Applied Environmental Microbiology
Dr. Gargi Singh
Department of Civil Engineering
Indian Institute of Technology, Roorkee

Lecture – 27
Microbial Symbiosis II

Dear students, in this class, we will continue what we talked about in previous class which is microbial symbiosis, but our interest now will be on the higher order of life forms particularly and specifically mammals. We will start with ruminant my mammals and then we will move on to human beings. So, let us get started here. Now in the figure on the left, you have 4 plots.

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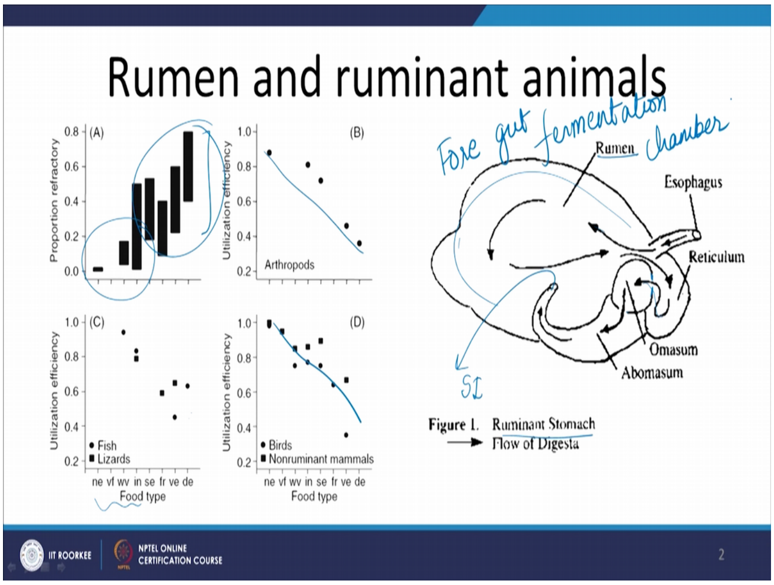

Rumen and ruminant animals

Figure 1. Ruminant Stomach
→ Flow of Digesta

And if you note here on the first plot of this particular plate, you have as the different food types at present on the x axis and the proportion refractory. So, the proportion that is really hard to digest is increases or is different for the different kinds of food types. So, for example, the food on the right most have higher amount of refractory in them than the food types present in the on the left side of the x axis.

So, if we look at different kinds of organisms and how much how well and how efficiently they can degrade the 2 different kinds of foods the one here which have very low or negligible amount of refractory food proportion and the ones that are very high in the refractory portion of the food. So, if you look at fish and lizard we noticed that they can digest very well the food here which is which has very low refactor refractory proportion, but their efficiency drops by up to sixty percent for the fish when the refractory portion rises up. Similarly for arthropods, we notice a quite linear decrease as the portion of refractory or difficult to digest part of the food increases again in birds and mammals that are non ruminant like ourselves.

We notice that high fiber food or food that is hard to digest will reduce the efficiency of digestion and this is where the stomach of ruminant; orga-ruminant living beings such as cows and rabbits really helps them digest their food better especially when it has high concentration or higher proportional refractory materials now what will be your typical refractory materials in food; obviously, cellulose and maybe some amount of lignin or hemicellulose.

Now, this is very relevant when we talk about being such as termites which feed on wood or rabbits which feed mostly on grass and on different kinds of leaves also with herbivores such as cows and buffalos now they have evolved to have a special kind of stomach which we just abbreviate and we call it rumen and now we are going to notice today in this class we are going to study how the microbial composition of the rumen effects the or encourages or allows degradation of cellulose and other complex organics there are otherwise very hard to digest.

For example, if we humans who are non ruminant mammals eat grass, we will not get will hardly get any calories out of it now depending on the grass it might have some sugars and we will take benefit of that sugar, but we will not be able to digest the fibers or cellulose same is the case with dogs, but when it comes to ruminant beings they can make energy out of it. So, let us look at the rumen of a cow and try to get an understanding of how a rumen works in general.

So, this is your Esophagus. So, Esophagus is your basically the food pipe through which the food travels in. So, now, the cow has eaten some grass and it will go through the Esophagus into the rumen reticulum chamber. So, this big chamber here is rumen reticulum chamber now it goes in the rumen reticulum chamber to stay here it will ruminate for a while which means a different kind of degradation happening here and then it will be vomited by back to the mouth where the cow or buffalo will chew the card. So, they will chew it again send it back again and then this process will continue the process of food coming in from mouth to rumen reticulum chamber and then going back to the mouth until the food is small enough for Esophagus into the Omasum Abomasum; Omasum and Abomasum portions of the stomach.

Now, this ports are usually much much smaller than what is depicted here for in schematic, but the point is that every time the food ruminates in the rumen and is chewed again by the cow buffalo or the ruminant mammal there is some level of degradation that happens apart from the mechanical chewing done by the cow and this degradation is carried out mostly by enzymatic action of microbes and to some degree in enzymatic action of enzymes released by the mammal itself now once the food is has been chewed to smaller levels enough plenty of degradation has happened then the food will pass into Omasum and Abomasum and it will go into the small intestine. So, this is your small intestinal tract.

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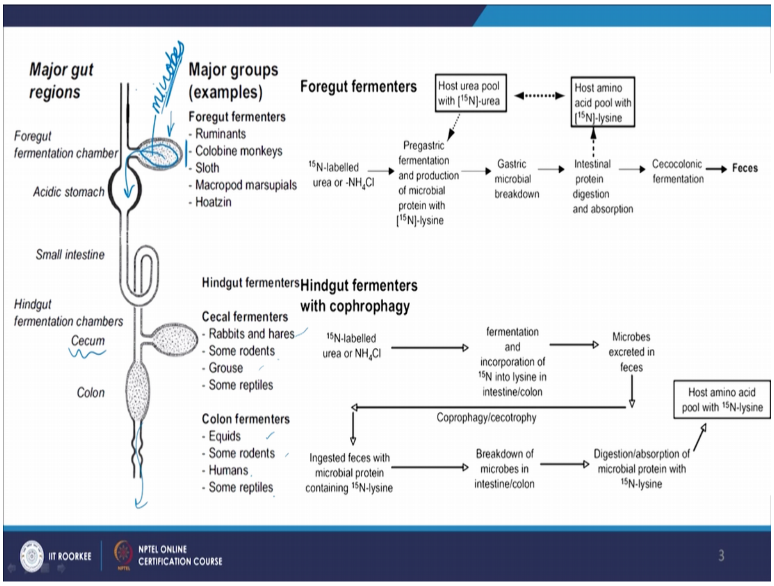

Major groups (examples)

Foregut fermenters
- Ruminants
- Colobine monkeys
- Sloth
- Macropod marsupials
- Hoatzin

Hindgut fermenters

Cecal fermenters
- Rabbits and hares
- Some rodents
- Grouse
- Some reptiles

Colon fermenters
- Equids
- Some rodents
- Humans
- Some reptiles

So, if we look at this cartoon of the digestive tract of ruminant and mammal we notice that they have a foregut fermentation chamber then they have an acidic stomach which very much like our acidic stomach they have a small intestine followed that follows acidic stomach again very much like our digestive system and then in their hindgut or in where we have large intestine they have something called Cecum which is their hindgut fermentation chamber some fermentation also happens in the colon and then the excretion of the waste.

So, they have basically 2 fermentation chamber one is the foregut fermentation chamber the other is hindgut fermentation chamber. So, the big refractory components of food that would degraded and chewed up to small enough quantities in the rumen now will either ferment in the foregut fermentation chamber or in hindgut fermentation chamber now we have 2 types of ruminants one are the foregut fermentated fermenters which ferment mostly in their foregut and then they are hindgut fermenters which ferment mostly in their hind.

Now, the examples of foregut would be the most ruminants monkeys sloth marsupials such as kangaroo hoatzin the in hindgut fermenters we have again 2 different kinds of hindgut fermenters one they will ferment in the Cecum and the other in the colom. So, in the colon so, the Cecum fermenters would be rabbits hares some rodents such as rats grouse and some reptiles the colon fermenters would be equids some rodents humans like ourselves and reptiles.

So, for foregut fermenters they foregut is microbrush because that is where they are fermenting. So, the microbes here after they have fermented the food what they will do is they will uptake the daughter products or during the fermentation they will take the daughter products and there is lot of microbes that are not produced because this is nutrient rich. So, microbial growth is exponential there and they have lot of the microbial growth increases very high concentration of microbial proportion.

Now, when the food the fermented food goes to the acidic stomach, it does not go alone it carries some biomass with it. So, when the biomass comes here in the acidic stomach the biomass gets acid hydrolyzed and thus the cow or the animal not only gets food from the food that it eat the grass or whatever it eat, but also benefits also has the additional benefit of eating the microbial biomass that grew up on in upon the fermentation daughter products that also fermentation that happen in the foregut chamber, this advantage is not present with hindgut fermenters like ourselves for us hindgut fermenters, we do not have that advantage for us the food does not pass through a foregut fermentation chamber. Many of us; most of us do not have one food directly goes to the acidic stomach undergoes initial degradation and then undergoes Cecum fermentation or colon fermentation.

Now, all the biomass that is produced here in the Cecum or in the colon is excreted out directly and that is one reason why our fecal matter is. So, rich in microbes now think about this if the human body or other hindgut fermenters could actually capitalize the biomass and the poop then we would require much less food this advantage is only present for foregut fermenters not for the hindgut fermenters. So, in this particular study there whose picture is there on the right side of the slide what they did was they added urea or ammonium chloride with isotope of heavier isotope of nitrogen.

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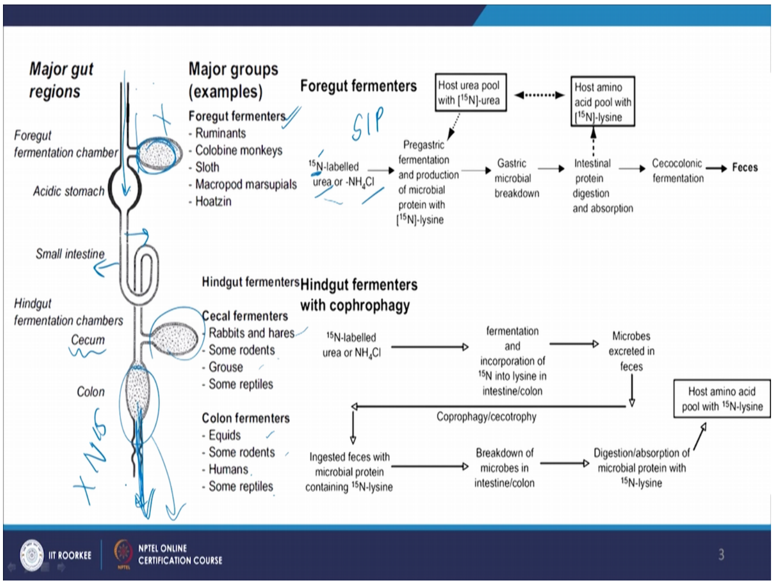
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Colon fermenters
- Equids
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So, these were high heavier urea or heavier ammonium chloride this is a stable isotope probing a technique in which I add a heavier isotope of the either carbon nitrogen or any other element that I know will be up taken by biomass and then as it goes through different parts of the digestive system I can look at radioactivity and I can get an idea of there than this nitrogen is being removed.

So, we noticed that in foregut fermenters when the labelled nitrogen urea or labelled ammonium chloride and label nitrogen source was added it was fermented here. So, there was lot of labelled nitrogen that was detected here and then in acidic stomach again it was detected and then it was assimilated in the small intestine and it became part of the cow became part of the foregut fermenter and there was hardly any hardly any heavier nitrogen observed in the poop and hindgut fermenter there will be a different situation in hindgut fermenter because there is no because there is no foregut fermentation we will notice and when the fermentation happens in the hindgut either in Cecul, Cecum or in colon we noticed that a lot of nitrogen that has been degraded becomes part of biomass is not adsorbed is not absorbed by the body and is released in the excreta.

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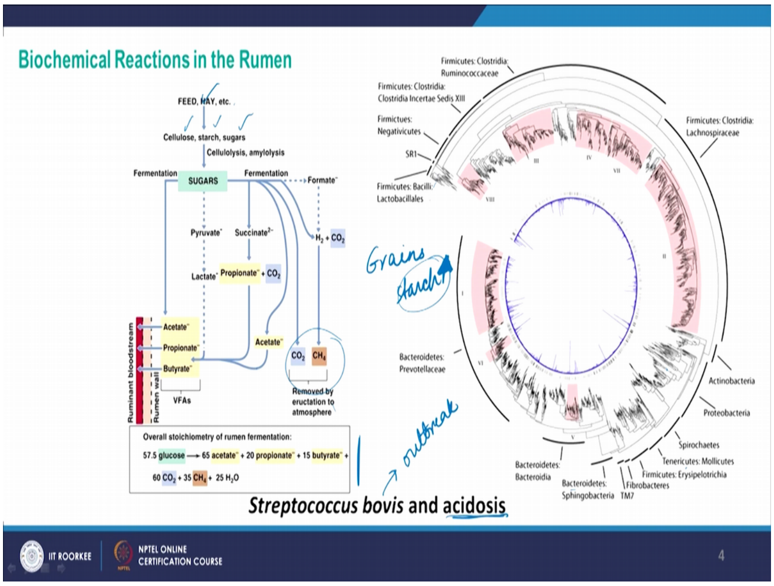

Biochemical Reactions in the Rumen

Overall stoichiometry of rumen fermentation:

$$57.5 \text{ glucose} \longrightarrow 65 \text{ acetate}^- + 20 \text{ propionate}^- + 15 \text{ butyrate}^- + 60\ CO_2 + 35\ CH_4 + 25\ H_2O$$

Streptococcus bovis and acidosis

Now, look at there what happens in the rumen where not a fermentation happens for ruminants the foregut fermenters usually we have the feed which is hay grass or other things they have the very rich in cellulose instarch and sugar. So, we they undergo cellulolysis amylolysis. So, the break down into simpler sugars in rumen so, this is all bacterial degradation. So, here we are talking about our foregut fermenters which are our ruminants.

So, if you remember this rumen this is your foregut fermentation chamber. So, it under breaks down into sugar then into pyruvate lactate and then we will go to the ruminant bloodstream or it sugar can directly go to the ruminant blood bloodstream depending on the kind of sugar it can it will undergo fermentation in form formate or methane or carbon dioxide or acetate if its acetate or propionate that will go inside the bloodstream and if it is carbon dioxide and methane the cow will excrete it out. So, this is called eructation and then this is the overall stoichiometry of your rumen fermentation.

Now, the microbes that that inhabit and they that cattle that in a way carry out these chemical reactions are very diverse as is very clear by the panel on the right where you notice that the each of these dendogram is one kind of microbe that has been detected using the third generation sequencing technique we will call them metagenomics because we do not require to culture them. We notice that there are lot of clostridia that represent in the rumen in the rumen of a cow and that is why we initially believe that most of the cellulose degraders are present in clostridia or in firmicutes which is still that which is still relevant because most of the cluster cellulose degrading organisms or the one that carry out the hydrolysis of cellulose that we know are present and firmicutes.

So, we have firmicutes bacilli lactobacilli negativicutes clostridia ruminococcaceae all of them are firmicutes and then we had other kind of firmicutes then some actinobacteria proteobacteria some are proteobacteria spirochaetes tenericutes some bacteroidetes sphingobacteria and so, this is the very diverse and yet very localized microbial community of rumen.

Now, now important thing is this is the rumen microbiology you already how does it affect the health of the animal now here is the thing if there is any rapid change in the rumen microbiology that can affect the health of the animal for example, if instead of feeding the cow hay or cellulose rich food I started feeding then grain rich food now the grains they have red; they have very different chemical composition than cellulose rich hay the sugars are is there the protein content is different sugar content is different and more importantly they are very high starch content now because of this very high starch content what happens is that streptococcus bovis grows rapidly in rumen.

In fact, it is almost like a streptococcus bovis outbreak in that rumen it colonizes and becomes a dominant member of the rumen and it starts degrading starch and then reserves in acidosis acidic environment and rumen which irritates the rumen may even lead to internal hemorrhage and kill the cow kill the ruminant animal. So, not what we eat will affect, the microbial community will affect the daughter products and that will affect the health of the cow or the animal.

So, this is the first glimpse that we have here of how there are food effects a microbial community in the gut then this is the gut of the cow the foregut fermentation chamber and that affects our health.

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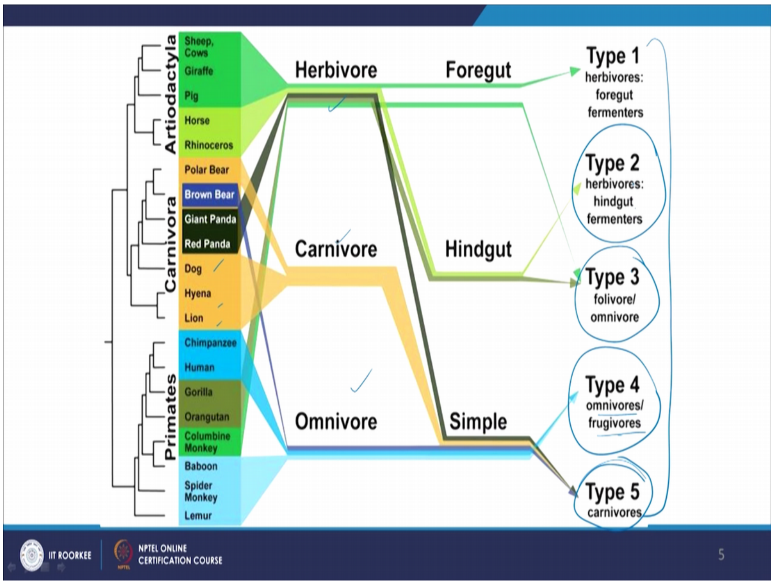

So, now if we look at other microbes other beings that have hindgut fermentation or just simple digestive system we can divide them into no now we are talking about food how food affects our microbial community in the fermentation chamber in our health. So, if we look at different kinds of animals which is different kinds of food for example, we will have herbivores carnivores and omnivores now the omnivores.

Typically tend to have they come they tend to come in the type 4 of kind of gut microbial communities which is typical with omnivores and frugivores once either it foods carnivores have a very different microbial community omnivores and frugivores are very different community now again among herbivores we have 2 different either hindgut or foregut now within hindgut and foregut, we will have 2 which are remember that whether the degradation is happening in the Cecum fermentation chamber or in the colon that will affect the kind of microbial community. So, if we look at the mammals and we look at their microbial community in the digestive tract.

We notice at least five distinct types and all depend upon the kind of food they are eating whether they are dog hyena lion if they are carnivore carnivorous right they will all have similar microbial community and among the herbivore we have foregut fermenters and hindgut fermenters their microbial communities are different and among the hindgut community members also we have 2 different classes of community microbial community.

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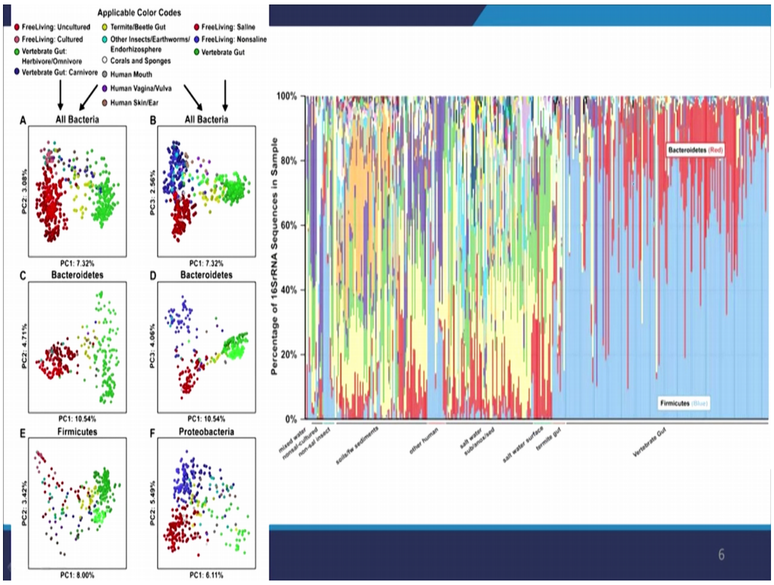

Now, now again this is a very nice study where they looked at different kinds of guts of different organisms and not just good, but also for example, human mouths human skin human vulva and they noticed that how the microbial communities are different and are similar. So, very nice study I will make this slide available. So, take a good look and try to understand what kind of information we can generate from the next generation sequencing tool on the right panel is an equally interesting a plot from a recent study where you notice how microbial diversity varies from different kind of environments.

So, this is a vertebrate guard and its mostly dominated by firmicutes and then there are some bacteroidetes right and then this is the human other human samples we have considerable amount of firmicutes and some amount of bacteroidetes and other microbes in our soil and sediment have very diverse microbial communities compared to human gut and human microbiome and compared to the vertebrate carter and. So, we and also termite gut because there is lot of cellulose degradation happening in termite gut it also has plenty of firmicutes some bacteroidetes and other microorganisms.

Now, the thing to notice that if we notice this is the proportion for human lot of that is a this is human the first blue is the human gut then we have lot of clostridium hardly any bacteroidetes and other microbes. Now this is not does not mean that every human will have similar microbial community structure. In fact, what we know is that microbial community structure varies from one human to another.

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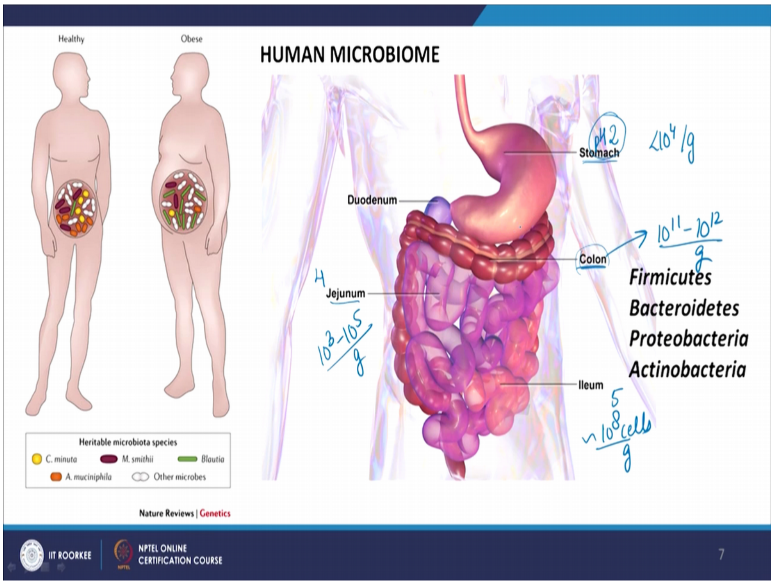

HUMAN MICROBIOME

Firmicutes
Bacteroidetes
Proteobacteria
Actinobacteria

And that brings me to here if you talk about human microbiome not only it is the amount of human microwave community present in different parts of our digestive system vary, but also the composition varies from one person to another. In fact, the recent studies in human microbiome and the internal environment of human system it shows that depending on how a microbial community changes within will impact our health and our well being for example, now we know that the healthy the microbe the gut microbial community of a healthy individual is distinct from the gut microbial community of an obese individual and we will talk about how it is distinct and how much it is distinct.

So, the human gut can be human digestive system can be broadly classified into or at least where the most of digestion is happening stomach duodenum ileum jejunum and colon in stomach the p h is around 2 and we have less than 10 to power 4 microbes per gram of digestive pulp. So, if we take a pulp from stomach we will have less than 10 to power of 4 cells per gram of the digestive pulp then the next step would be the food entering the jejunum in jejunum the p h is between around 4 and we will have anywhere from 10 to power three to 10 to power 4 microbial cells per gram of the pulp.

Now, if we go to ileum; ileum the p h rises to five and we will have anywhere from 10 to power eight around 10 to power eight cells per gram of the digestive pulp and now if we go to our colon or our large intestines will have very high number of microbes ranging from 10 to power 11 to 10 to power 4 cells per gram. So, we can see that where the fermentation is happening we are the colon fermenter hindgut fermenters we will have lot of microbes present compared to stomach in other places.

Now this is very important not only how many microbes are present, but who is present for example, if helicobacter pylori is present in stomach it can cause some severe hyperacidity and in it survives very valiant at stomach p h by the way and it can also cause ulcers the acidic ulcers which are very painful we also know that it has this particular microbe h pylori has very high co correlation with stomach cancer. So, this is one instance where we know that presence or absence of a particular microbe if it is just like normally in with very less in very less number it will not affect a lot, but if it colonizes a lot will have acidity peptic ulcers and even stomach cancer.

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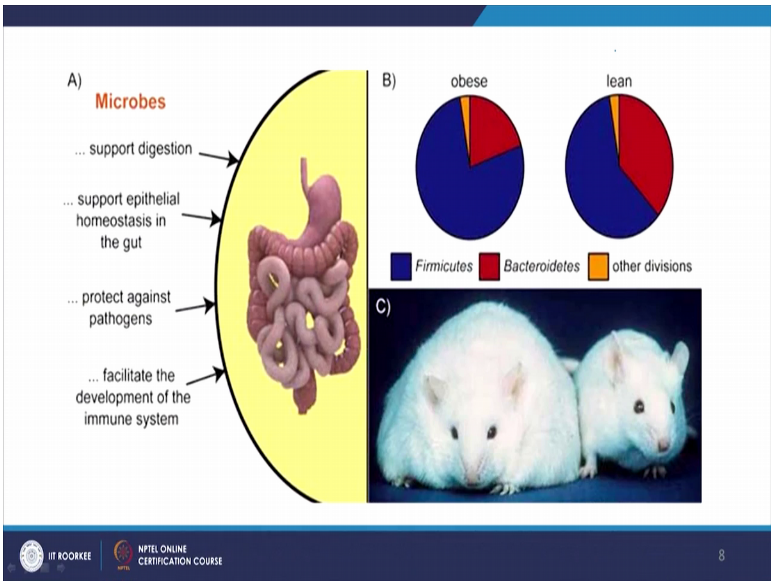

A)

Microbes

... support digestion

... support epithelial homeostasis in the gut

... protect against pathogens

... facilitate the development of the immune system

So, now most of the studies for human digestive system are not carried on humans, but they are carried on poor mice. Now we know this thing that we look at microbial community in the gut of mice and we know found out that the microbes they support digestion because they help in fermentation they carry out fermentation they support the epithelial homeostasis in gut. So, the epithelium system and how it remains in homeostasis they protect us against pathogens because if you have a rich in diverse community present in your gut and in intruding pathogen in your gut then if there is very high chances that the in the native microbial community in your gut will out compete the pathogen you will not have an infection; however, if you eating antibiotics for some reason because of some discrepancy in diet or other environmental stressors the microbial community has become weak diversity has lost has been lost and the members that are abundant are not really very robust then a pathogen has a higher chances of colonizing you are intestine out competing other microbes.

So, our gut microbes actually protect this pathogens against pathogens and they help us develop a better immune system also there is more study we will talk about it if not in this lecture in the next lecture this shows how the gut microbiota actually influences our mood and our well being. So, now, we looked at fat mice and thin mice and we found out that the fat mice the obese mice. So, they both were fed similar diet same diet they were given same amount of exercise, but some of them became really fat obese some of them were healthy lean. So, we noticed that the microbial community in the fat guy mice was very rich and firmicutes and not. So, much in bacteroidetes the lean mice had a slightly more proportion of bacteroidetes; i fact, twice as much.

So, we then this is when people started suspecting that obesity perhaps has not to do with our microbial community structure in the gut.

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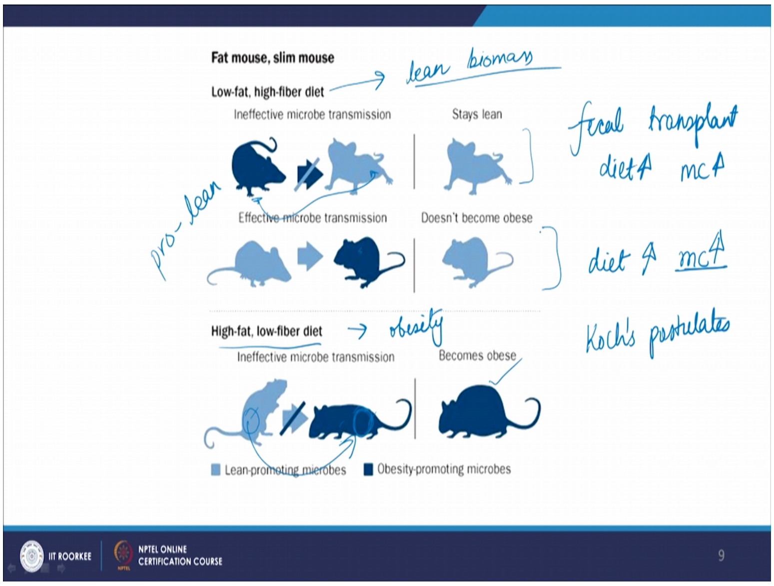

Fat mouse, slim mouse

Low-fat, high-fiber diet

High-fat, low-fiber diet

So, people did other kind of studies where they fed low fat high fiber diet which is typically associated with lean biomass. So, low fat high fiber diet will result in lean biomass typically. So, in this particular figure the dark mice here have microbes that that promote obesity the light mice has microbe they do not promote obesity.

So, when microbes were taken from the poop and this is by the way fecal transplant where the fecal matter from a particular animal is transplanted into the gut of the other animal then this particular transplant was done from a rat that had microbes which promote obesity that is high firmicutes less bacteroidetes into a mic into a rat that had microbes that promote leanness that is more bacteroidetes and the diet was promoting leanness then there was the microbes the microbes stayed proleanness and the rat stayed lean. So, we notice that if diet is prolean and the microbial community is also prolean then the rat was lean.

In other case we took a rat and we took a rat that has microbes which are prolean. So, the rit that the prolean as in when I say prolean I am talking about microbes that microbe community that is rich in bacteroidetes which we know is typical of lean mouse and we put it in a put this transplanted this microbial community in a rat that was going to get obese because it had microbes that are pro obesity we noticed that it did not become obese. So, if diet is pro leanness, but the micro, but the rat has a tendency to become fat to become obese and if it is not given any transplant then it will become fat, but if it is given transplant if microbes that promote leanness of injected in it then it becomes lean very good.

Now, in other case we notice that if we feed a high fat high fat low fiber diet which results more often than not into obesity and we did not we did not do the transplant from a microbial community of a lean microbial community of a lean rat into the fat rat of the microbial community that encouraged fatness then the rat became obese on the other hand when we did to the transplant then we took microbial community that promoted leanness and transplanted into rat that had microbial community that promoted obesity it did not become obese.

So, this was a very nice practice of if you remember cause postulates that we talked in first few lectures and with this. This was more details than what is represented in the schematic people actually proved scientists prove that microbial community and diet both have very important impact. In fact, microbial community may have slightly more imp benefit and impact on the biomass of the rat.

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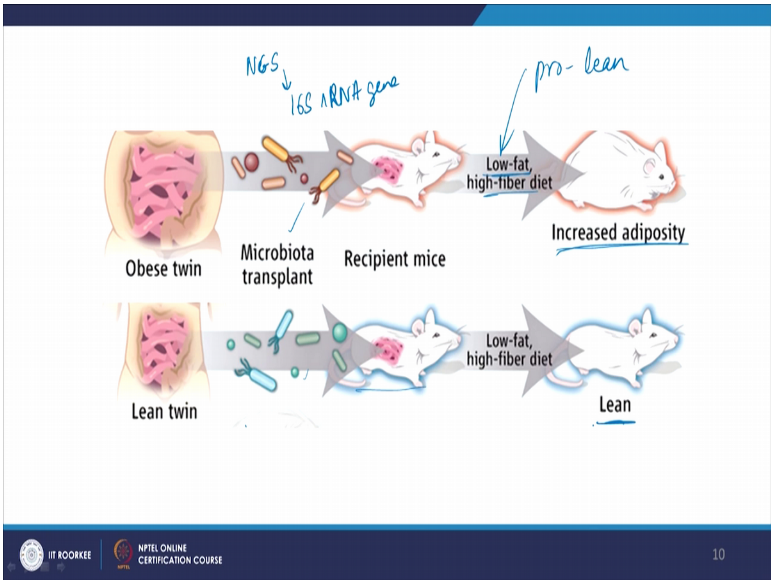

Now, the similar experiments are being done in human beings we take 2 twins. So, the twins have everything same, but one of them is obese another is twin another is lean. So, if we take microbes from we know that the microbes in the obese twin are different from microbes in the lean twin and we have done this using next generation sequencing tools techniques on 16 SRRNA gene and when we take the microbial community from the obese twin and put it in a healthy recipient rat and we feel it feed it low fat high fiber diet.

So, the diet is proleanness the diet wants you to become lean, but the microbial community has come from a fat person and sooner or later you become you have increased adiposity and the rat becomes fat on the other hand when we took microbes from a lean twin and put it in a mouse that and put it in a mouse that was fed the same diet we notice that the mouse remain lean and healthy. So, even in human beings we are noticing that our microbial community will decide our body mass index.

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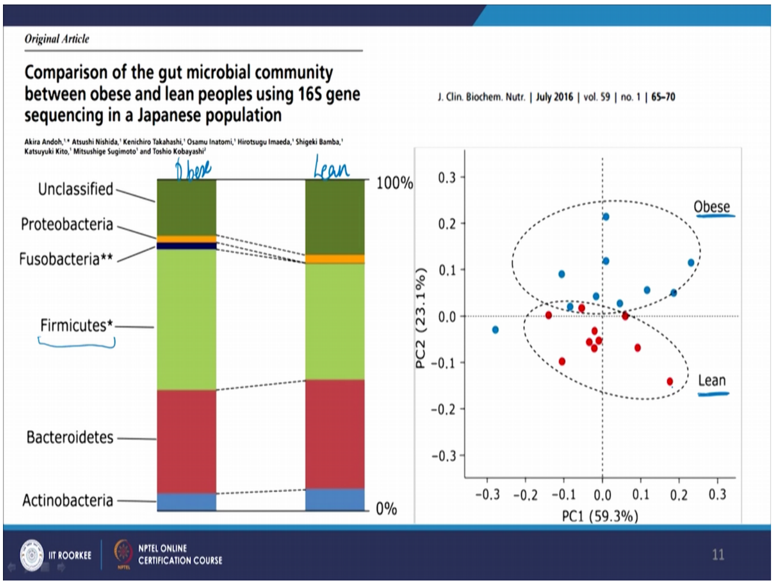

Original Article

Comparison of the gut microbial community between obese and lean peoples using 16S gene sequencing in a Japanese population

Akira Andoh,[1,*] Atsushi Nishida,[1] Kenichiro Takahashi,[1] Osamu Inatomi,[1] Hirotsugu Imaeda,[1] Shigeki Bamba,[1] Katsuyuki Kito,[1] Mitsushige Sugimoto[1] and Toshio Kobayashi[2]

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Now, in fact, recently in Japan there was a study where they compared the gut microbial community of obese and lean people using 16 SRRNA gene sequencing in Japanese population and they found out now that the that they found out that the becteroidetes in lean population is slightly more than in obese population not a lot not a not a very dramatic difference, but a slight difference there is a difference definitely significant difference in the concentration levels relative levels of firmicutes between lean and obese the important part is that the microbial community of obese Japanese volunteers in further study was very different compared to the lean microbial community in lean members again signifying that the microbial community varies and when it varies the health of the body were vary too.

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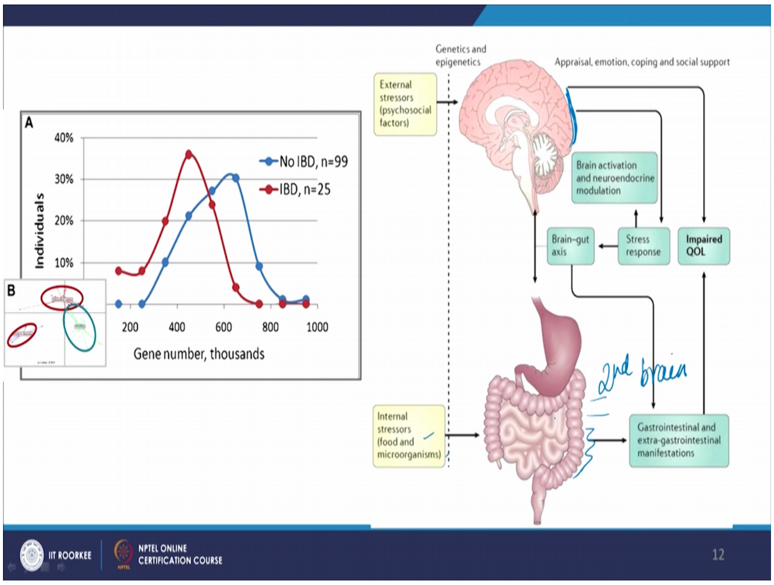

And then this is not just related to obesity or body mass index, but it also has to do with other diseases such as the typical being the irritable bowel syndrome. So, initially people were concerned, why some patients are having such irritable bowel and are so sick and is this a pathogen when they were trying to identify the pathogen they found out that though they cannot delineate any particular pathogen the microbial community structure.

In the excreta of patients suffering from IBS is very different from the healthy population and then they found out that the way whether it because of the stressors which may be change in food or change in microbes or other drugs when the microbial community in the gut changed a lot, then the irritable bowel syndrome Saturn depending on who is being promoted now not only the microbial community changed, but also the gene functional genes changed. So, the kind of processes whether it is methanogenesis fermentation or whatever is happening in the last intestine in small intestine changed for patients with IBS and healthy people and that is one of the hope of treatment for IBS patients which is fecal transplant.

So, either we can identify what were the triggers what were the stresses that changed the healthy microbial community into unhealthy I b s prone microbial community and if there is a way to shifted it back to healthy microbial community or we can actually take fecal matter from fecal microbial community from a healthy individual and transplanted into an a sick patient and then improve their improve their health the other part where this other avenue of research where research is being carried out on how our internal environment and microbial colonization of our internal environment affects our health and well being is how it actually affects our brain.

Now, we know that there is a brain gut access and there are lot of neurons after the number of density of neurons in the brain the highest density of neuron would be in our small intestine and large intestine. So, this is called the second brain now our gut is our second brain.

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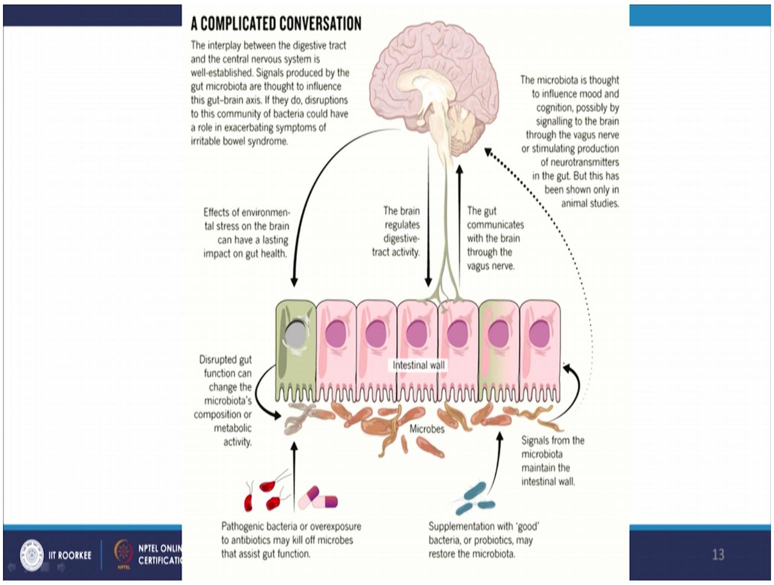

A COMPLICATED CONVERSATION

The interplay between the digestive tract and the central nervous system is well-established. Signals produced by the gut microbiota are thought to influence this gut–brain axis. If they do, disruptions to this community of bacteria could have a role in exacerbating symptoms of irritable bowel syndrome.

The microbiota is thought to influence mood and cognition, possibly by signalling to the brain through the vagus nerve or stimulating production of neurotransmitters in the gut. But this has been shown only in animal studies.

Effects of environmental stress on the brain can have a lasting impact on gut health.

The brain regulates digestive-tract activity.

The gut communicates with the brain through the vagus nerve.

Disrupted gut function can change the microbiota's composition or metabolic activity.

Signals from the microbiota maintain the intestinal wall.

Pathogenic bacteria or overexposure to antibiotics may kill off microbes that assist gut function.

Supplementation with 'good' bacteria, or probiotics, may restore the microbiota.

Now, depending on what kind of here depending on what kind of microbes are colonize here depending on what kind of chemical reactions are happening here what kind of transmitters are being produced here the messages transmitted from gut to brain change and that changes are moved. So, we have well established that there is a very strong interplay between our digested track and our central nervous system.

So, the stomach will inform the on how things are should we be happy or not and then the brain will inform the stomach, we are under stress of you are happy or not and that is the reason why under stress when people will have Diarrhea and why people after they are fed they feel very happy or when they are not fed they feel crappy. So, we have scientific evidence for this and in fact, this is now being used for treating diseases medical psychological diseases and also its used for training the mind to improve the health of the digested tract. So, dear students this is all for today in next class we will move on and we will look at other public health challenges that are linked very closely to microbiology not instance of pathogenic microbiology, but environmental microbiology and we will foray into antimicrobial resistance.

Thank you very much.