Oral Biology
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Lecture - 15
Oral microbiome

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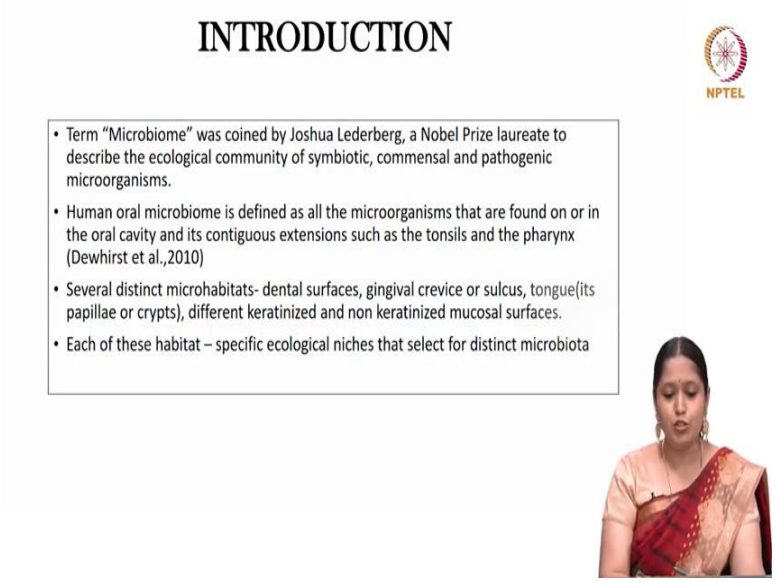

Welcome to today's lecture. Today's lecture is on topic Oral Microbiome. So, the term oral microbiome was coined by Joshua Lederberg, a Nobel Prize laureate, he described it as ecological community of symbiotic commensal and pathogenic microorganisms. Human oral microbiome is defined as all the microorganisms that are found in or on the oral cavity and its contiguous extensions like tonsils as well as pharynx.

Several distinct microhabitats include dental surfaces, gingival crevice or sulcus, tongue, papillae, different keratinized, non keratinized mucosal surfaces and each of these habitats belongs to specific ecological niches that select for distinct microbiota.

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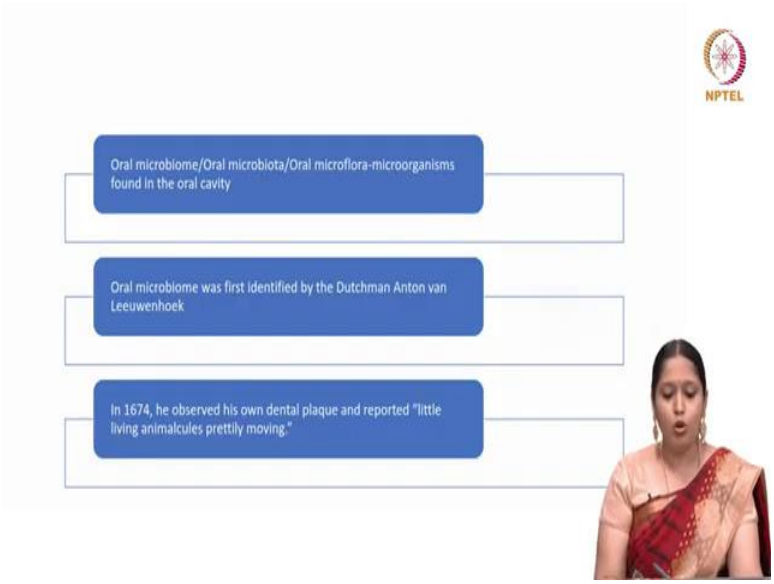

Oral microbiome is also known as oral microbiota or oral microflora. It refers to organisms found in the oral cavity. Oral microbiome was first identified by Anton van Leeuwenkoek. In the year 1674 he observed his own dental plaque and he reported it as tiny little living animalcules prettily moving.

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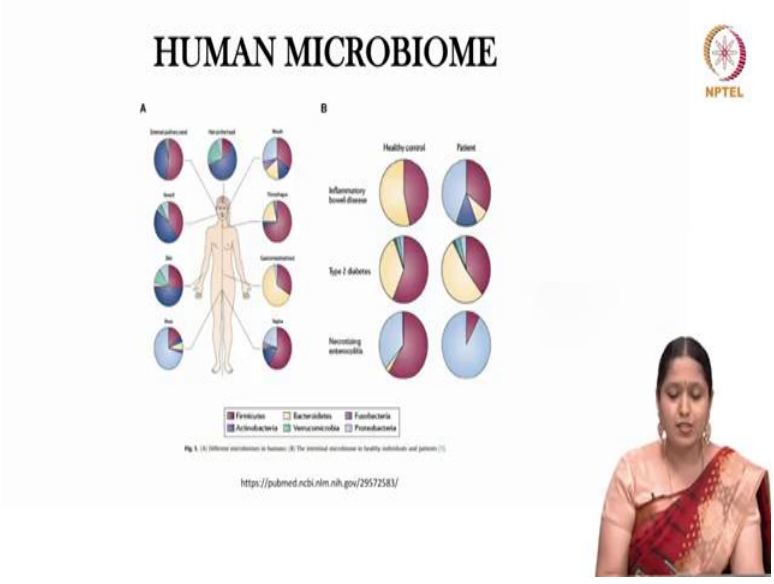

So, this is a picture of human microbiome, it shows different types of microbiomes present in human body like hair, on the head, oesophagus gastrointestinal, tract, genital organs, skin, nostril as well as the external auditory canal.

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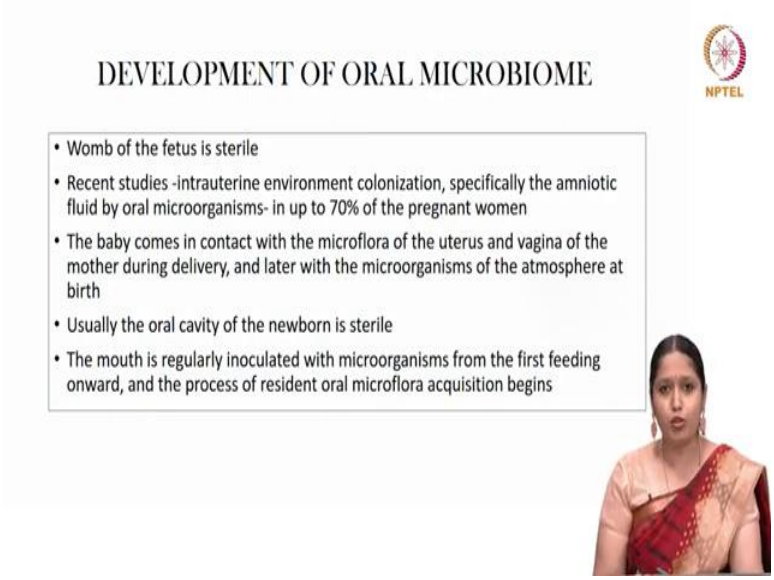

Now, let us see the development of oral microbiome. So, womb of the foetus is initially sterile. Recent studies have shown that intrauterine environment colonization was present especially in the amniotic fluid in almost 70 percentage of pregnant women. The baby comes in contact with the microflora of the uterus and vagina during delivery and later with the microorganisms present in the atmosphere at birth.

Usually, the oral cavity of the new born is sterile the mouth is regularly inoculated with microorganisms from the first feeding onwards and the process of resident oral microflora acquisition begins at this stage.

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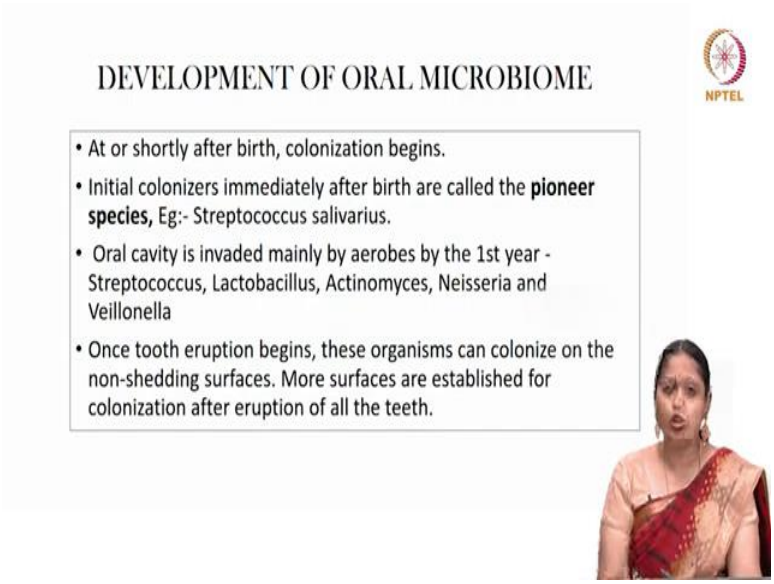

At birth or shortly after birth colonization begins. Initial colonizers immediately after birth are called as pioneer species. So, for pioneer species example is streptococcus salivarius. Oral cavity is invaded by aerobes by the 1st year which includes Streptococcus, Lactobacillus, Actinomyces, Neisseria and Veillonella. Once tooth eruption begins the organisms can colonize on the non-shedding surfaces. More surfaces are established for colonization after eruption of all teeth.

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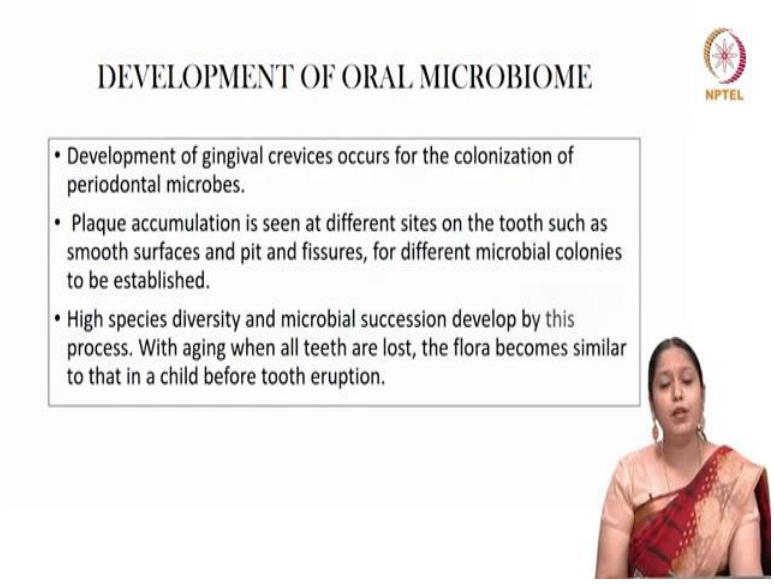

So, development of gingival crevices occurs at the later stage this is because for the colonization of periodontal microbes. Plaque accumulation is seen at different areas of the tooth like smooth surfaces as well as pit and fissures. High species diversity and microbial succession develop at this process. Once with aging all the teeth are lost then the oral microflora will become similar to that of the child before tooth eruption.

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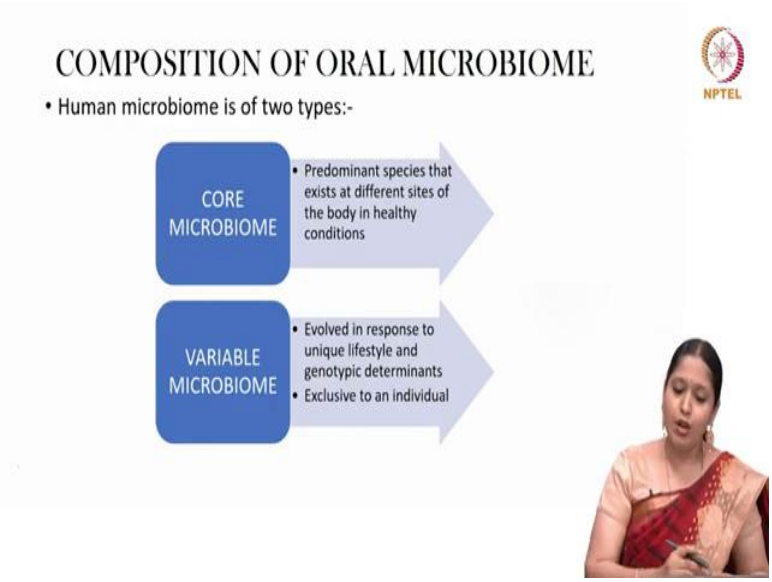

Now, coming to the composition of oral microbiome. Human microbiome is of two types; core microbiome and variable microbiome. Core microbiome represents the predominant species that exist at different sites of the body in healthy individuals. Variable microbiome is exclusive to an individual and it is evolved in response to unique lifestyle and genotypic determinants.

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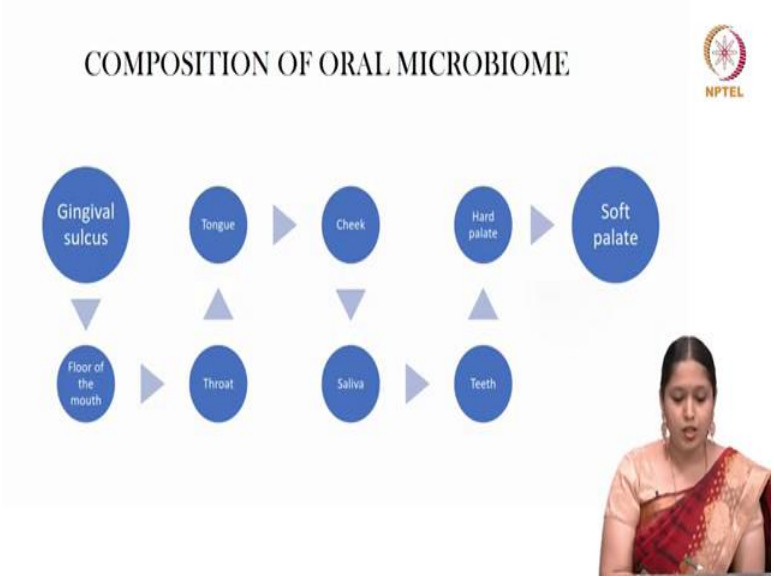

So, oral microflora or microbiome, they are seen in gingival sulcus, tongue, cheek, hard palate, soft palate, floor of the mouth, throat, saliva and teeth.

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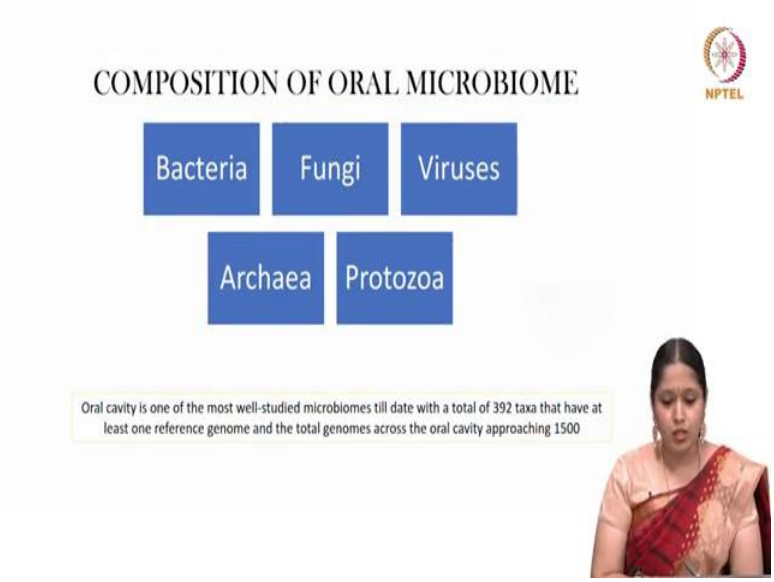

So, it also includes bacteria, fungi, viruses archaea and protozoa. Oral cavity is one of the most well studied microbiomes till date with a total of 392 taxa that have at least one reference genome and the total genomes across the oral cavity approaching 1500.

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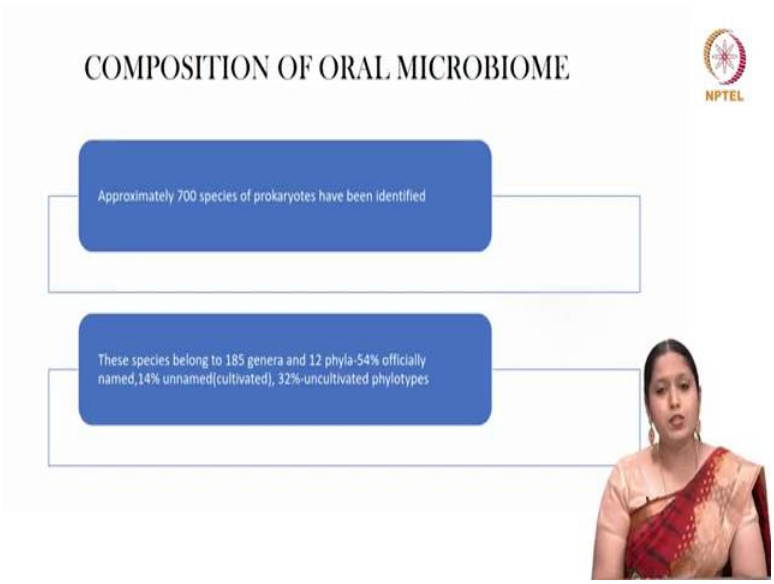

Approximately 700 species of prokaryotes have been identified. These species belong to 185 genera, 12 phyla and 54 percentage are officially named, 14 percentage are cultivated and 32 percentage are uncultivated phylotypes.

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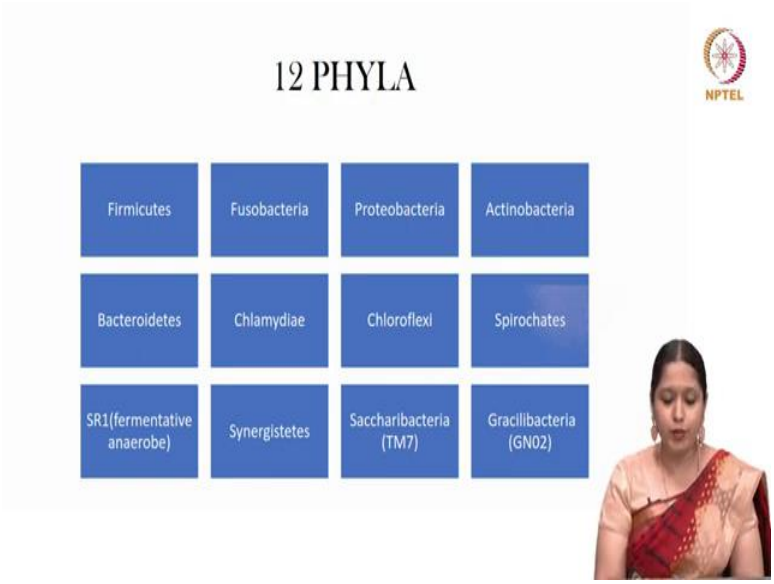

So, the 12 phyla include firmicutes, fusobacteria, proteobacteria, actinobacteria, bacteroidetes, chlamydiae, chloroflexi, spirochates, SR1 which is fermentable anaerobe, synergistetes, saccharibacteria and gracilibacteria.

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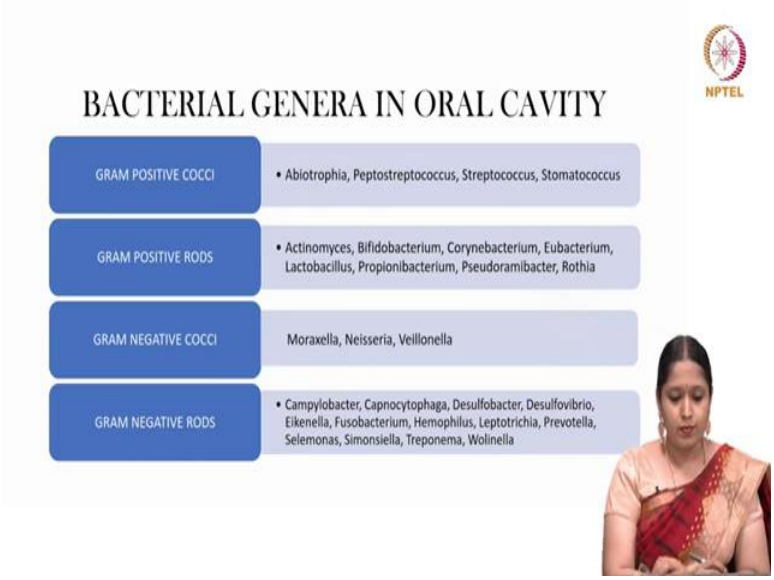

So, the bacterial genera in oral cavity includes gram positive cocci, gram positive rods, gram negative cocci and gram-negative rods. The gram-positive cocci include peptostreptococcus, abiotrophia, streptococcus and stomatococcus. Gram positive rods includes actinomyces, bifidobacterium, corynebacterium, lactobacillus and others.

Gram negative cocci; it includes Moraxella, Nisseria and Veillonella and gram negative rods include campylobacter, Capnocytophaga, Desulfobacter, Desulfovibrio, Eikenella species, fusobacterium, treponema and other species. So, these are the gram-positive cocci, bacilli and gram negative cocci and bacilli.

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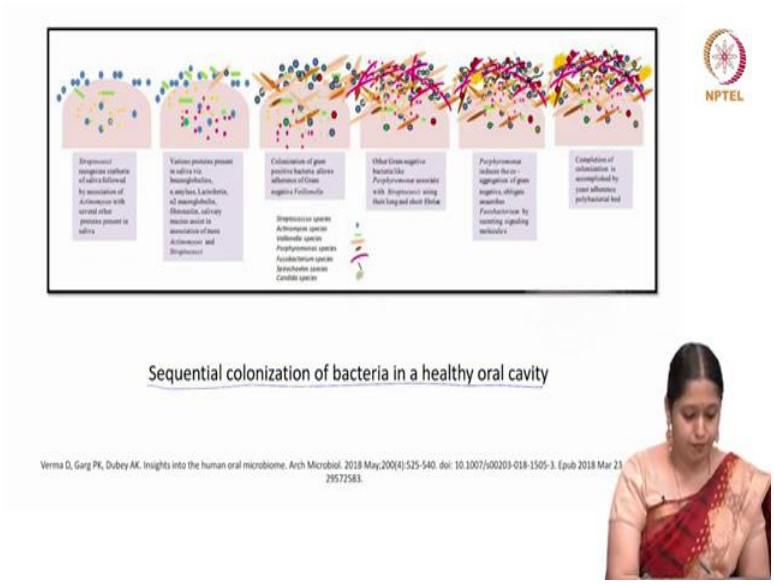

So, this picture shows the sequential colonization of bacteria in a healthy oral cavity. So, initially streptococci will recognize the statherin which is present in saliva followed by association of actinomyces with several other proteins present in the saliva.

So, the initial colonizers are streptococci later on actinomyces will come and various proteins present in the saliva apart from statherin like immunoglobulins, alpha amylase, lactoferrin, alpha 2 macroglobulin, fibronectin, salivary mucins also assist in association of more actinomyces and streptococci.

So, after the colonization of gram-positive bacteria there it allows adherence of gram negative bacteria which is Veillonella,other gram negative bacteria like Porphyromanas also associate with Streptococci. Later on Porphyromanas will produce signalling molecules which induces co-aggregation of gram negative obligate anaerobes. Later once completion of colonization is accomplished by yeast adherence which leads to poly bacterial bed. So, this is a sequential colonization of bacteria in a healthy oral cavity.

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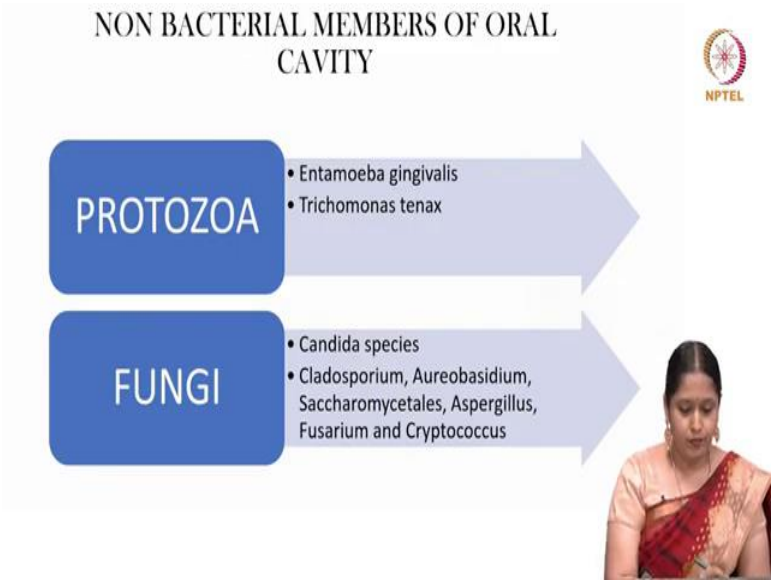

So, till now we have discussed about the bacterial members of the oral cavity, next includes the non-bacterial members of oral cavity which includes protozoa and fungi. Under protozoa comes Entamoeba gingivalis and Trichomonas tenax and fungi mostly Candida species, apart from that Cladosporium, Saccharomycetales, Aspergillus Cryptococcus, Aureobasidium and Fusarium are also seen.

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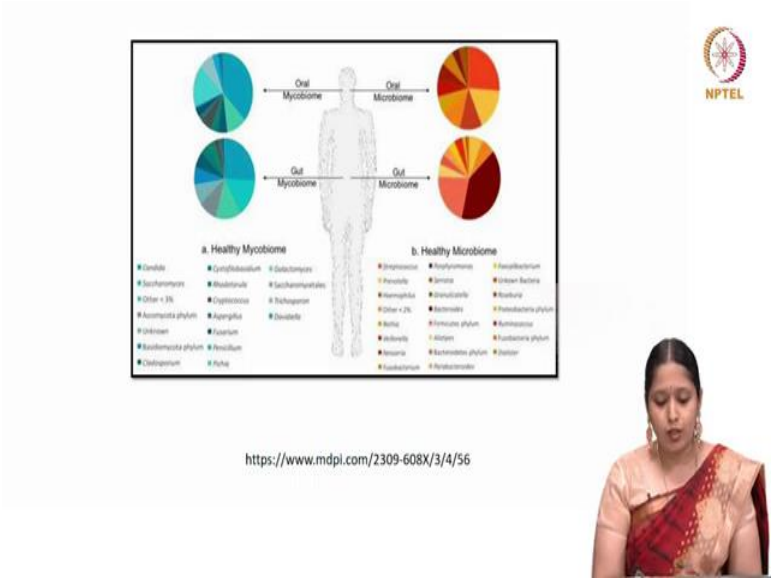

So, this picture shows a healthy mycobiome as well as the healthy microbiome. So, healthy microbiome as I mentioned before it includes streptococcus species and various

other bacterial species and healthy mycobiome which is the fungal component predominantly includes Candida species and other species like Cryptococcus, Pencillium, Saccharomycetales as well as Ascomycotoma phylum.

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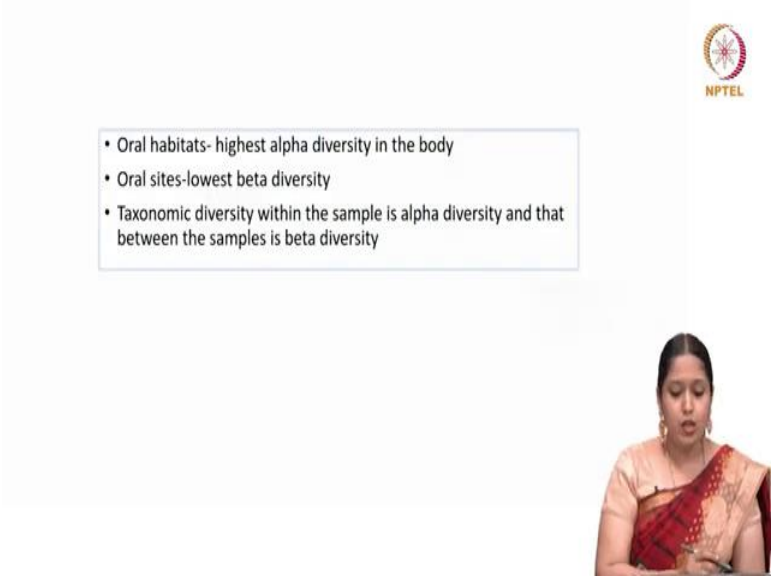

So, oral habitats have always got a highest alpha diversity in the body and it has got a lowest beta diversity. So, taxonomic diversity within the sample is called as alpha diversity and between the sample is called as beta diversity. Oral habitat has got highest alpha and lowest beta diversity.

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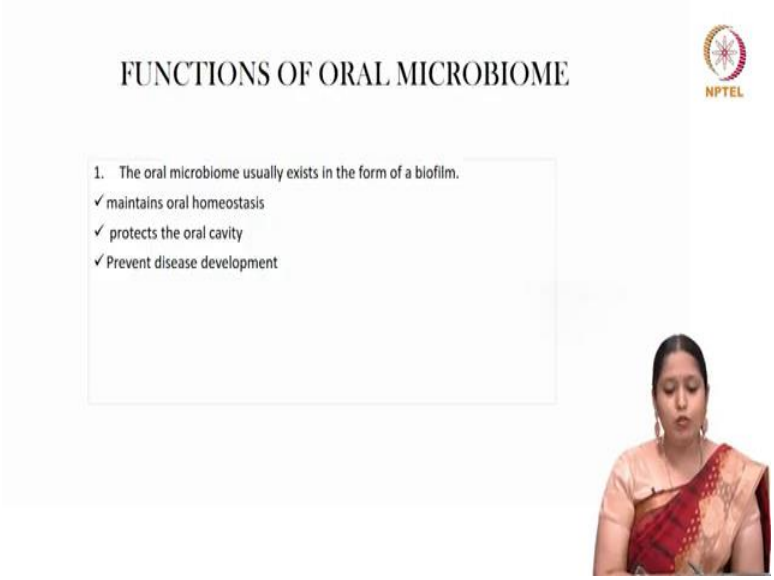

Now, let us see the functions of oral microbiome. So, oral microbiome usually exists in the form of a biofilm,it maintains oral homeostasis, it protects the oral cavity and it prevents disease development.

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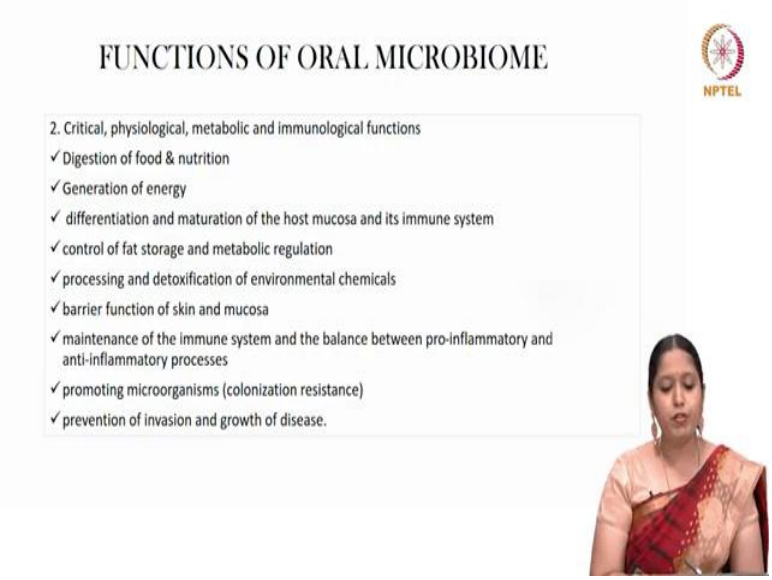

Apart from that it also performs critical physiological, metabolic and immunological functions which includes digestion of food and nutrition, generation of energy differentiation and maturation of host mucosa and the immune system, control of fat storage and metabolic regulation.

Processing and detoxification of environmental chemicals, barrier function of skin and mucosa, maintenance of the immune system and the balance between pro inflammatory and anti-inflammatory processes, colonization resistance and prevention of invasion as well as the growth of the disease. So, these are the functions of oral microbiome.

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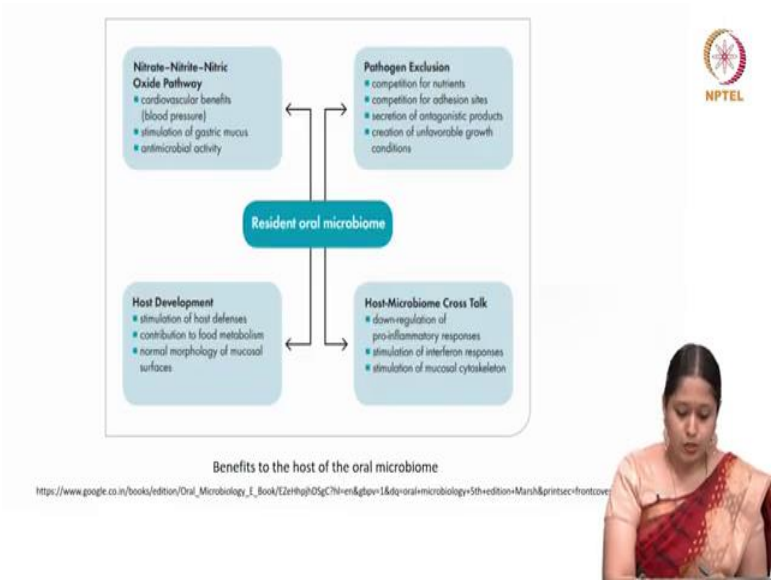

So, what are the benefits to the host by the oral microbiome? It includes numerous benefits like cardiovascular benefits which helps in controlling blood pressure, stimulation of gastric mucus and the most important function is the antimicrobial activity. Apart from that the resident oral microbiome also helps in pathogen exclusion which competes for the nutrients, adhesion sites, antagonistic products as well as creation of unfavourable growth condition.

And it also helps in host development by stimulation of host defences and it also contributes to food metabolism and normal morphology of the mucosal surfaces, also it helps in host microbiome crosstalk.

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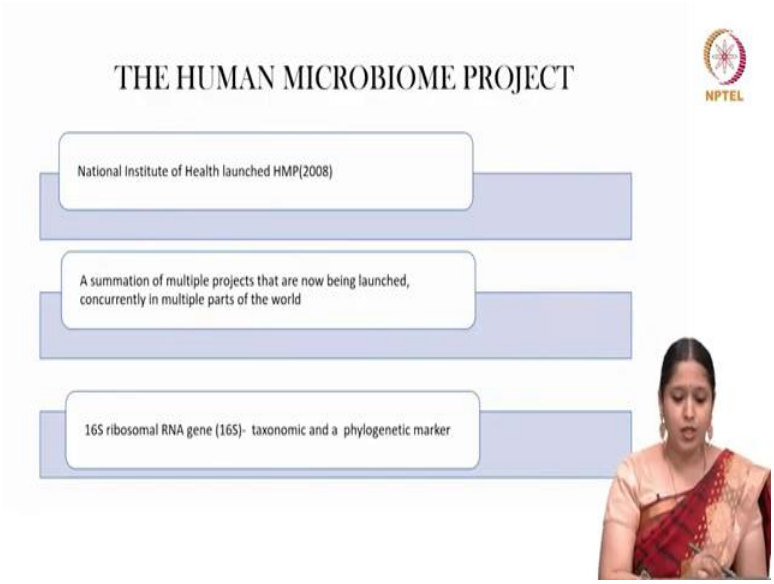

There is something called as human microbiome project which was first launched in the year 2008 by National Institute of Health, it is nothing, but a summation of multiple projects that are now being launched concurrently in multiple parts of the world. And they consider 16S ribosomal RNA as the taxonomic as well as the phylogenetic marker.

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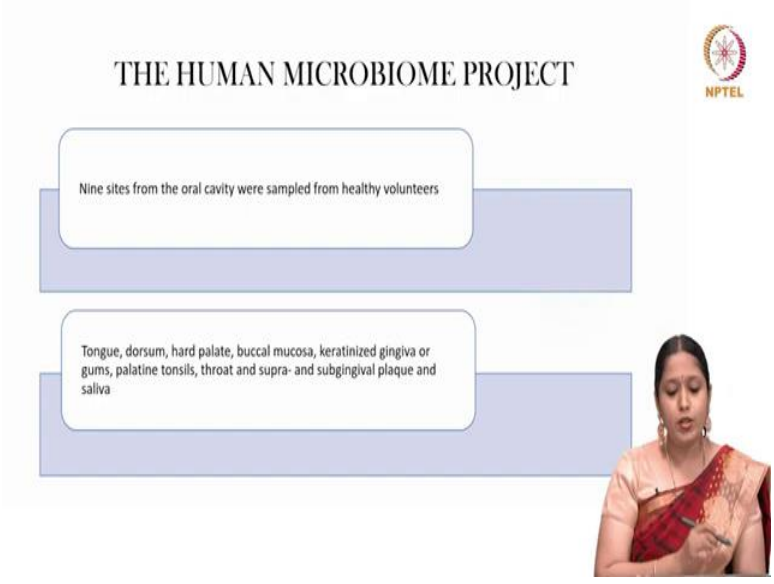

So, nine sites from the oral cavity were sampled from healthy volunteers and those sites include tongue, dorsum of tongue, palate, hard palate, buccal mucosa, keratinized gingiva, palatine tonsils, throat, supra gingival and subgingival plaque as well as saliva.

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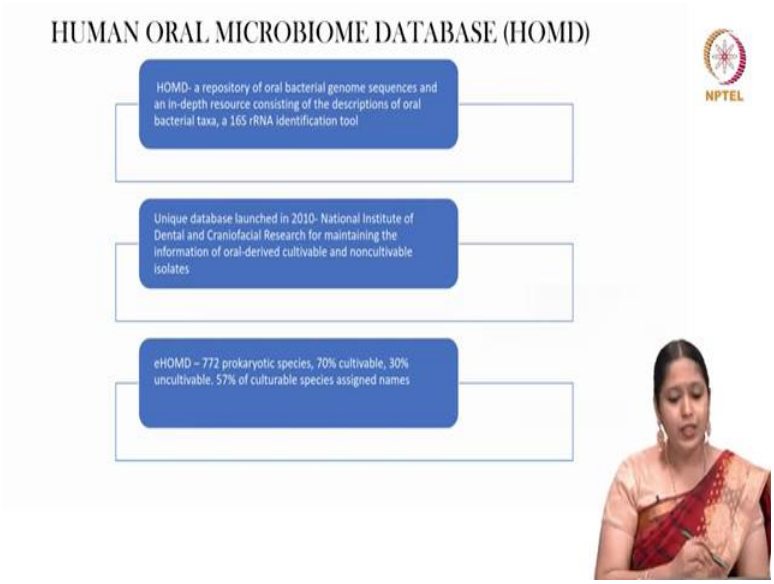

Another thing is human oral microbiome database. This is a unique database which was launched in the year 2010 by the National Institute of Dental and Craniofacial Research especially for maintaining the information of the oral derived cultivable and non cultivable isolates.

So, a human oral microbiome database is a repository of oral bacterial genome sequences and an in depth resource consisting of the description of oral bacterial taxa, a 16S rRNA identification tool.

There is something called as expanded human oral microbiome database which is also called eHOMD. It consists of 772 prokaryotic species, 70 percentage of cultivable, 30 percentage of uncultivable and 57 percentage of culturable species are assigned names.

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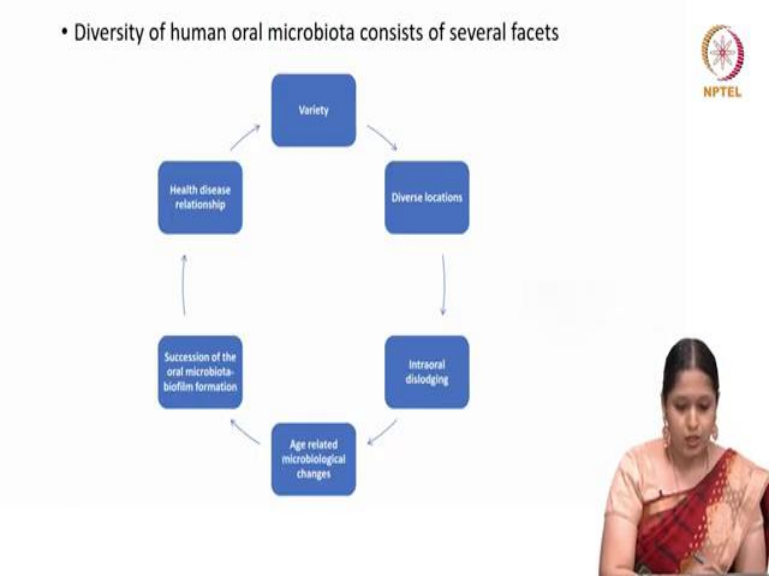

So, diversity of human oral microbiota consists of several facets. First is variety as I mentioned before there are huge variety of micro-organisms inside the oral cavity, then diverse locations when you come to locations these microorganisms are present on soft tissue as well as hard tissue as well as on dentures and implants.

Next is intraoral dislodging-certain microbiomes are particular to a specific niche, but what happens is certain microorganisms like streptococcus mutans has the ability to dislodge from one place to another. So, they are seen in multiple sites. So, this is one of the properties, that is intraoral dislodging. Next is age related microbiological changes succession of oral microbiota that is biofilm formation and health disease relationship. So, these are all the different facets.

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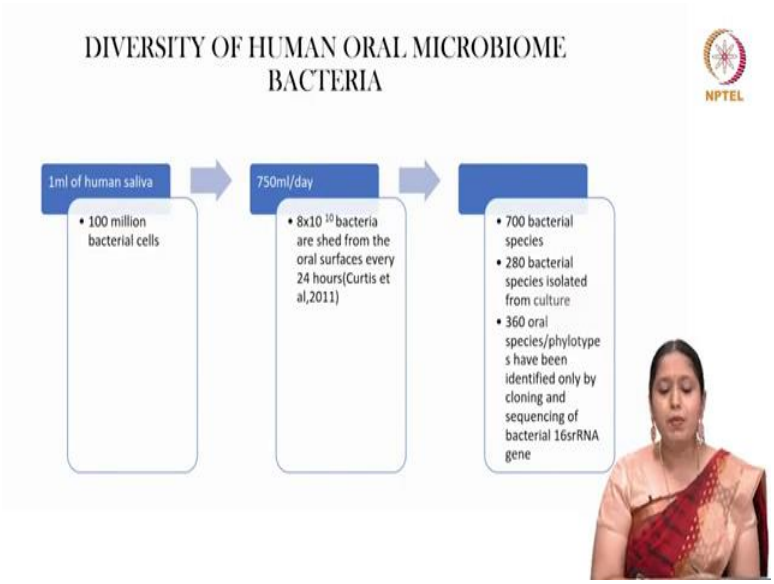

So, 1 ml of human saliva contains approximately 100 million bacterial cells and 750 ml of saliva is secreted per day. $8x10^{10}$ bacteria are shed from the oral surfaces every 24 hours and there are 700 bacterial species, 280 bacterial species isolated from culture, 360 oral species or phylotypes have been identified only by cloning and sequencing of bacterial 16S rRNA gene.

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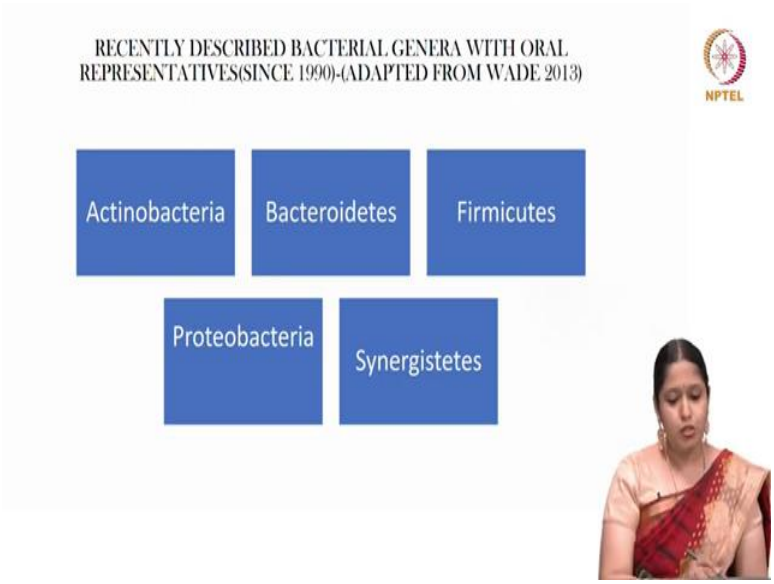

So, this list includes recently described bacterial genera with oral representatives adapted from Wade 2013 which includes Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria and Synergistetes.

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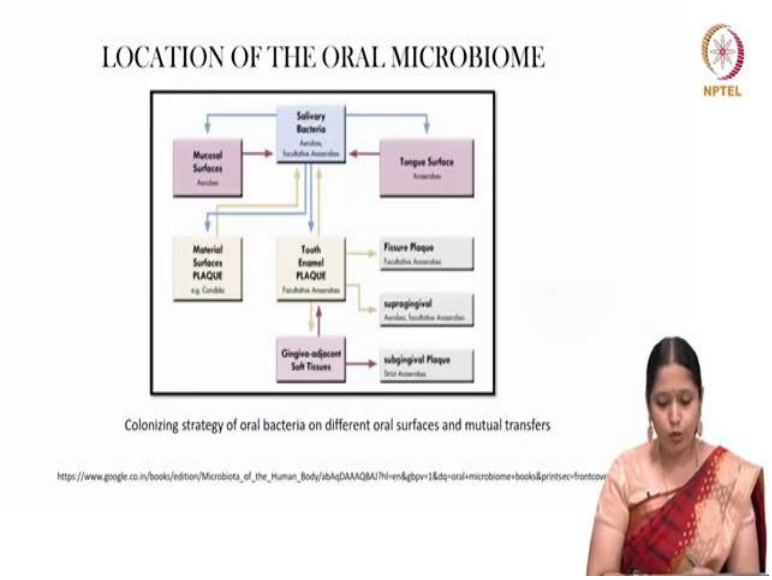

Now coming to the location of oral microbiome, this picture shows the colonizing strategy of oral bacteria on different oral sites. So, salivary bacteria mainly include aerobes and facultative anaerobes tongue surface has got anaerobes, fissure plaque it has got facultative anaerobes, supra gingival it includes aerobes and facultative anaerobes tooth enamel has got a plaque.

So, it has got a facultative-anaerobes gingiva adjacent soft tissues under that subgingival plaque has got strict anaerobes. And also, material surfaces like plaque have got Candida species and mucosal surfaces has got aerobes.

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Now, what are the properties? Properties include high species diversity, surface attachment or co adhesion, obligate anaerobes, fastidious nutrition or unculturable nature, slow growth as well as identification.

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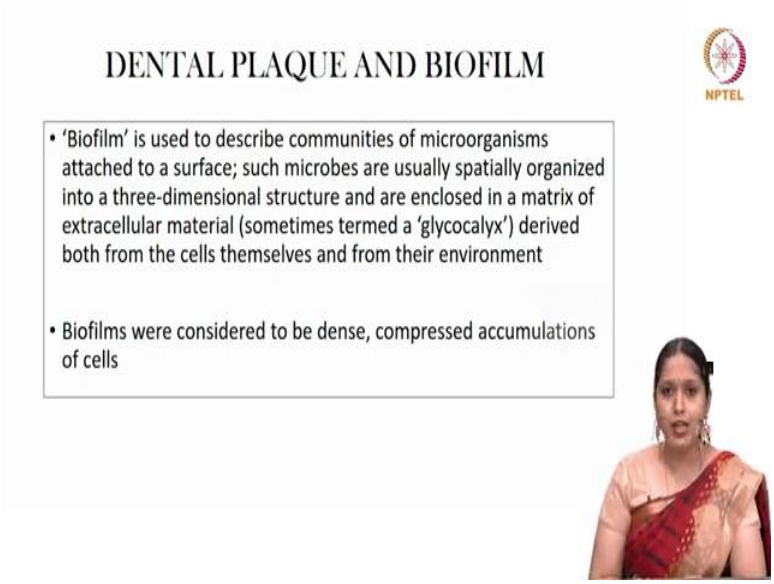

Now, let us see the dental plaque and biofilm. So, a biofilm is described as a communities of microorganisms attached to a surface such as microbes that are usually spatially organized in a three-dimensional structure and they are enclosed in a matrix of extracellular material which sometime termed as a glycocalyx and they are derived both

from the cells themselves as well as the environment. So, biofilms were considered to be a dense compressed accumulation of cells.

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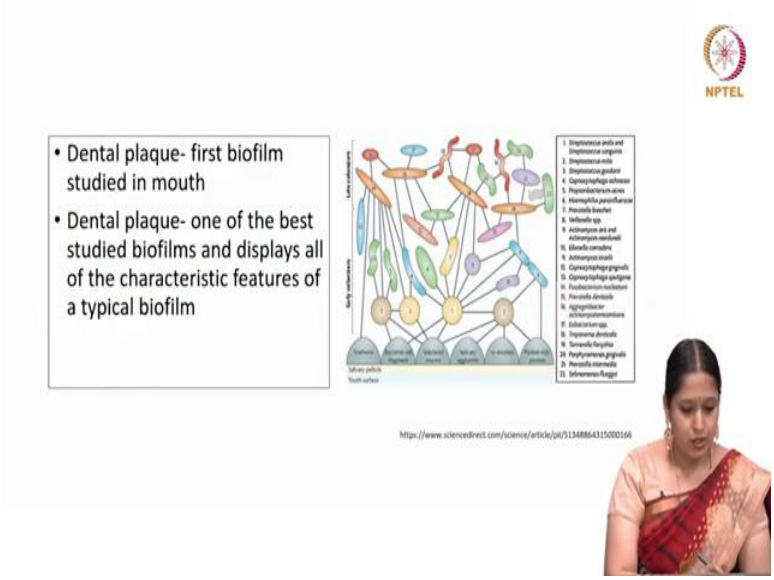

Dental plaque is considered to be the first biofilm studied in the mouth. So, dental plaque is one of the best studied biofilms and displays all the characteristic features of a typical biofilm. So, in this picture you can see the tooth surface which is covered by salivary pellicle and the saliva has got various proteins and other materials like statherin,bacterial cell fragment,mucins, salivary agglutinants, alpha amylase and proline rich protein.

So, all these components will cause association of microorganisms first in early colonizers which predominantly includes streptococcus and then comes the late colonizers.

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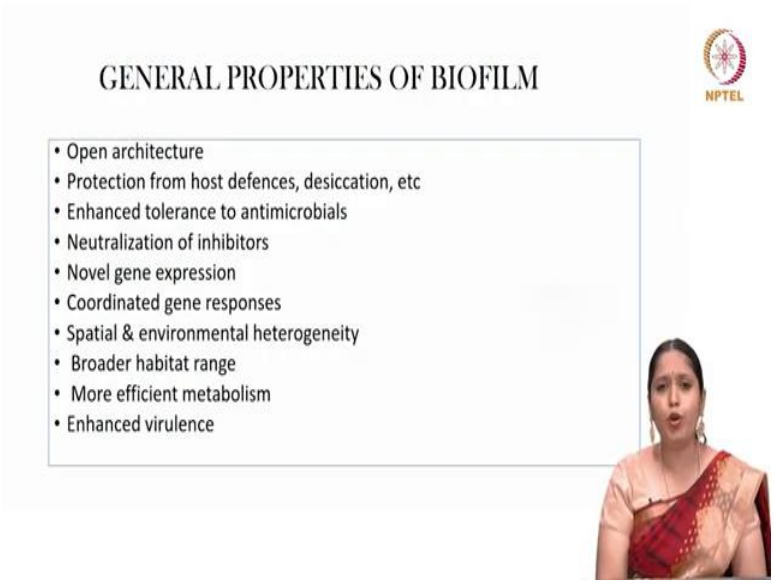

So, what are the general properties or ideal properties of a biofilm; it includes open architecture, protection from host defence, enhanced tolerance to antimicrobials, neutralization of inhibitors, novel gene expression, coordinated gene responses, spatial and environmental heterogeneity, broader habitat range, more efficient metabolism and enhanced virulence. So, these are all the ideal properties of a biofilm.

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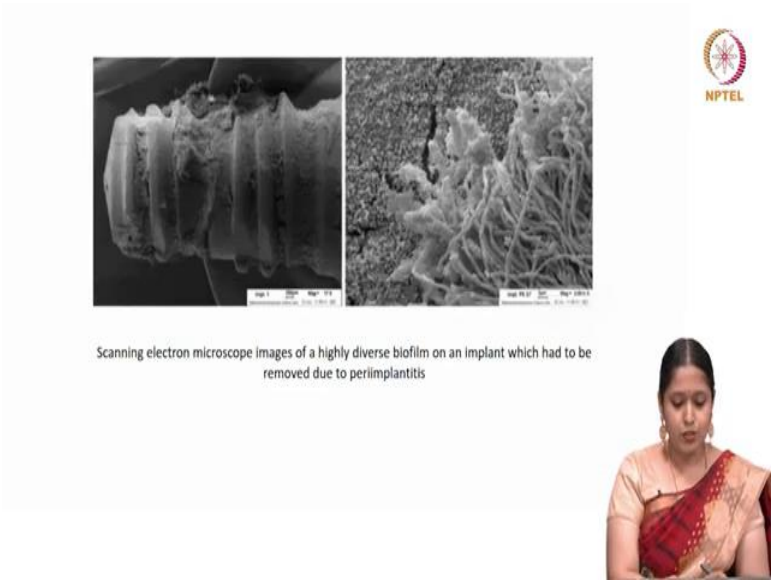

So, this is scanning electron microscopic picture of a highly diverse biofilm which is seen on an implant dental implant and later on this dental implant has to be removed because it caused periimplantitis.

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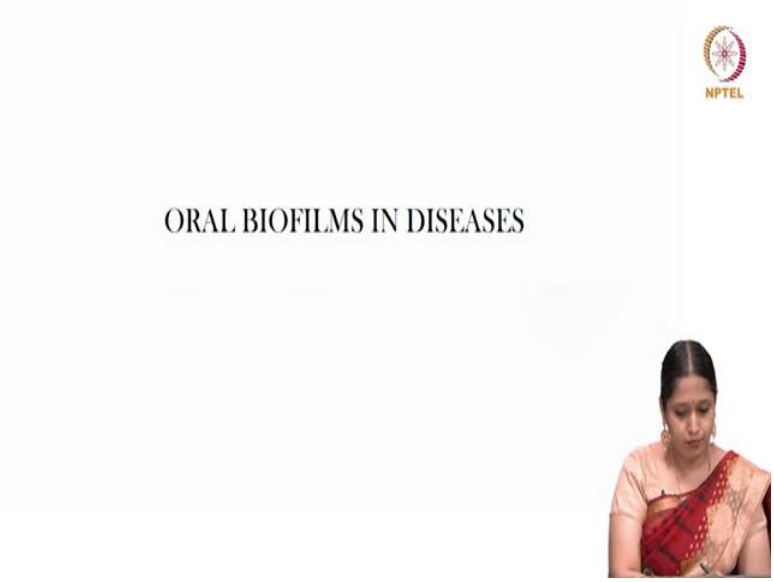

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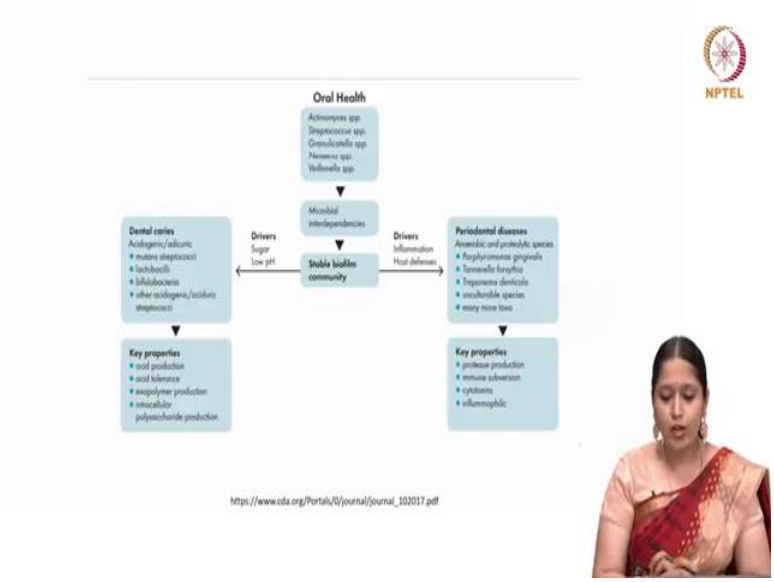

Now, let us see oral biofilms in diseases. So, normal healthy oral flora includes actinomyces,streptococcus and neisseria and veillonella species and it forms a stable biofilm community. But whenever there is a drive, external drivers like sugar as well as low pH what happens is it leads to a condition called as dental caries.

So, in dental caries- acidogenic or aciduric bacteria will be more predominant which includes mutant streptococci, lactobacilli, bifidobacteria and other acidogenic or aciduric streptococci.

So, the key properties will be there will be acid production, acid tolerance, exopolymer production and intracellular polysaccharide production, On the other side if another drivers or external stimuli comes like inflammation and host defence it leads to periodontal diseases, where there will be anaerobic as well as proteolytic species like Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola and other unculturable species. And the key properties will be protease production, immune subversion, cytotoxins and inflammophilic.

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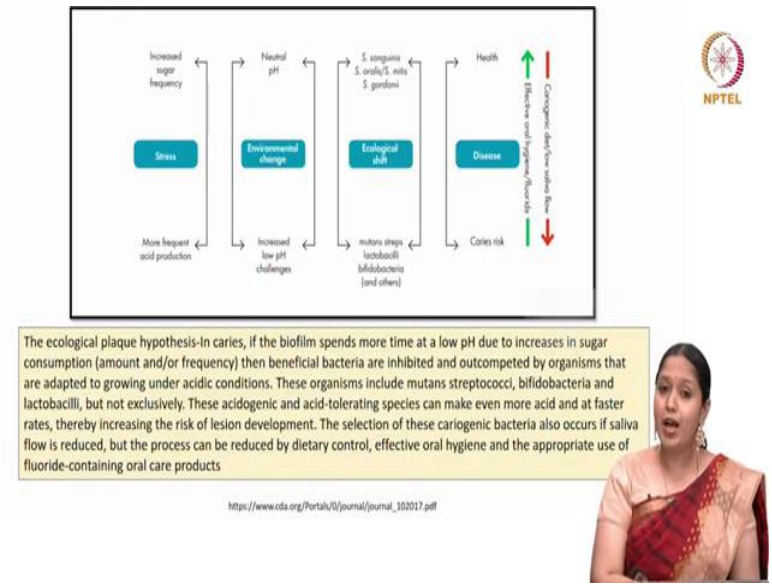

So, let us see the ecological plaque hypothesis how it is applicable to caries. So, in caries what happens is the biofilm spends more time because of the lower pH as a result of increased sugar intake, what happens is there will be reduction in the pH, the bacteria will produce acid which causes reduction in pH as a result it also depends upon increase in the sugar consumption either in the amount or in the frequency of sugar consumption.

So, what happens is the beneficial bacteria are inhibited and it will be out competed by organisms there are adapted to growing in acidic condition. So, these organisms mainly include mutant streptococci bifidobacteria and lactobacilli. So, these acidogenic and acid tolerating species can make more acid and at a faster rate this leads to condition called as

dental caries or tooth decay. So, what happens is because of stress there will be increased sugar frequency and there will be more frequent acid production.

Also there will be environmental change from neutral pH to low pH, there will be ecological shift from normal oral microflora to acid producing microflora or with those microflora which can tolerate under low pH like mutans streptococci, lactobacilli and bifidobacteria which leads to the disease from the normal health condition to caries risk condition.

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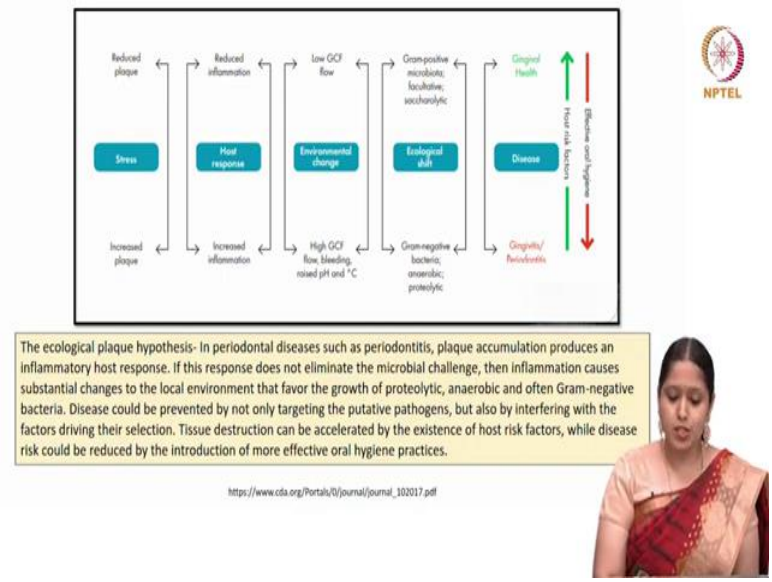

So, the ecological plaque hypothesis for the periodontal diseases. In conditions such as periodontitis plaque accumulation produces an inflammatory host response, always remember plaque accumulation causes inflammatory host response, if this response does not eliminate the challenge what happens is the inflammation causes substantial changes to the local environment that favour the growth of proteolytic, anaerobic and offer gram negative bacteria.

So, if the host immune response does not work there will be more number of proteolytic anaerobic as well as gram negative bacteria. So, what happens in case of periodontal disease? Stress will cause increased plaque and also host response from reduced inflammation to increased inflammation and environmental change from low gingival crevicular fluid flow to high GCF flow,bleeding and increased pH and there will be

ecological shift from gram positive microbiota facultative to gram negative bacteria anaerobic and proteolytic.

So, the normal gingival health is altered and it leads to periodontal diseases that is gingivitis and periodontitis. So, this is the ecological plaque hypothesis in relation to periodontitis.

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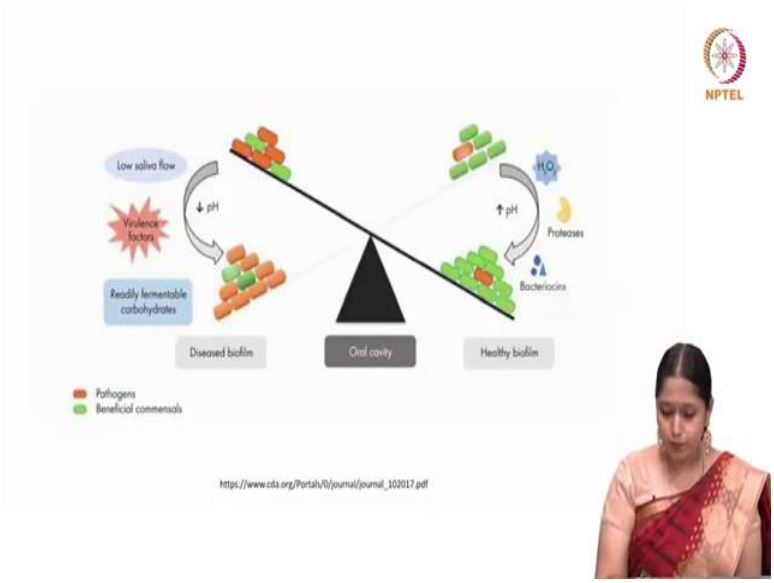

So, this picture also explains the healthy biofilm and a diseased biofilm. So, healthy biofilm has got more number of beneficial bacteria and there will be more pH and it is in a healthy condition, but what happens is because of low pH and more intake of sugar and it causes low pH and there will be acid production.

As a result, there will be more amount of readily fermentable carbohydrates and it forms a diseased biofilm. So, diseased biofilm has got lesser number of beneficial commensals and more number of pathogens.

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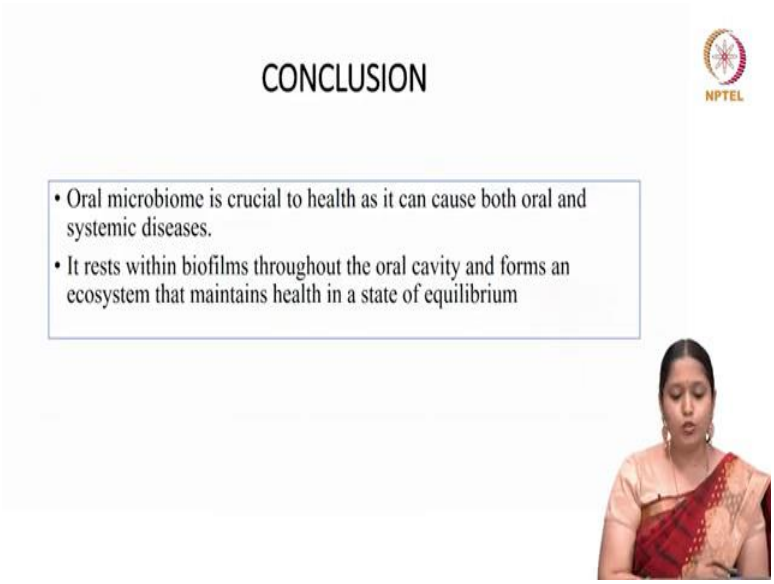

So, to conclude oral microbiome is crucial to health because it can cause both oral as well as systemic diseases. If any alteration happens in the oral microbiome or the biofilm there will be disease. It rests within the biofilms throughout the oral cavity and it forms an ecosystem that maintains health in a state of equilibrium.

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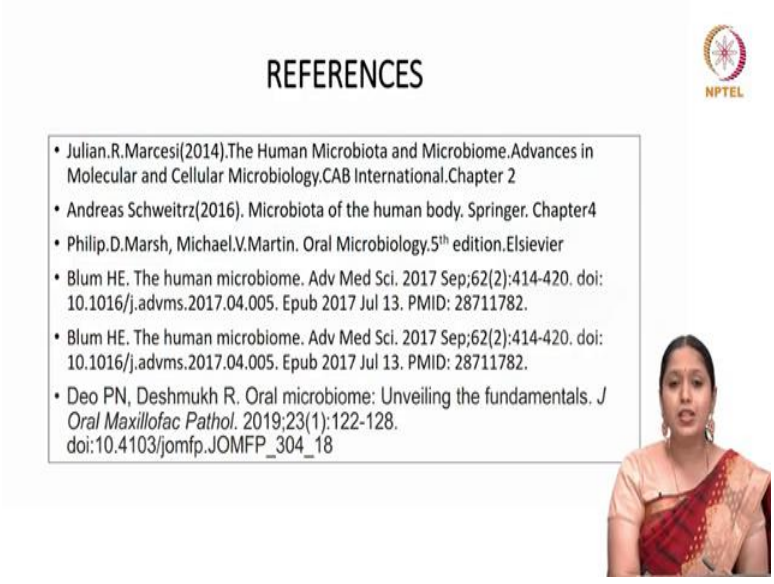

So, these are my references.

Thank you.