
Lecture
(Prepared by Inzhevatkina S.M., Department of Microbiology and Virology of Russian National Research Medical University NI Pirogov)
Human Microbiota

The human body is colonized by a vast number of microbes, collectively referred to as the human microbiota (the genome of the human microbiota is called microbiome). Human microbiota is complex association of symbiotic microorganisms formed during evolitional process.

Microorganisms inhabit skin and mucous membranes of a human’s body (mucosa, conjuctiva, respiratory, urogenital, and gastrointestinal tracts). M/os belong to bacteria, archaeae, and fungi. Viruses are also present (mostly phages, whose hosts are prominent members of microbiota).

Human microbiota stays in the dynamic stable balance.
Human Microbiota

The human microbiota consists of an estimated 100 trillion cells, at least 10 times the number of human cells.

Humans have been proposed to be “meta-organisms” consisting of 10-fold greater numbers of bacterial than animal cells that are metabolically and immunologically integrated.

The human meta-organism includes approximately $10^{14}$ prokaryotic organisms, with a biomass of >1 kg.
Composition of Human Microbiota

The population composition is remarkably stable at different anatomic locations along the gut, but absolute numbers vary greatly, ranging from $10^{11}$ cells/g content in the ascending colon to $10^{7-8}$ in the distal ileum and $10^{2-3}$ in the proximal ileum and jejunum. Anaerobes are several orders of magnitude more abundant than aerobes in the bacterial community, and a majority of the population (60%-90%) are representatives of 2 phyla: the *Bacteroidetes* and *Firmicutes*. There are more than 2000 species of commensal bacterial organisms within our bodies, the vast majority in the gut, and only about 100 known species of pathogen (≈5% of total number of m/o).
Normal Microbiota

- **NOSE**
  - Mass of microbes: 10g
  - Typical resident: *Streptococcus*

- **MOUTH**
  - Mass of microbes: 20g
  - Typical resident: *Streptococcus* (cheek), *Neisseria* (teeth)

- **VAGINA**
  - Mass of microbes: 20g
  - Typical resident: *Lactobacillus*

- **SKIN**
  - Mass of microbes: 200g
  - Typical resident: *Staphylococcus* (oily areas), *Corynebacteria* (moist areas)

- **GUT**
  - Mass of microbes: 1000g

**Microbial cells outnumber your own cells 10 to 1 and have a total mass of >1.2kg**
Normal Microbiota is divided by location:

1. Skin microbiota
2. Microbiota of conjunctiva
3. Microbiota of nasopharynx and mouth
4. Microbiota of respiratory tract
5. Microbiota of gastrointestinal tract
6. Microbiota of genitourinary tract
7. Microbiota of ear
Symbiotic microorganisms inhabiting the same area of a body form one ecosystem. Most of the community is autochthonous, meaning indigenous and stable, although allochthonous, or transient, members are also to be found (certainly most enteric pathogens fall into this category).

All microorganisms of one ecosystem may be arranged into 2 groups:

- **Indigenous or resident microbiota**
  - groups of microorganisms regularly found at the same age

- **Transient microbiota**
  - nonpathogenic or potentially pathogenic microorganisms that inhabit the area for a short period
Normal Microbiota of Skin

Resident m/o
- Gram positive cocci: 
  Micrococcus  
  Staphylococcus  
- Gram positive rods: 
  Corynebacterium  
  Propionibacterium  
  Brevibacterium

Transient m/o
- Gram positive cocci: 
  Sarcina
- Gram positive rods: 
  Bacillus  
  Actinomyces
- Yeasts: 
  Candida
The dermis and subcutaneous tissue are normally sterile.
Skin Microbiota

The predominant m/o of human’s skin are aerobic and anaerobic gram positive cocci and rods.

Limitation factors of skin microflora:
- pH of skin
- Secretion of fatty acids
- Secretion of lysozyme
- Presence of oxygen

*Staphylococcus aureus*  
Gram stain
Staphylococcus on skin
Micrococcus
Gram staining
Propionibacterium Actinomyces

Gram positive rods can be regularly detected in normal skin microbiota
Corynebacterium
Gram staining
Bacillus
Gram staining
Sarcina
Gram staining
Candida (Loeffler stain)
yeasts

*Candida*

Scanning electron microscope
• **Upper respiratory tract** -- G+ and G- cocci, G+ rods. Also some pathogens

• **Lower respiratory tract** -- usually sterile
Normal Microbiota of Nasopharynx and Oral Cavity

**Resident**

- **Gram positive cocci:**
  - Streptococcus
  - Micrococcus
  - Staphylococcus
- **Gram negative cocci:**
  - Neisseria
  - Moraxella (resemble Neisseria spp.)
  - Veillonella
- **Gram positive rods:**
  - Corynebacterium
  - Lactobacillus
- **Gram negative rods:**
  - Bacteroides

**Transient**

- **Gram positive cocci:**
  - Peptococcus
- **Gram positive rods:**
  - Actinomyces
  - Bacillus
- **Gram negative rods:**
  - Fusobacterium
- **Yeast:**
  - Candida
Microbiota of a Mouth and Nasopharynx

Veillonella spp

Haemophilus influenzae

Bacteroides fragilis

Neisseria spp.
Streptococcus, Gram stain
Lactobacillus, Gram stain
Bacteroides, Gram stain
The Oral Cavity is subdivided into several ecosystem

- mucous membranes
- saliva and throat liquids
- dental plaque
- gum pocket

More than 700 species are identified in oral cavity by current data!
Structure of the Tooth

- Enamel
- Gum
- Dentin
- Pulp Chamber
- Root Canal Containing Pulp Tissue
- Supporting Ligament
- Accessory Canal
- Root End Opening
- Crown
- Root
- Bone
The Dental Plaque

The dental plaque consists mainly of gelatinous deposits of high-molecular-weight glucans in which acid-producing bacteria adhere to the enamel.

Glucans are produced by streptococci, in association with actinomycetes.

The dental plaque is associated with the first step of caries.
• Scanning electron micrograph of dental plaque. Many different kinds of bacteria composing the plaque exhibit specific attachments to the tooth and to each other.
Plaque accumulates calcium salts to form tartar

\[ \text{Ca}_3(\text{PO}_4)_2 \text{ scale (tartar) from human tooth} \]

http://www.buckman.com/eng/biofilm3.htm
Scale magnified 7,000x to show imbedded bacterial biofilm

http://www.buckman.com/eng/biofilm3.htm
The Dental Plaque Microbiota

**Strict aerobes**
- Leptothrix
- Micrococcus
- Actinomycetes

**Strict anaerobes**
- Bacteroides
- Porphyromonas
- Prevotella
- Fusobacterium
- Veillonella

**Facultative anaerobes**
- Streptococcus (*S. salivarius, S.sangius, S.mitis, S.mutans, etc.*)
- Staphylococcus
- Lactobacillus
- Spirochetes
- Coryneforming bacteria
Bacteroides (dental) Gram stain
NORMAL MICROBIOTA OF THE GI TRACT

• The body is like a donut, with the alimentary canal the ‘inside’ of the donut. Things inside the alimentary canal are not truly ‘inside’ the body.
Gastrointestinal Tract

- Nasopharynx
- Oral cavity
- Throat
- Esophagus
- Duodenum
- Ascending colon
- Cecum
- Appendix
- Stomach
- Jejunum
- Descending colon
- Rectum
The most heavily colonized area.

All microorganisms of GI tract are arranged into ecosystems:

Stomach and duodenum - $10^3$ m/o per gram
Jejunum and ileum - $10^4$-$10^7$ m/o per gram
Colon and rectum - $10^7$-$10^{12}$ m/o per gram

Bacteria constitute 60% of fecal mass
Microbiota of Gastrointestinal Tract

**Streptococci**

Major bacteria present in the gastrointestinal tract include:
- Esophagus: Lactobacilli
- Stomach: Lactobacilli
- Duodenum: Enterobacteria
- Jejunum: *Enterococcus faecalis*, *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Peptococcus*, *Peptostreptococcus*, *Ruminococcus*, *Clostridia*, Lactobacilli
- Ileum: Lactobacilli
- Colon: Lactobacilli
- Anus: Lactobacilli
Intestinal Microbiota of Adults
(more than 1000 species, less than 50% can be cultivated)

The main content of human microbiota is represented by 5 phyla of m/o: Firmicutes (more than 200 genera of G+ bacteria),

Bacteroidetes (order Bacteroidales includes 4 families: Bacteroidaceae, Prevotellaceae, Porphyromonaceae и Rikenellaceae).

Actinobacteria,
Proteobacteria
Verrucomicrobia

Archaea are also present in GI flora (Methanobrevibacter spp.)
Main groups of GI bacteria

• **1 dominant group (up to 43%)**
  - *Clostridium coccoides group*
  - *Clostridium, Eubacterium, Ruminococcus, Coprococcus, Dorea, Lachnospira, Roseburia and Butyrivibrio*

• **2- dominant group**
  - *Clostridium leptum group 19-25% Clostridium, Eubacterium, Ruminococcus, Anaerqfilum genera*
  - The basic species in the 2nd group are *Faecalibacterium prausnitzii 64%, Ruminococcus bromii (12%)*,

• **3- group**
  - *Cytophaga-Flavobacter-Bacteroides*

• 1- minor group *Bifidobacterium* about 10 %, but small children have *Bifidobacterium* as dominant group (up to 90%) belonging to saccharolytical bacteria

• 2 – minor group
  - *Lactobacillus, Lactococcus, Enterococcus, Streptococcus, Leuconostoc*
Clostridium, Gram stain
Clostridium, Gram stain
(spores are vivid -arrows)
Bifidobacterium longum
Gram stain
Bifidobacterium
under Scanning Electron Microscope
Bifidobacterium bifidum, Gram stain

G+ polymorphic rods
Non-pathogenic habitant of humans
Bifidobacterium adolescentis
Lactobacillus under Scanning Electron Microscope
Lactobacillus
Leuconostoc (cocci)
Intestinal Microbiota of Adults

Transient m/o

• Gram positive cocci: *Peptococcus*
• Gram positive rods: *Bacillus*
• Gram negative rods: *Enterobacteriaceae* (e.g., *E.coli*)
• Yeasts: *Candida*
**E. coli**
Gram stain

- Number $10^6$-$10^8$ per gram

- Synthesize 9 vitamins:
  K, B1, B2, B3, B5, B6, B9, B12, PP.
At present Streptococci group D are renamed *Enterococcus* spp.: *Enterococcus faecalis* under Scanning Electron Microscope
Enterococcus faecalis
*Helicobacter pylori*
(stomach ulcer causative agent)
*Helicobacter pylori*,
CAUSATIVE AGENT OF STOMACH ULCERS
Establishing Normal Microbiota

www.ehponline.org

www.solarnavigator.ne
Establishing Normal Microbiota

At birth, the gut is sterile and is colonized immediately, ultimately developing into a stable community, although there are marked variations in microbial composition between individuals.
3 sources of newborn's microbiota

- Mother’s intestinal and vaginal microbiota
- Microbiota of medical personal
- Microbiota of the environment
Acquisition of the Human Microbiome

- Initial inoculum from mother occurs during pregnancy
- Vaginal delivery –
  - microbiome develops species similar to mother’s vagina
- Cesarean section –
  - microbiome develops predominant species similar to skin flora of mother and hospital attendants
- Breast feeding provides bacteria from mother’s GI tract

Intestinal Microbiota of a Newborn
(the first month of life)

**Breast-fed baby**

The predominant resident m/o are different

*Bifidobacterium* spp. (about 90%) and
*Lactobacillus* spp., *Enterococcus* spp., *E. coli*, *Bacteroides* spp.

transient m/o:
*Klebsiella*, *Staphylococcus*, *Clostridium* spp.

**Bottle-fed baby**

The predominant resident m/o are *Bifidobacterium* and *Lactobacillus* spp.
associated with *Bacteroides* spp., *Clostridium* spp., *Enterococcus* spp., *E. coli*

transient m/o:
*Enterobacter*, *Klebsiella*, *Proteus*, *Citrobacter*, *Staphylococcus*, *Bacillus*, *Candida* spp.
Genitourinary Tract

- Urinary Tract
  - Kidneys
  - Ureters
  - Urinary bladder
  - Urethra
- Male and Female Reproductive Systems
Normal Microbiota of Genitourinary Tract

Urethra

**Resident m/o**
- Gram positive cocci:  
  - *Streptococcus*
  - *Staphylococcus*
  - *Peptostreptococcus*
- Gram negative cocci:  
  - *Veillonella*

**Transient m/o**
- Gram negative rods:  
  - *Prevotella*
  - *E. coli*
- Yeasts:  
  - *Candida*
Normal Microbiota of Genitourinary Tract

**Vagina**
 Resident m/o

- **Gram positive cocci:**
  - *Streptococcus*
  - *Staphylococcus*

- **Gram negative cocci:**
  - *Veillonella*

- **Gram positive rods:**
  - *Lactobacillus*

- **Gram negative rods:**
  - *Prevotella*

Different species of *Lactobacillus* are predominant in reproductive period
Normal Microflora of Genitourinary Tract

Vagina
Transient m/o

- Gram positive cocci:
  Streptococci group B
  \((S.\text{agalactiae})\)
- Gram positive rods:
  \(Bifidobacterium\)
  \(Coryneforming\ bacteria\)
- Gram negative rods:
  \(Fusobacterium\)
- Yeasts:
  \(Candida\)
Clue cells are vaginal squamous epithelial cells coated with the anaerobic gram-variable coccobacilli Gardnerella vaginalis and other anaerobic bacteria causing bacterial vaginosis.
• «Clue cells»
• Mobiluncus spp.
Microbiota of Ears

Middle ear and inner ear sterile.

Outer ear and auditory canal contain same types of m/o as are found on the skin. The most common m/o colonizing the outer ear is coagulase-negative *Staphylococcus*. Other m/o colonizing the skin have been isolated from this site, as well as potential pathogens such as *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and the *Enterobacteriaceae*. The last group has also been associated with disease at this site.
Microbiota of Conjunctiva

Conjunctiva is colonized with coagulase-negative staphylococci (especially *S. epidermidis*), diphtheroids (*Corynebacterium xerosis*) and viridans streptococci as well as rare numbers of microorganisms found in the nasopharynx (*Moraxella* and *Neisseria* spp.). The conjunctival flora is normally held in check by the flow of tears, which contain antibacterial lysozyme.
Microbes occupy ecological niches
Qualitative and Quantitative Compound of Normal Microbiota

depends on:

- *State of Health*
- *Age and sex*
- *Type of a diet*
- *Climate conditions*
- *Ecological conditions*
Significance of Human Microbiota

Colonization resistance means that commensal bacteria ensure pathogen displacement by biofilm formation on the surface of mucous membranes, thus, nutrient and receptor competition and inhibition of pathogens by lactic and acetic acid secretion, production of bacteriocins and other substances.

Through the production of short-chain fatty acids, resident bacteria positively influence intestinal epithelial cell differentiation and proliferation, and mediate other metabolic effects. Microbial colonization increases the uptake of nutrients (e.g., glucose) in the intestine.
Significance of Human Microbiota

Normal microbiota stimulates the development and activity of the immune system. Innate immune responses to the commensal flora educate the immune system and influence adaptive responses to exogenous antigens. Surface enterocytes secrete many immune mediators in response to microbial antigens, including antibacterial peptides, immunoglobulin A (IgA) and chemokines. The endotoxins liberated by gram-negative microorganisms of microbiota may help the defense mechanism of the body by triggering the alternative complement pathway, as long as they are not produced in excessive amounts.
Significance of Human Microbiota

Normal gut microflora is involved in production of many substances, necessary for the host, especially vitamins K and group B (e.g., B$_{12}$), releases organic acids, aids in the absorption of nutrients and breakdown products, and participates in conversion of bile pigments and bile acids. Gut microbiota produce enzymes which metabolize some nutrient substrates (e.g., oligosaccharides by *Bifidobacterium* spp. and polysaccharides by *Bacteroides* spp.)
Significance of Human Microbiota

Some microorganisms can metabolize dietary carcinogens and improve ion absorption. Also intestinal flora is important in peristalsis, regulation of gas contents and redox potential of the gut. It participates in formation of water-salt balance.

Molecular mimicry of host molecules, whereby bacteria display surface molecules resembling those of the surface of the host, could contribute to immune hyporesponsiveness to some resident bacteria (e.g., commensal strain *Bacteroides fragilis* confers a competitive survival advantage, which allows these bacteria to reside in close contact with intestinal enterocytes that are covered with similar fucosylated glycoproteins and glycolipids).

Negative effects of microbiota: The experimental reports of carcinogenicity to be of questionable value for cyclamate due metabolic activity of microbiota.
Biofilm Forming Mechanism

- Host cell membrane
- Receptor
- Adhesin
- Bacterial cell
Photo of Biofilm

Lactobacillus
# Ratio Anaerobes:Aerobes

<table>
<thead>
<tr>
<th>Site</th>
<th>Total Bacteria (per/ml or gram)</th>
<th>Ratio anaerobes:aerobes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upper Airway</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal Washings</td>
<td>$10^3$-$10^4$</td>
<td>3-5:1</td>
</tr>
<tr>
<td>Saliva</td>
<td>$10^8$-$10^9$</td>
<td>1:1</td>
</tr>
<tr>
<td>Tooth Surface</td>
<td>$10^{10}$-$10^{11}$</td>
<td>1:1</td>
</tr>
<tr>
<td>Gingival Crevice</td>
<td>$10^{11}$-$10^{12}$</td>
<td>1000:1</td>
</tr>
<tr>
<td><strong>Gastrointestinal Tract</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>$10^2$-$10^5$</td>
<td>1:1</td>
</tr>
<tr>
<td>Small Bowel</td>
<td>$10^2$-$10^4$</td>
<td>1:1</td>
</tr>
<tr>
<td>Ileum</td>
<td>$10^4$-$10^7$</td>
<td>1:1</td>
</tr>
<tr>
<td>Colon</td>
<td>$10^{11}$-$10^{12}$</td>
<td>1000:1</td>
</tr>
<tr>
<td><strong>Female Genital Tract</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocervix</td>
<td>$10^8$-$10^9$</td>
<td>3-5:1</td>
</tr>
<tr>
<td>Vagina</td>
<td>$10^8$-$10^9$</td>
<td>3-5:1</td>
</tr>
</tbody>
</table>
Functions of Human Microbiota

<table>
<thead>
<tr>
<th>Protective functions</th>
<th>Structural functions</th>
<th>Metabolic functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogen displacement</td>
<td>Barrier fortification</td>
<td>Control IEC differentiation and proliferation</td>
</tr>
<tr>
<td>Nutrient competition</td>
<td>Induction of IgA</td>
<td>Metabolize dietary carcinogens</td>
</tr>
<tr>
<td>Receptor competition</td>
<td>Apical tightening of tight junctions</td>
<td>Synthesize vitamins e.g., biotin, folate</td>
</tr>
<tr>
<td>Production of anti-microbial factors e.g., bacteriocins,</td>
<td>Immune system development</td>
<td>Ferment non-digestible dietary residue and endogenous</td>
</tr>
<tr>
<td>lactic acids</td>
<td></td>
<td>epithelial-derived mucus</td>
</tr>
</tbody>
</table>

Commensal bacteria

- IgA
- Short-chain fatty acids
- Mg²⁺, Ca²⁺, Fe²⁺
- Vitamin K, Biotin, Folate

Ion absorption
Salvage of energy
Dysbiotic Microbiota

A dysbiotic microbiota is an ecological disorder of the bacterial community; the concept is often associated with the pathogenesis of several immunodeficiency diseases.
Diagnosis of Alterations of Human Microbiota

• Cultural (bacteriological) method - qualitative and quantitative examination (time-consuming)

• Molecular-genetics methods:
  Polymerase Chain reactions (PCR, RT-PCR)
  New molecular phylogenic approaches based on sequencing of bacterial 16S ribosomal RNA, or in higher-resolution, bulk genomic sequencing of entire communities/environments, allow census-based, non-culture-dependent inventories or “metagenomics” of the entire microbiota population composition and their component genes, which can be tabulated and correlated with host phenotypes.
Bacteriological Method (Culture)

Tenfold dilutions of sample are prepared from any particular specimen of human ecological niche (skin, stomach, rectum, etc.). All dilutions are inoculated on selective or differential-diagnostic media for typical indigenous and transient m/o. For example, if we study feces, we use:

- MRS medium for *Lactobacillus*;
- Blaurock medium for *Bifidobacterium*;
- Salt agar for *Staphylococcus*;
- TSN medium for *Clostridium*;
- Endo or MacConkey agar for *Enterobacteriaceae* family;
- Sabouraud’s dextrose agar for *Candida*, etc.

Each type of medium needs particular time and conditions (aerobic, anaerobic, etc.) for incubation.

Then, subsequently, we calculate the number of colonies, estimate the concentration of each m/o and identify up to species of the m/o all types of colonies from each medium.
Growth of *Candida* on Sabouraud’s dextrose agar
Polymerase Chain Reaction
Specialized Equipment for PCR
Results of PCR will be visible in gel electrophoresis
Sequencing of gene 16S rRNA detects the nucleotide sequence of isolated pure cultures. Gene 16S rRNA is conserved and serves as universal marker for identification of species. Full databases are developed for 16S rRNA gene sequence of prokaryotes. It is currently assumed that two strains are members of the same species if their 16S rRNA gene sequence identity is >99%, and it may provide the first indication that a novel species has been isolated if an identity of <98.7% is found.
MALDI-ToF Mass-Spectrometry

Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-ToF mass-spectrometry) is a soft ionization method used in mass-spectrometry. Large fragile macromolecules (e.g., carbohydrates, proteins, deoxyribonucleic and ribonucleic acids) can be analyzed by the method. MALDI-ToF mass-spectrometry is easy and rapid technique and needs minimal amount of specimen. The pure culture of microorganisms is mixed with the matrix solution on the surface of metal or plastic slide. Evaporation of the solution induces formation of crystal from the mixture. The slide is placed in vacuum, and then short pulses of laser light are focused on the sample. Sample and matrix begin to volatilize. Arrival at the end of the flight tube is recorded by high speed recording device.
Biopreparations Used for Correction of Dysbiosis

- **Probiotics** – contain *pure culture of a resident live m/o* ("Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host.”)

- **Prebiotics** – contain *nutrient substrate* (growth factor) which are necessary for growth of some resident m/o (usually oligosaccharides and their derivates and can be obtained from artichokes, onions, chicory, garlic, corn, oatmeal, haricot beans, and peas. The properties of prebiotics are ensured by inulin, fruit oligosaccharides, galactosaccharides & raffinose).

- **Symbiotics** - contain *nutrient substrate* and *pure culture of a resident m/o*
Mechanism of Action of Probiotic Strains

1. They compete with pathogens in adhering to receptor sites (human-origin strains);
2. They compete for nutrients that are essential to their multiplication;
3. They produce lactic acid, which promotes a balanced intestinal pH;
4. They produce antimicrobial substances that inhibit the growth of pathogens;
5. They generate multiply enzymatic activities that help to stimulate the immune system.
Examples of Probiotics

Lactic acid-producing bacteria:

1. Lactobacillus spp. (Lactobacillus bulgaricus, Lactobacillus rhamnosus GG, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus salivarius, Lactobacillus casei, Lactobacillus johnsonii, and Lactobacillus gasseri)

2. Bifidobacterium spp. (Bifidobacterium lactis, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium thermophilum, and Bifidobacterium pseudolongum).

In western medicine: Streptococcus salivarius subsp. Thermophilus, Enterococcus faecalis, Saccharomyces boulardii (yeast) are applied.
Multicomponent Probiotic Formulation

Probiotic can contain several species of microorganisms simultaneously (e.g., *Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum* and *Saccharomyces boulardii; Bifidobacterium longum, Bifidobacterium breve, Lactobacillus rhamnosus* and *Lactobacillus acidophilus (Primadophilus Bifidus)*). Such preparations have increased efficiency in treatment of diarrhea.
Example of Probiotics
Example of Probiotics
Example of Probiotics
Example of Probiotics
Example of Probiotics

[Image of a probiotic supplement bottle]
Note!!!

Never use probiotics which contain *Bacillus spp.* (they had been initially prepared for treatment of diarrhea in cattles);

*E.coli* (they cause excessive contents of coliforms in gut, normal count of them not more than 1% of gut microflora);

metabolites of any microbes (toxic metabolites can’t improve the state of the microflora and only cause inflammation and diarrhea).
Bacillus spp.
Hemolysis on blood agar
Foodstuffs
Gnotobiology is the science which studies the mechanisms of microbial influence on the host’s immune system on a model of germ-free animals. Germ-free animals are obtained by hysterectomy or caesarian section. Germ-free animals have no their own microflora — sterile animals. Germ-free animals live in sterile conditions. Germ-free animals get sterile food. Germ-free mice, rats, guinea pigs, monkeys, chicken are available for gnotobiologic research.
Properties of Germ-Free Animals

Germ-free animals are more susceptible to infection and have reduced vascularity, digestive enzyme activity, muscle wall thickness, cytokine production and serum immunoglobulin levels, smaller Peyer's patches and fewer intraepithelial lymphocytes.

Reconstitution of germ-free mice with an intestinal microbiota is sufficient to restore the mucosal immune system.

**Example:** Colonization of germ-free mice with a single species, *Bacteroides thetaiotaomicron*, affects the expression of various host genes that influence nutrient uptake, metabolism, angiogenesis, mucosal barrier function and the development of the enteric nervous system.