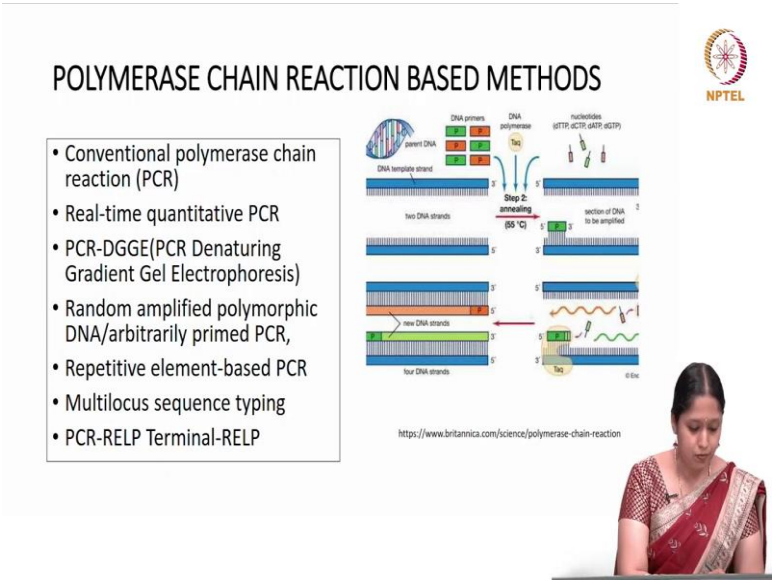

There are different methods of polymerase chain reaction, which includes Conventional polymerase chain reaction, Real time quantitative PCR, PCR DGGE which is PCR Denaturing Gradient Gel Electrophoresis, Random amplified polymorphic DNA or Arbitrarily primed PCR, Repetitive element-based PCR, Multilocus sequence typing as well as PCR restricted length polymorphism terminal RELP. So, these are all the different types of polymerase chain reaction-based methods.

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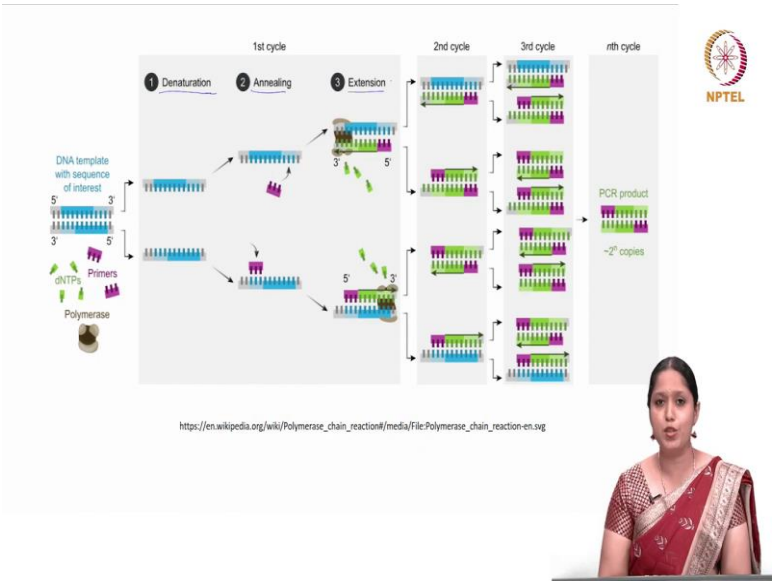

So, there are different stages in the polymerase chain reaction. First step is denaturing, second is annealing and third is extension and later on there will be little bit extension or elongation.

So, first step is denaturation. Under denaturation what happens is the chamber is increased to a temperature of 98 degree Celsius or 20 to 30 seconds so that the nucleic acid, the nuclear material is denatured as a result the double stranded DNA splits up into two single strands.

Followed by denaturation, the next step is annealing here the temperature is reduced to 50 degree Celsius and it is maintained for 40 seconds and after annealing comes the next step, which is called as extension or elongation.

So, these are all the various steps of polymerase chain reaction. So, under polymerase chain reaction we will be able to detect various bacterial species and apart from bacterial species it also helps in identification of virus, which includes HIV and other types of viruses.

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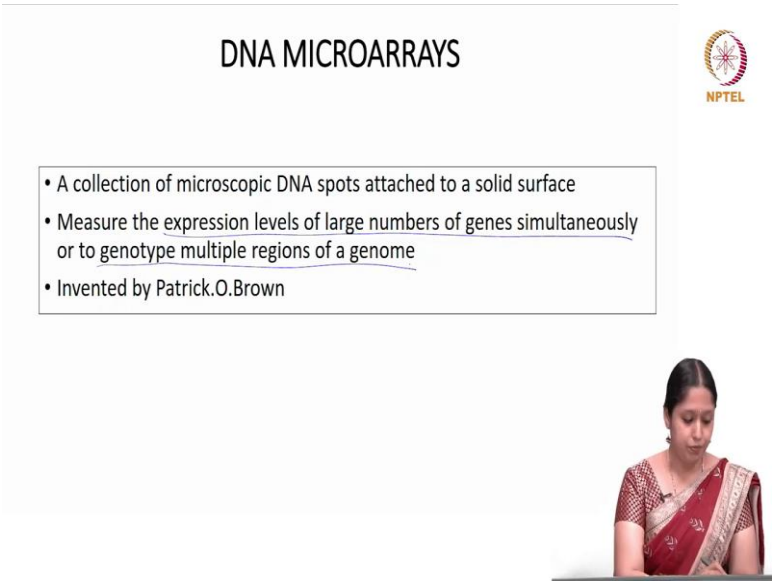

Next comes the DNA microarrays. So, DNA microarrays are nothing, but a collection of microscopic DNA spots attached to the solid surface. It measures the expression levels of large number of genes simultaneously or to genotype multiple regions of a genome.

So, the main function of DNA microarray is, it will measure the expression levels of large number of genes simultaneously. So, we will be able to simultaneously measure the expression of large number of genes and also you can find the genotype of multiple regions of a genome.

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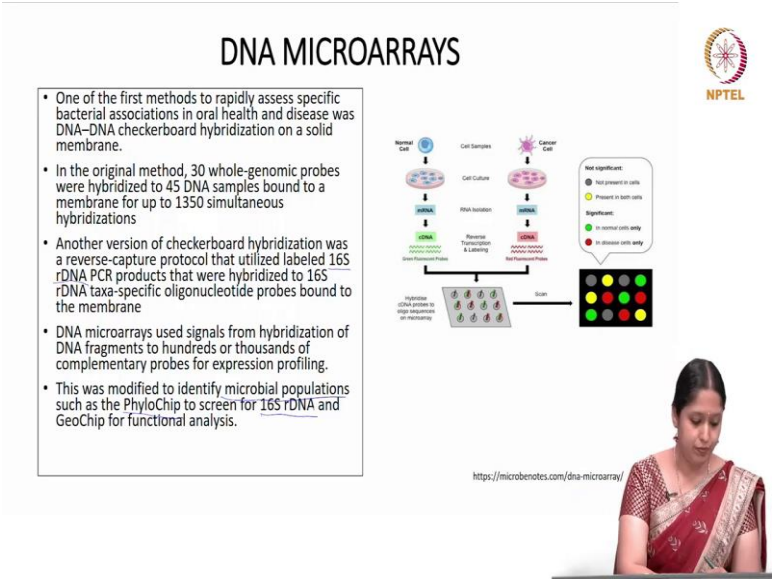

So, this technique was invented by Patrick O Brown. So, one of the first methods to rapidly assess the specific bacterial association in oral health and disease was DNA-DNA checkerboard hybridization on a solid membrane. So, in the original method what happened is 30 whole genomic probes were hybridized to 45 DNA samples bound to a membrane for up to 1350 simultaneous hybridization.

Another version of checkerboard hybridization was a reverse capture protocol that utilized labelled 16S rDNA PCR products that were hybridized to 16S rDNA taxa specific oligonucleotide probes bound to a membrane. So, DNA microarrays it uses signals from hybridization of DNA fragments to hundreds or thousands of complementary probes for expression profiling. So, you should remember this.

Later on, this was modified to identify microbial populations such as phylochip to screen for 16S rDNA and GeoChip for functional analysis. So, PhyloChip is used for screening 16S rDNA and GeoChip is used for functional analysis. This is a modification for identifying the microbial population.

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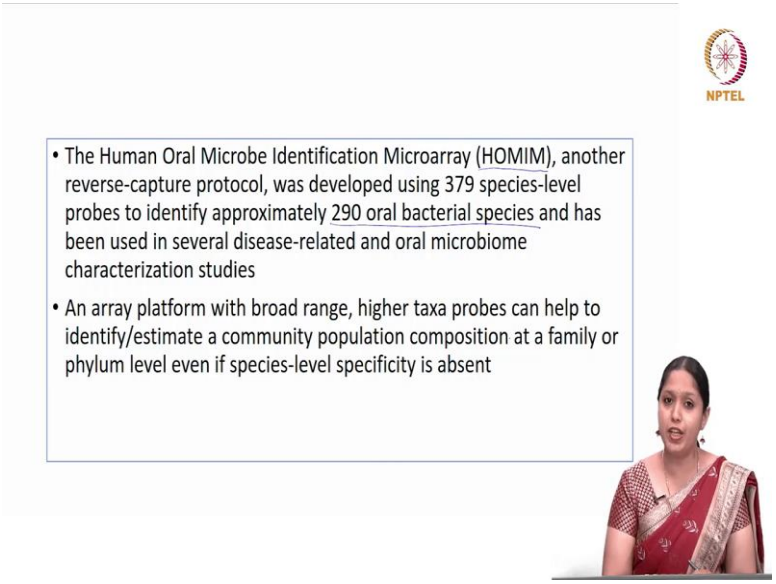

The Human Oral Microbiome Identification Microarray which is also known as HOMIM. HOMIM it is another reverse capture protocol which was developed using 379 species level probes to identify approximately 290 oral bacterial species and it has been used in several disease related and oral microbiome characterization studies.

So, HOMIM is Human Oral Microbiome Identification Microarray. An array of platform with a broad range and higher taxa probes, it can help to identify or estimate a community population, composition at a family or phylum level even if species level specificity is absent. So, this is a main thing of HOMIM that is Human Oral Microbiome Identification Microarray.

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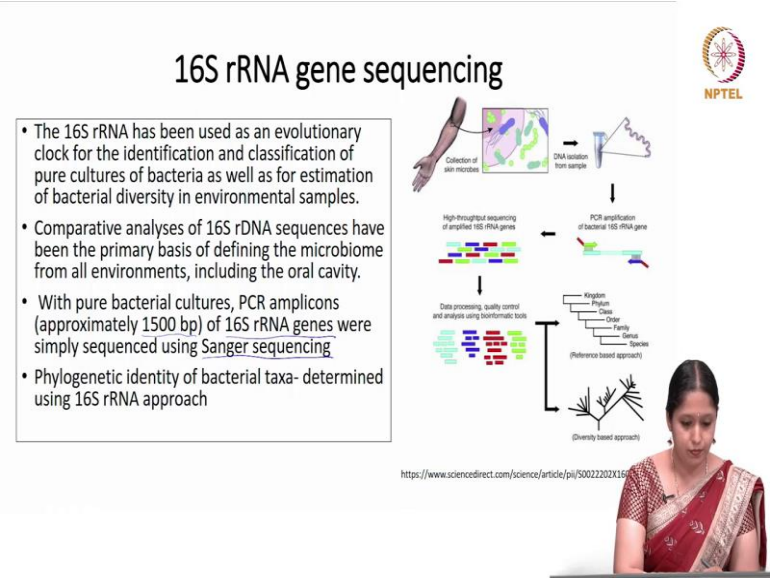

So, next comes another important methodology which is 16S rRNA gene sequencing. So, the 16S rRNA gene sequencing-it has been used as an evolutionary clock for the identification and classification of pure cultures of bacteria as well as it also helps in estimation of bacterial diversity in the environmental samples.

So, the comparative analyses of 16S rDNA sequences have been the primary basis of defining the microbiome from all environment including the oral cavity. So, what happens is with pure bacterial cultures, PCR amplicons which approximately 1500 base pair of 16S rRNA genes were simply sequenced using a method called Sanger sequencing.

So, with pure bacterial culture PCR amplicons of approximately 1500 base pairs of 16S rRNA genes. They were sequenced using a method called Sanger sequencing. So, phylogenetic identification of bacterial taxa is determined using this approach of 16S rRNA.

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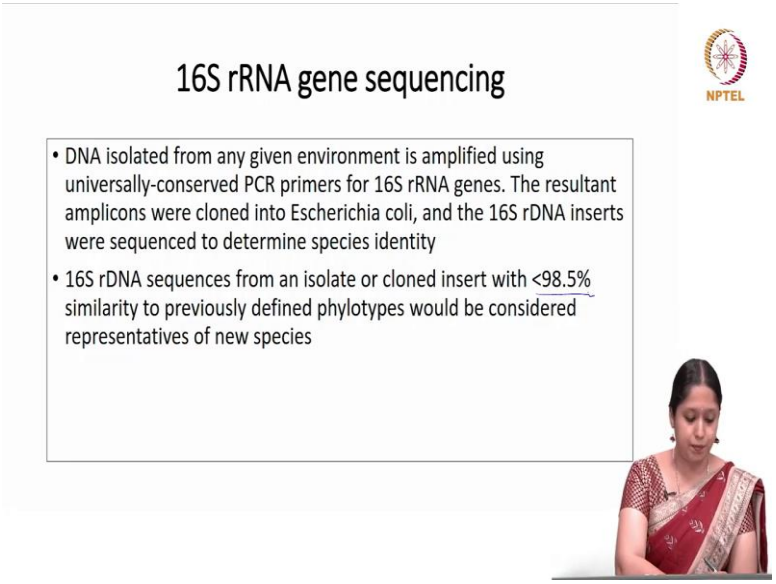

So, DNA isolated from any given environment is amplified using universally conserved PCR primers for 16S rRNA genes. The resultant amplicons were later cloned to E-coli and then the 16S rDNA inserts were sequenced to determine the species identity. So, the 16S rDNA sequences from an isolate or cloned insert with less than 98.5 percentage similarity to previously defined phylotypes would be considered representatives of new species. So, it is an important procedure that is 16S rRNA gene sequencing.

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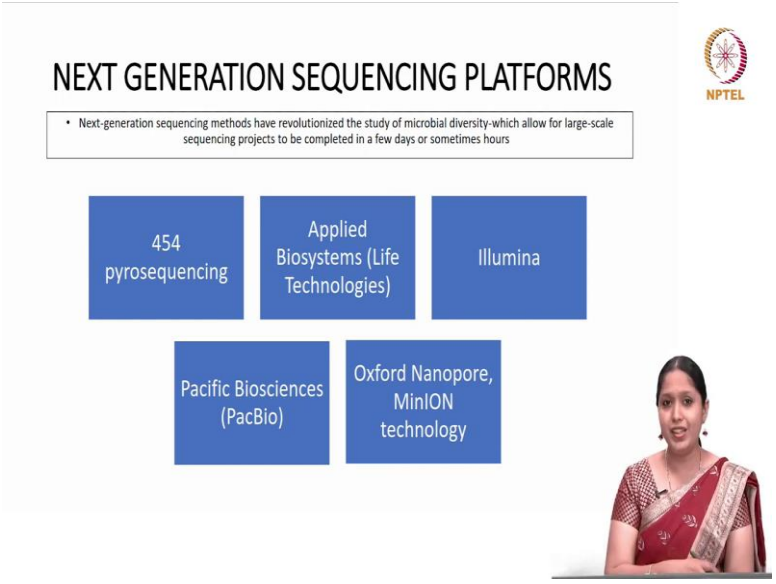

So, next thing is the next generation sequencing platform. So, the next generation sequencing platforms is a revolutionized method for the study of microbial diversity which allow for large scale sequencing projects to be completed in a few days or sometimes within few hours.

So, it is a new methodology that is next generation sequencing platform under that comes various 5 types which includes 454 Pyrosequencing, Applied Biosystems, Illumina, Pacific Biosciences and Oxford Nanopore that is minion technology MinION technology. Let us see each and everything in detail.

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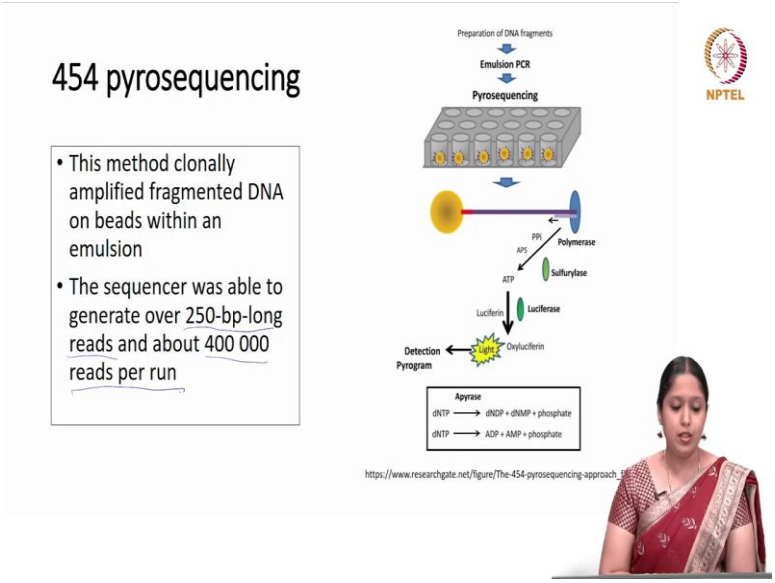

So, first comes the 454 pyrosequencing. So, this method clonally it amplified fragmented DNA or on beads with an emulsion. So, it amplifies the, main function of the 454 pyrosequencing is it clonally amplified fragmented DNA on beads with an emulsion. The sequencer was able to generate over 250 base pair long reads and about 400000 reads per run. So, this is about 454 pyrosequencing.

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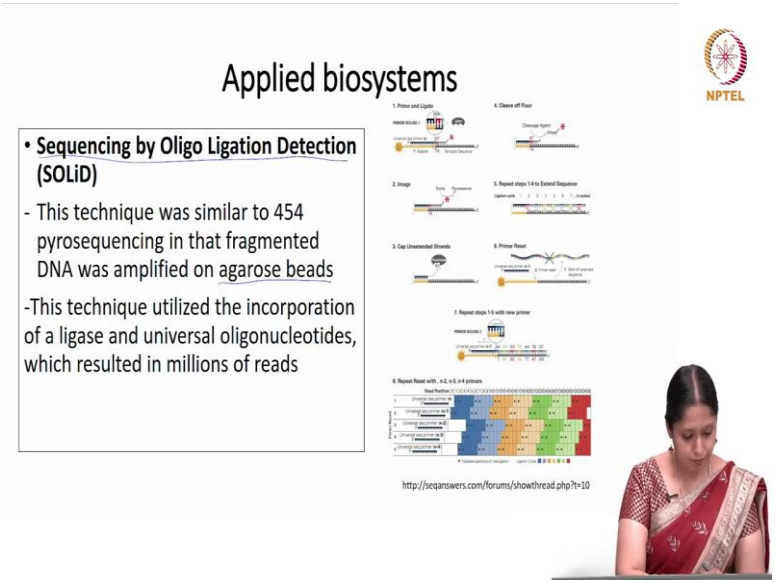

Next comes the Applied Biosystems. So, under Applied Biosystems it is of two types. First one is solid, SOLiD means Sequencing by Oligo Ligation Detection. So, SOLiD is nothing, but sequencing by Oligo Ligation Detection. This technique is similar to 454 pyrosequencing, which we saw before right just now before.

In that the fragmented DNA was amplified on agarose beads. Earlier it was fragmented DNA was amplified in an emulsion, here the fragmented DNA was amplified on agarose beads. This technique utilized the incorporation of a ligase and universal oligonucleotides, which results in millions of reads. So, this is one type of applied biosystem that is solid or sequencing by oligo ligation detection.

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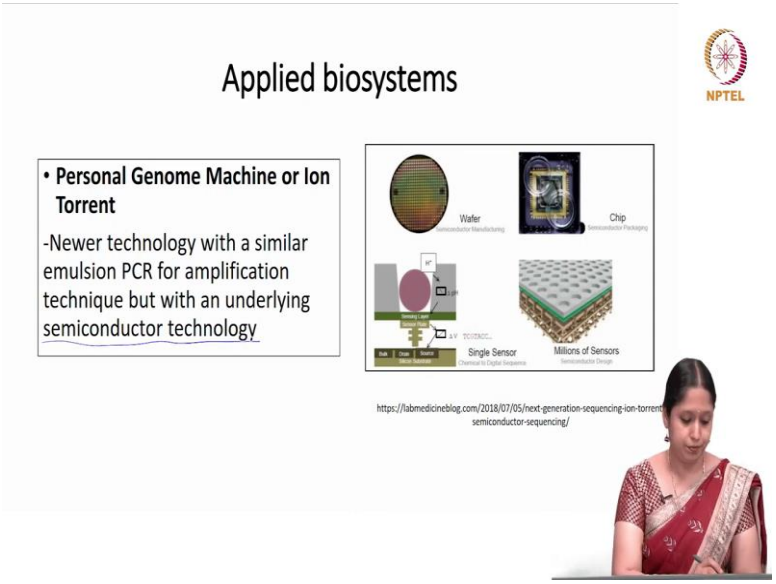

So, next comes the Personal Genome Machine or Ion Torrent. So, personal genome machine or Ion torrent is a newer technology with a similar emulsion PCR for amplification technique, but with an underlying semiconductor technology. So, important part here is it uses a semiconductor technology personal genome machine or Ion torrent.

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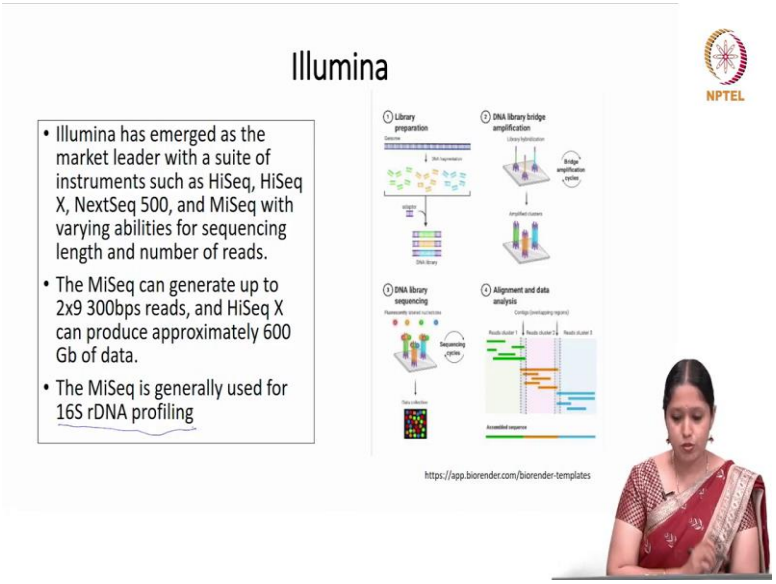

So, the next type of the next generation sequencing methodology is Illumina. Illumina has emerged as a market leader with the suite of instruments such as HiSeq, HiSeq X,

NextSeq 500 and MiSeq, with varying abilities for sequencing length and number of reads.

The MiSeq can generate up to 2x9 300 base pairs reads and HiSeq X can produce approximately 600Gb of data. The MiSeq is generally used for 16S rDNA profiling. So, this is about Illumina.

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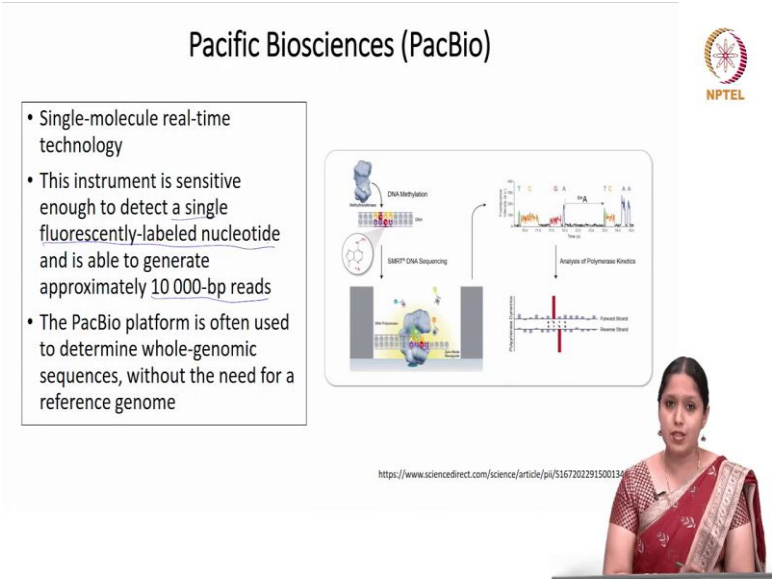

Next comes the Pacific Biosciences which is another type of next generation sequencing platform. So, it is a single molecule real time technology and this instrument is sensitive enough to detect a single fluorescently labelled nucleotide and is able to generate approximately ten thousand base pair reads.

So, it is a very sensitive technology and it can detect a single fluorescently labeled nucleotide and it is able to generate approximately 10000 base pair reads. So, the PacBio or pacific biosciences is often used to determine the whole genomic sequences without the need for a reference genome. So, this procedure does not make use of reference genome. So, it is used to determine the whole genomic sequences.

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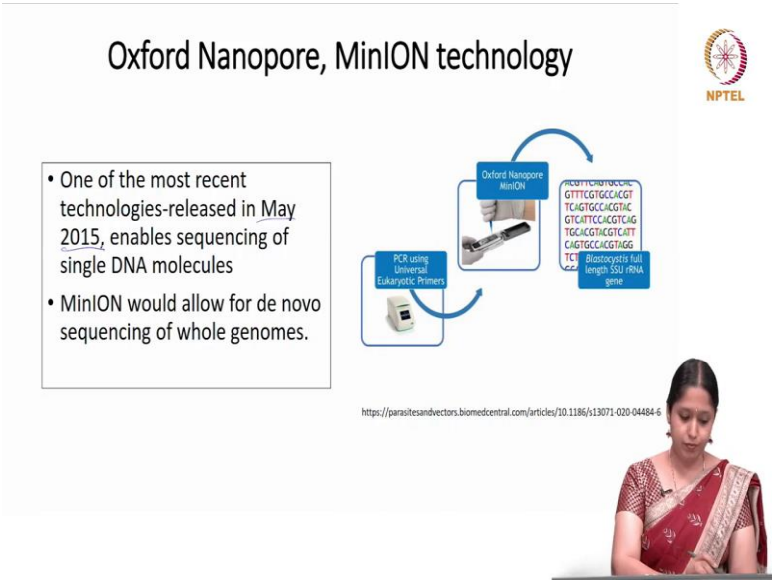

Next comes the Oxford Nanopore or MinION Technology. It is one of the most recent technologies which was released in the year May 2015. It enables sequencing of single DNA molecules. MinION would allow for de novo sequencing of whole genomes.

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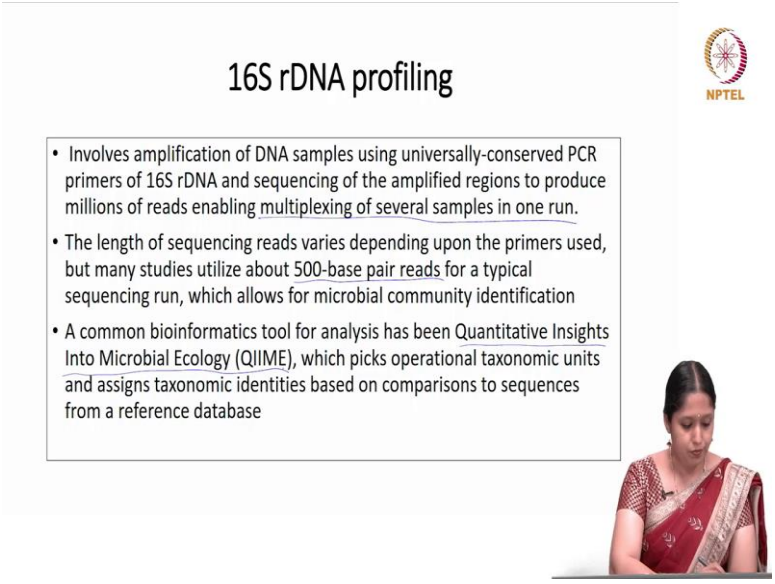

So, next procedure comes the 16S rDNA profiling. It involves amplification of the DNA samples using universally conserved polymerase chain reaction primers of 16S rDNA and sequencing of the amplified regions to produce millions of reads enabling multiplexing of several samples in one run.

So, this is one of the important advantages of this procedure, it enables multiplexing of several samples in one run. The length of sequencing read varies depending upon the primers used, but many studies in utilize about 500 base pair reads for a typical sequencing run, which allows for microbial community identification.

So, a common bioinformatics tool for analysis has been called as a Quantitative Insights into Microbial Ecology or QIIME. So, QIIME is Quantitative Insights into Microbial Ecology. It picks operational taxonomic units and it assigns taxonomic identities based on comparisons to sequences from a reference database. So, there is something called as HOMINGS. HOMINGS refers to Human Oral Microbiome using Next Generation Sequencing.

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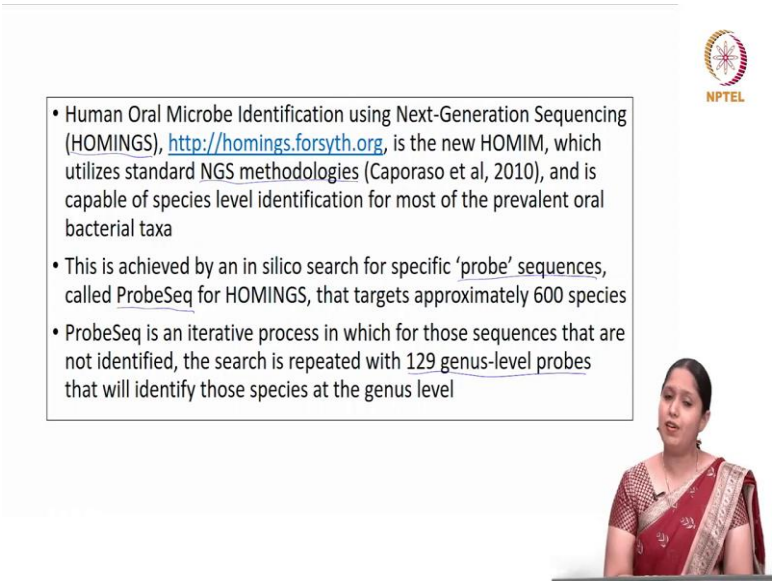

So, HOMINGS is Human Oral Microbe Identification using the Next Generation Sequencing and you can see a link here and when you click that link it will go automatically to this page of HOMINGS and you can see the new species level identification of the bacterial taxa and all you will be able to see.

So, it is a new Human Oral Microbiome Immuno Array which utilizes standard NGS methodologies. It utilizes standard next generation sequencing methodologies and it is capable of species level identification of most of the prevalent overall bacterial taxa and this is achieved by an in-silico research for specific probe sequences called ProbeSeq for HOMINGS that targets approximately 600 species. So, ProbeSeq is an iterative process

in which those sequences that are not identified the search is repeated with 129 genus level probes and it will also help to identify those species at the genus level as well.

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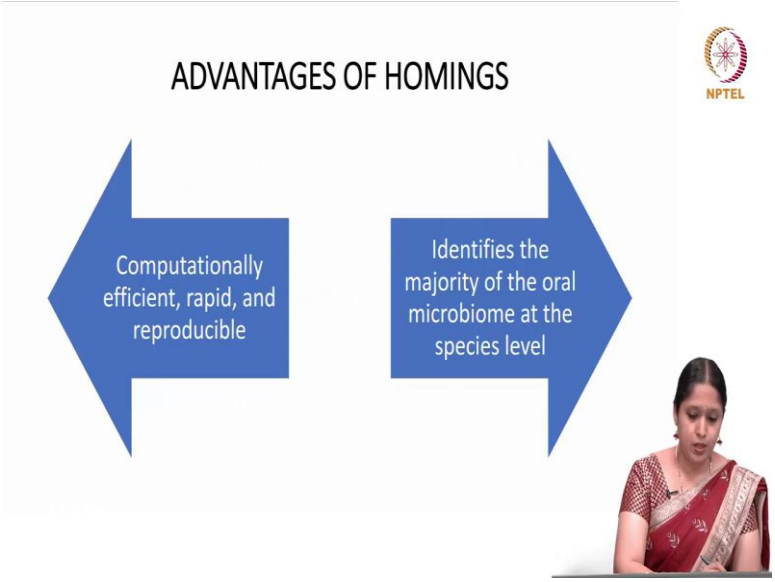

So, what are the advantages of HOMINGS- it is computationally efficient, rapid as well as it is reproducible. And another advantage is it identifies the majority of oral microbiome at the species level. So, the next procedure which is used for the evaluation of oral microbiome is the Whole Genome Shotgun Metagenome Sequencing.

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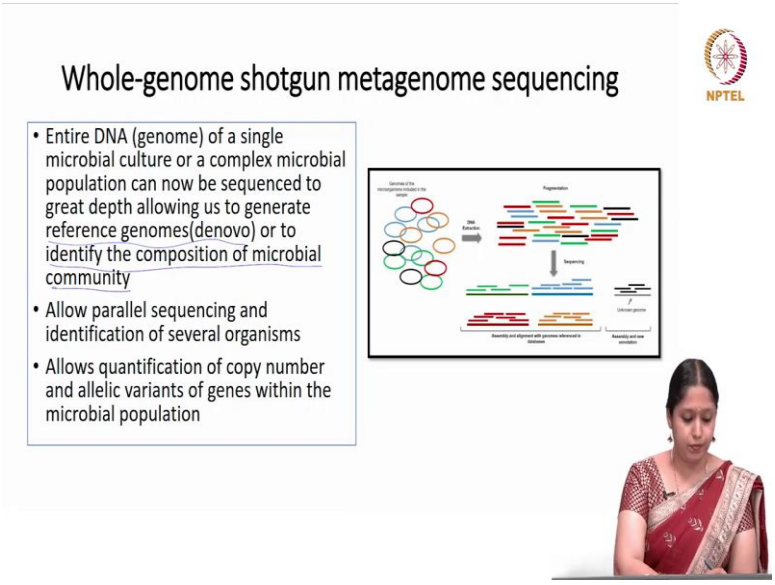

So, what happens in this procedure is the entire DNA or genome of a single microbial culture or a complex microbial population can be sequenced to a great depth allowing us to generate reference genomes denovo or it also helps to identify the composition of microbial community.

So, using this procedure either the entire DNA of a single microbial culture or a complex microbial population can be sequenced to a greater depth. It allows parallel sequencing and identification of several other microorganisms. It also allows quantification of copy number and allelic variants of genes within the microbial population.

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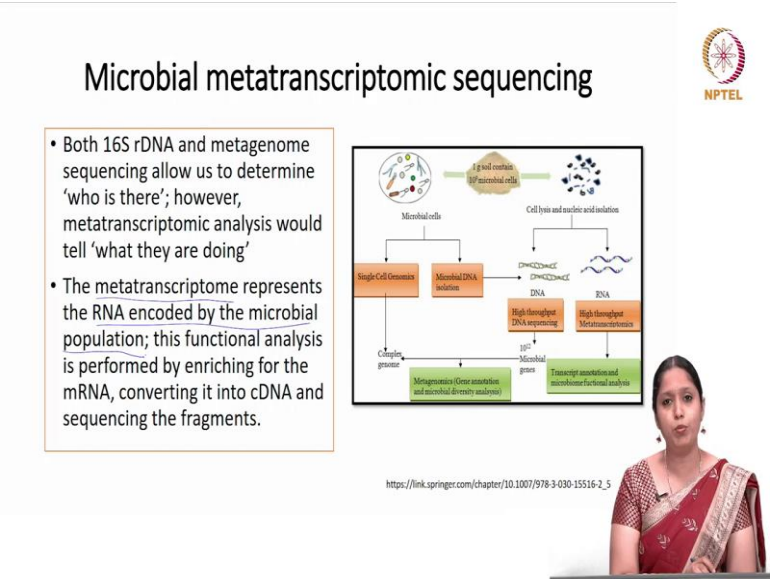

So, next procedure is the Microbial Metatranscriptomic Sequencing. So, the both 16S rDNA as well as the meta genomic sequencing which I mentioned before it allows us to determine only who is there, but when you want to know what they are doing that procedure can be done with the help of this microbial metatranscriptomic sequencing.

So, what happens is the metatranscriptome represents the RNA encoded by the microbial population. So, it represents the RNA encoded by the microbial population, thus the functional analysis is performed by enriching for mRNA and converting it into complementary DNA and sequencing the fragments. This is called as a Microbial Metatranscriptomic Sequencing.

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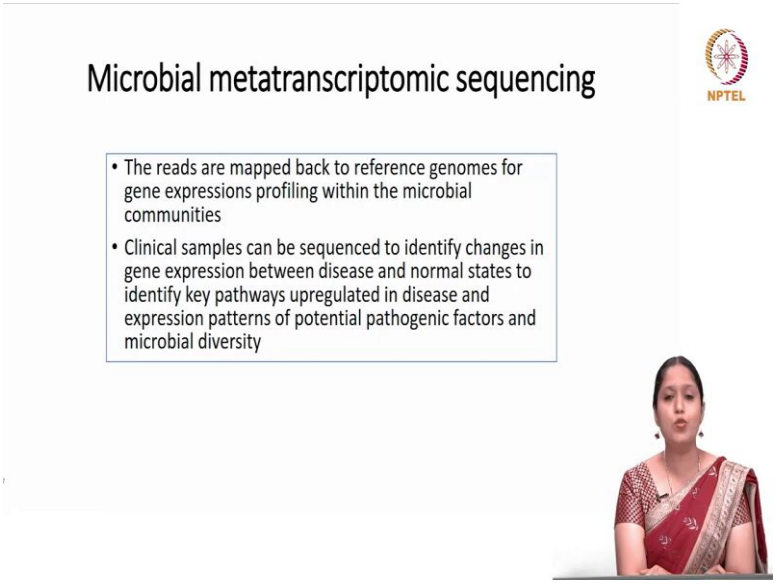

The reads are mapped back to reference genomes for gene expression profiling within the microbial communities. So, the clinical samples can be sequenced to identify changes in gene expression between the disease and normal state and it also helps to identify the key pathogens or key pathways upregulated in the disease and expression patterns of potential pathogenic factors as well as the microbial diversity. So, these are all the different methodologies right from the traditional method to the most advanced method, which are used for the evaluation of oral microbiome.

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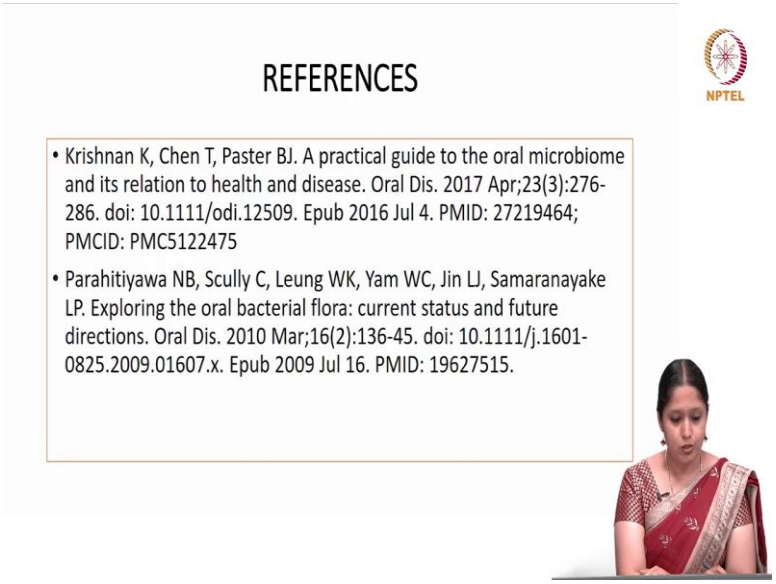

So, these are the references.

Thank you.