



# Recent Advancement in Probiotic and Prebiotics for health promotion

**Prof. Rama Chaudhry**

Department of Microbiology, All India Institute of Medical Sciences, India



# Origin of Probiotics

- Dr Elie Metchnikoff, a Russian biologist first introduced the concept of probiotics in 1908
- Diet rich in fermented foods leads to a longer and healthier life.
- He suggested that *Lactobacilli* might counteract the putrefactive effects of gastrointestinal metabolism.
- The first microbe used specifically for this purpose was *Lactobacillus bulgaricus*



# Probiotics ?

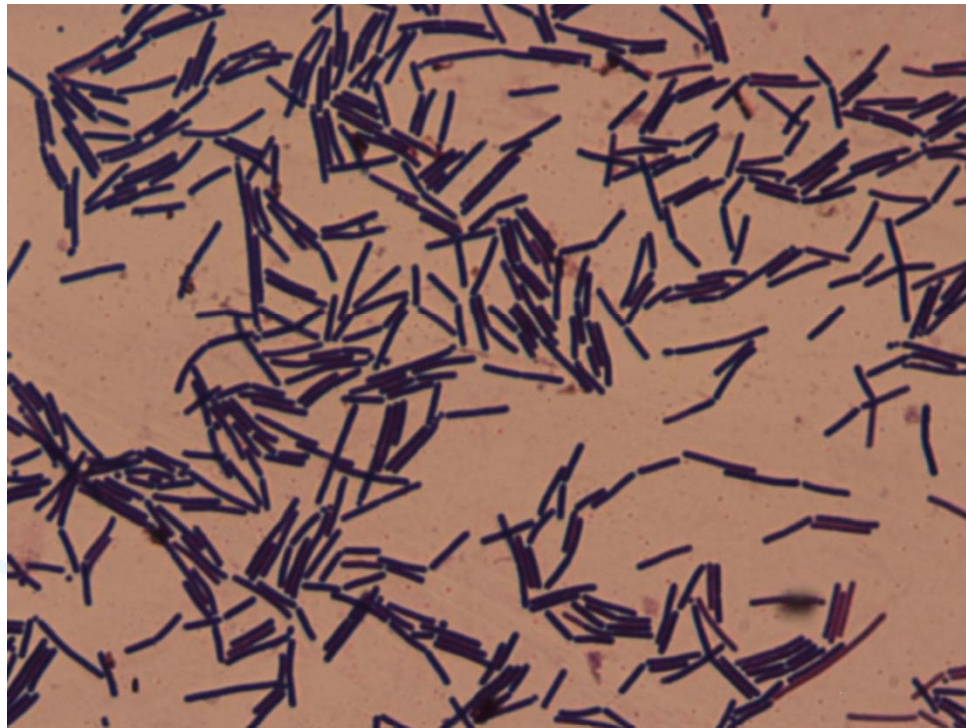
- Probiotic candidates, are live microbial food supplements.
- Non-pathogenic and non-toxicogenic
- Retain viability during storage and survive passage through the stomach & small bowel
- Capable of colonizing the intestinal tract to influence human health.





# Probiotic

## Thin long Rods



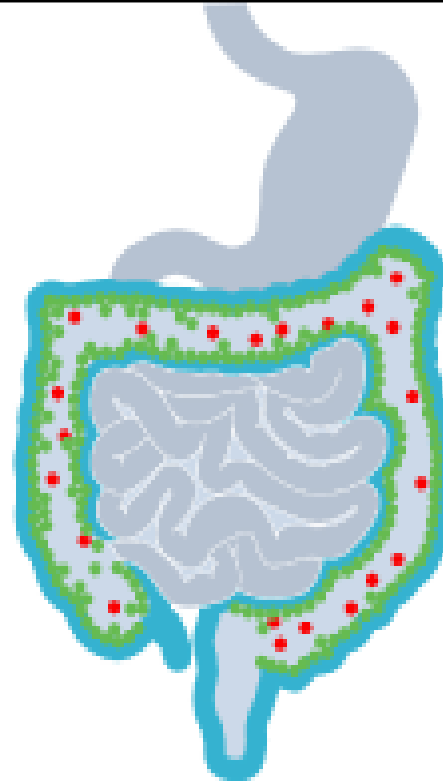


# Probiotic function

Prevent potentially harmful bacteria attaching to gut lining

Reduce the availability of nutrients for potentially harmful bacteria

Excrete antimicrobial substances



Bring down the pH in the colon

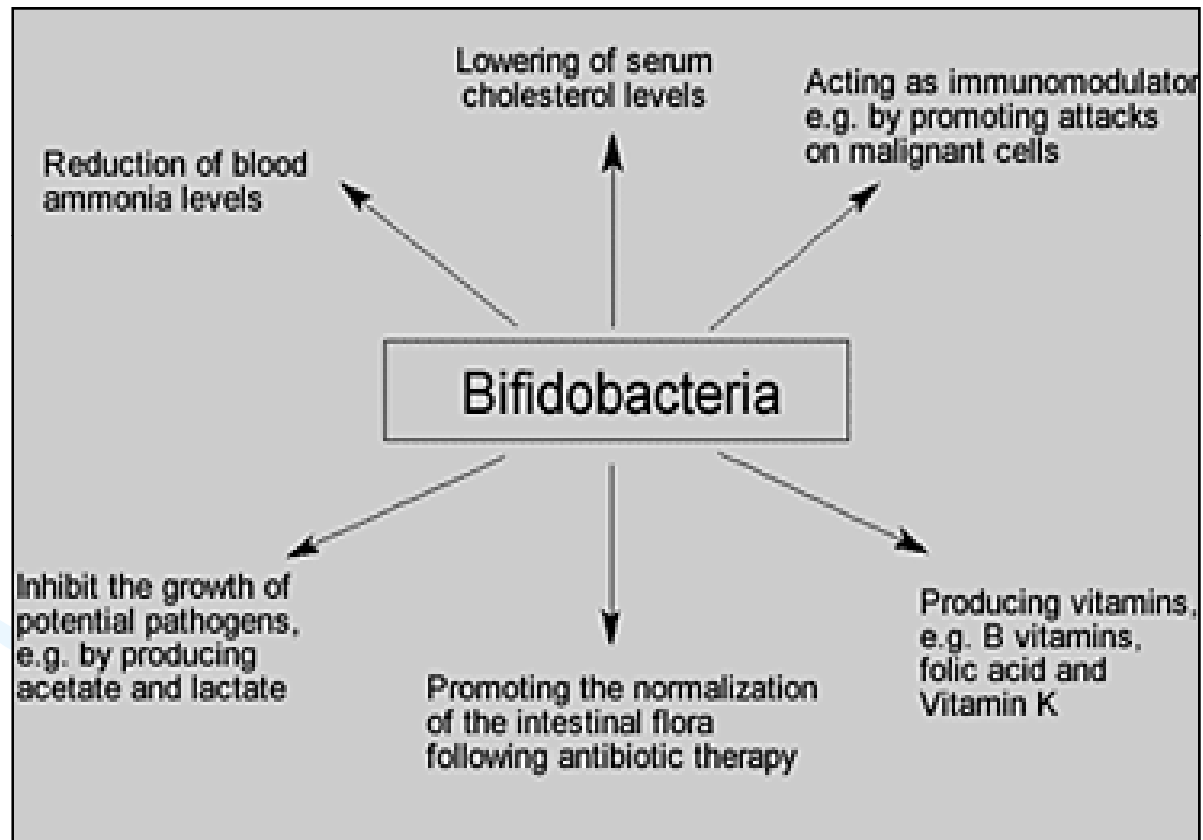
Improve mucous production from cells lining the gut

Ongoing support of the immune system

- Friendly bacteria
- Harmful bacteria



# Beneficial effects of Probiotic bacteria





# An effective, probiotic

- To survive exposure to gastric and bile acid
- Adherence to the intestinal epithelium
- Persistence and multiplication
- Inhibitory activity against multiplication of enteropathogens / target bacteria.
- Effective in treatment of acute GI infections
- Resistant to most antibiotics



- Produce Antimicrobial Compound

- ✓ Organic acids
- ✓ Hydrogen peroxide
- ✓ Carbon dioxide
- ✓ Diacetyl
- ✓ Acetaldehyde
- ✓ bacteriocins

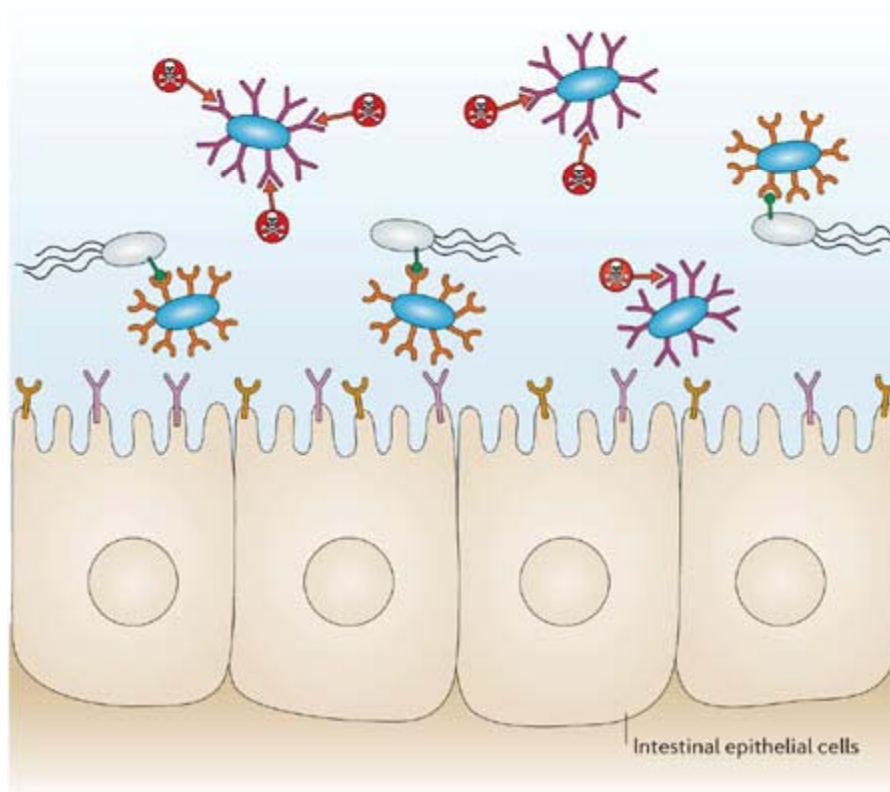
- To be safe, noninvasive, noncarcinogenic and nonpathogenic

- Coaggregate to form a normal balanced flora.





# MICROBIAL INTERFERENCE THERAPY



Copyright © 2006 Nature Publishing Group  
Nature Reviews | Microbiology

The strategy is based on interfering with pathogen–receptor or toxin–receptor interactions by engineering the expression of host-receptor mimics on the surface of a harmless bacterium, so that this 'designer probiotic' strain (blue) can bind and neutralize toxins in the gut lumen or interfere with the adherence of pathogens (white) to the intestinal epithelium



# Gastrointestinal infections

- DIARRHOEA rotavirus, travellers, community acquired, antibiotic associated(AAD), *Clostridium difficile* colitis
- Inflammatory Bowel Syndrome
- Crohn's disease, ulcerative colitis



## Gastrointestinal

- Prevents or reduces duration of diarrhea (rotavirus)
- Prevents or reduces Clostridium difficile infections.
- Induces remission of inflammatory bowel disease.
- Prevents recurrence of ulcerative colitis manifestation.
- Reduces symptoms in irritable colon
- Reduces the incidence of colonic cancer



- **Stomach**

- ✓ Prevents or reduces Helicobacter infections

- **Pancreas**

- ✓ Prevents as reduces septic manifestations in pancreatitis

- **Liver**

- ✓ Reduces clinical manifestations, mortality and extent of cellular damage in toxic liver injury.

- **Skin and body surfaces**

- ✓ Reduces atopic eczema manifestations in children
- ✓ Reduces biofilm



*Agarwal R, Sharma N, Chaudhry R. et.al.*

## *STUDY at AIIMS*

Probiotic - Lactobacillus GG (LGG)

**Colonization with LGG - 21% of infants ( <1500g ) v/s 47% (>1500g)**

**Probiotic treatment -<1500g                      significant increase by day 21.**

Increased Gram (+) and anaerobic species.

No difference in species number in controls. (P < 0.05).

**LGG preparation – well tolerated.**



## Long-term colonization of a *Lactobacillus plantarum* synbiotic preparation in the neonatal gut.

Panigrahi P, Pardia S, Pradhan L, Mohapatra SS, Misra PR, Johnson JA, Chaudhry R, Taylor S, Hansen NI, Gewolb IH.

### BACKGROUND:

The hospital-based trial to determine the colonizing ability, tolerance, and impact on the stool flora of 7 days of administration of a synbiotic supplement to a neonatal cohort,

### RESULTS:

Nineteen infants received the active study supplement and 12 infants received the placebo for 7 days.

*L. plantarum* was cultured from the stools of 84% of the treated infants after 3 days of treatment, and from 95% of infants on day 28 after birth.

The number of bacterial species was significantly higher on days 21 and 28 in the synbiotic preparation group vs. placebo ( $P = 0.002$  &  $0.03$ ).

The supplement group had significantly higher gram-positive colony counts on days 14 ( $P = 0.002$ ) and 28 ( $P = 0.04$ ).

The supplement was tolerated well.

### CONCLUSIONS:

The synbiotic preparation colonized quickly after 3 days of administration and the infants stayed colonized for several months after therapy was stopped. There was an increase in bacterial diversity and gram-positive organisms and a reduction of gram-negative bacterial load in the treatment group.



## Treatment of acute diarrhoea in infants/children with a mixture of *Lactobacillus rhamnosus* strains – a randomized, double-blind, placebo – controlled trial.

### AIM:

- To determine whether *L. rhamnosus* strains (573L/1, 2,3) would be effective in shortening infectious diarrhoea.

### METHODS:

- 87 children (age range: 2 months to 6 years) with infectious diarrhoea were administered a mixture of 3 *L. rhamnosus* strains- dose  $1.2 \times 10^{10}$  CFU, or placebo, BD x 5 days. Primary outcome – measure was the duration of diarrhoea.
- Secondary measures were duration of parenteral rehydration, adverse events, and GI colonization by above strains.

### RESULTS:

- Reduced mean duration of diarrhoea in cases
- Duration of parenteral rehydration also reduced
- Gut colonization 80% at 5 days, 41% at 14 days
- No adverse events were noted.



# Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of 5 different preparations.

## OBJECTIVE:

- To compare the efficacy of 5 probiotic preparations used in treating acute infectious diarrhoea in children. Aged 3-36 months.

## INTERVENTION:

- ORS (control group)
- *Lactobacillus rhamnosus strain GG*
- *Saccharomyces boulardii*
- *Bacillus clausii*
- Mix of *L delbrueckii var bulgaricus*, *Strep thermophilus*, *L. acidophilus*, *Bifidobacterium bifidum*
- *Enterococcus faecium*

Contd....2





# OUTCOME:

## Primary Outcome

- Duration of diarrhoea
- Daily number and consistency of stools

## Secondary outcome

- Duration of vomiting and fever
- Safety of preparation

## RESULTS:

- 571 children received the preparations.
- The children who received *L.rhamnosus GG*, and those who received the mixture of 4 strains had shorter duration of diarrhoea, less number of stools/day.
- The remaining preparation did not affect primary outcomes. Secondary outcomes were similar in all groups.



## Effect of *Lactobacillus rhamnosus* GG in persistent diarrhea in Indian children: a randomized, double-blind controlled trial.

### AIM:

- To evaluate the role of LGG as probiotics in persistent diarrhea (PD) in children of North Bengal – a hospital based study.

### STUDY :

- 235 children admitted (over 2 years) with PD were randomized to receive either ORS or ORS + LGG (60 million cells/ dose) for 7 days.

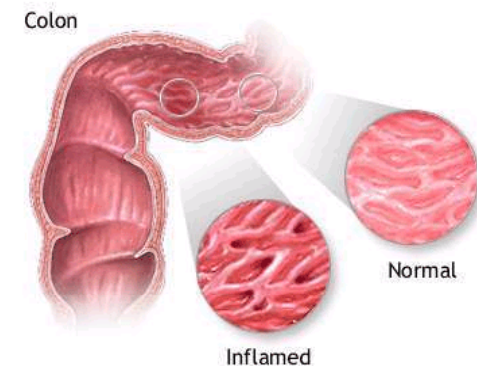
### RESULTS:

- Mean duration of diarrhoea was significantly lower in cases than controls.
- Average duration of hospital stay was also significantly lesser in cases.
- No complication was observed with LGG.



# Probiotics & Inflammatory Bowel Diseases

- Pathogenesis of IBD is not known
- An aggressive Immune Response to the resident luminal flora, rather than - altered flora
- Malin et al 1995 first time demonstrated increased no.of IgA secreting cells following probiotic therapy.
- Giatler et al - *E. coli* , *B. vulgatus* Increased & *Bifidobacteria* decreased in cases of active crohn's disease





# Probiotics on *C. difficile* Diarrhoea

- *C. difficile* leading cause of AAD
- 5-66% of Pts. have recurrent disease
- 20% recurrences after standard antibiotic therapy
- Protective. micro flora is initially damaged by antibiotic treatment
- “first hit” leaves the host susceptible to colonization and subsequent Infection by *C. difficile*.
- “second hit” occurs when the infected host is treated with Van/Met. further destroying susceptible bacterial flora.





# Probiotics on *C. difficile* Diarrhoea

## Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of *Clostridium difficile* disease.

- From 25 randomized controlled trials (RCTs), probiotics significantly reduced the relative risk of AAD (RR = 0.43, 95% CI 0.31, 0.58,  $p < 0.001$ ).
- From six randomized trials, probiotics had significant efficacy for CDD (RR = 0.59, 95% CI 0.41, 0.85,  $p = 0.005$ ).

### CONCLUSION:

- A variety of different types of probiotics show promise as effective therapies for these two diseases.
- meta-analyses, three types of probiotics (*Saccharomyces boulardii*, *Lactobacillus rhamnosus* GG, and probiotic mixtures) significantly reduced the development of antibiotic-associated diarrhea.
- *S. boulardii* was effective for CDD.



## Table The Probiotic effect of lactic acid bacteria (LAB) in human and animal health

Medical target	Example strain	Reference
Prevent food allergy	<i>L. rhamnosus</i> GG	Sutas et al., 1996
Block formation of biogenic amines	<i>L. lactis</i> ESI 561 <i>E. faecalis</i> INIA 4-07 <i>E. faecalis</i> EFS 2 <i>L. acidophilus</i>	Joosten et al., 1996
Overcome lactose intolerance	LAB	Gilliland and Kim, 1984
Prevent diarrhea (antibiotic-induced, rotavirus, travellers, community acquired, <i>Clostridium difficile</i> colitis)	<i>L. rhamnosus</i> GG <i>L. acidophilus</i> LB	Fooks et al., 1999 Heyman, 2000 Oksanen et al., 1990 Simakachorn et al., 2000
Reduce intestinal disorders and pouchitis.	LAB <i>L. rhamnosus</i> GG <i>L. acidophilus</i>	Sanders, 2003 Gionchetti et al., 2000 Kuisma et al., 2003 Canducci et al., 2000
Suppress side effects of <i>Helicobacter pylori</i> medication with antibiotics.		



Medical target	Example strain	Reference
Treat Crohn's disease, ulcerative colitis and inflammatory bowel disease (IBD) Stimulate anticarcinogenic activity	<i>L. rhmanosus</i> GG <i>B. infants</i> UGC35624 LAB LAB	Gupta et al., 2000 Von Wright et al., 2002 Marteau et al., 2002 Goldin, 1990
Treat coronary heart disease and anticholesterolaemic effects Control of human urinary tract infection and vaginosis.	<i>L. acidophilus</i> <i>L. acidophilus</i> <i>L. rhamnosus</i> (GG) <i>L. rhamnosus</i> GR-1	Hirayma and Rafter, 2000 Schaafsma et al., 1998 Gilliland et al., 1985 Kontiokari et al., 2001 Reid, 2001 Reid 2002
Reduced blood urea nitrogen level in uremic rats	<i>Sporo Sarcina Pustchurii</i> (SP)	Ranganathan et. Al 2006
Immunomodulating effect	<i>L. plantarum</i> 299 <i>L. rhmanosus</i> GG	Pathmakanthan S, et al., 2004 Schultz M, et al., 2003, Passi T, 2000



**Will the Probiotics be the  
magic bullet in the era of  
antibiotics resistance?**





# Antibiotic resistance: A global threat

- The emergence of pathogenic bacteria resistance to broad range of antibiotics.
- Most serious threat to public health worldwide.
- Bacterial resistance increases medical cost .



# PROBIOTICS- claimed clinical effects

## General

- Reduces the incidence and severity of sepsis in intensive care units
- Reduces the incidence and severity of sepsis in major surgery



# Prevention of Carcinogenesis & Tumor Growth

## *1st hypothesis*

Prevention or delay in tumor development by lactobacilli

- By binding to Mutagenic compound in the intestine.
- Decrease in absorption of these mutagenic heterocyclic amines.
- Reduced urinary excretion of mutagens after a test meal by 50% in 6 volunteers



## *Second hypothesis*

- Suppression of growth of Bacteria that convert Procarcinogens → carcinogens
- LGG ↓ activity of
  - β glucoronidase
  - Nitroreductase
  - Cholyglycine hydrolase
- Consumption of milk fermented *L. casei shirota* (4wks)
  - β glucoronidase
  - β glucosidase (activity in 10 subjects but not in 10 Controls)
- *Sapanhaak et al Eur. J. Clin. Nutr 1998;52:1-9*
- Aso et. al 1992 demonstrated delay in recurrence of Bladder tumors after *L. cassei* x 1yr. In 57% pt versus 83% in Control group (p <0.01)



# Selection of *Lactobacillus* species to recolonize the vagina with recurrent bacterial vaginosis

- Probiotic strains :

- ✓ *L. crispatus* (55730)
- ✓ *L. acidophilus* (61701 and 61880)
- ✓ *L. delbrueckii subsp. delbrueckii* (65407)

- Results:

- ✓ *L. crispatus* (55730) and *L. acidophilus* (61880) produced H<sub>2</sub>O<sub>2</sub>
- ✓ All strains produced highly acidic environment
- ✓ *L. acidophilus* (61880) strongly adherent to vaginal epithelial cells
- ✓ Isolates 61880 and 61701 selected as a probiotic candidates for vaginal recolonization



# Probiotics in HIV

## Use of probiotics in HIV-infected children: a randomized double blind controlled study.

- **AIMS**

- ✓ To determine whether the use of probiotics could improve the immune response determined by CD4 cells mm<sup>-3</sup> counts and reduce liquid stool episodes.

- **STUDY**

- ✓ A randomized double-blind controlled trial with 77 HIV-infected children (2-12 years) divided into two groups:
- ✓ 1. one receiving probiotics ( Bifidobacterium bifidum & Streptococcus thermophilus -2.5 x 10<sup>10</sup> colony forming units)
- ✓ 2. a standard formula (control group), for 2 months.
- ✓ The CD4 counts at the beginning and end of the study.
- ✓ The quality and number of stools were assessed by a questionnaire (watery to normal stool consistency).

- **RESULTS**

- ✓ an increase in the mean CD4 count in the probiotics group (791 cells mm<sup>-3</sup>)
- ✓ The change from baseline in mean CD4 cell count was +118 cells mm<sup>-3</sup> vs. -42 cells for children receiving the probiotic formula and control formula, respectively (p = 0.049).
- ✓ A similar reduction in liquid stool consistency in both the groups (p < 0.06),
- ✓ The incidence of loose-soft stools showed a small decrease in both groups (p < 0.955) and there was an increase in the incidence of normal stool consistency in both the groups (p < 0.01).
- ✓ Probiotics have immunostimulatory properties and might be helpful in the treatment of HIV-infected children.



# Immunomodulatory Effect

- Lactobacillus plantarum demonstrated beneficial immunomodulatory activity by increasing IL-10 synthesis and secretion in macrophages and T-cells derived from inflamed colon.
- This may provide a mechanism through which probiotic bacteria ameliorate inappropriate inflammation and induce tolerance.



# To study Immuno-modulatory effects of probiotic

- **Colonocytes**

- ✓ **Gastro intestinal epithelium -- rapid renewal.& shed into the fecal stream every day..**
- ✓ **Exfoliated epithelial cells are representative of entire colon**
- ✓ **Used for clinical investigations of disease processes in non- invasive manner.**
- ✓ **Exfoliated colonocytes express Immunoglobulin on cell surface (IgA.,IgG).**
- ✓ **Cytokeratins indicating epithelial lineage as well as colon specific antigen.**

- **Aim of the work.**

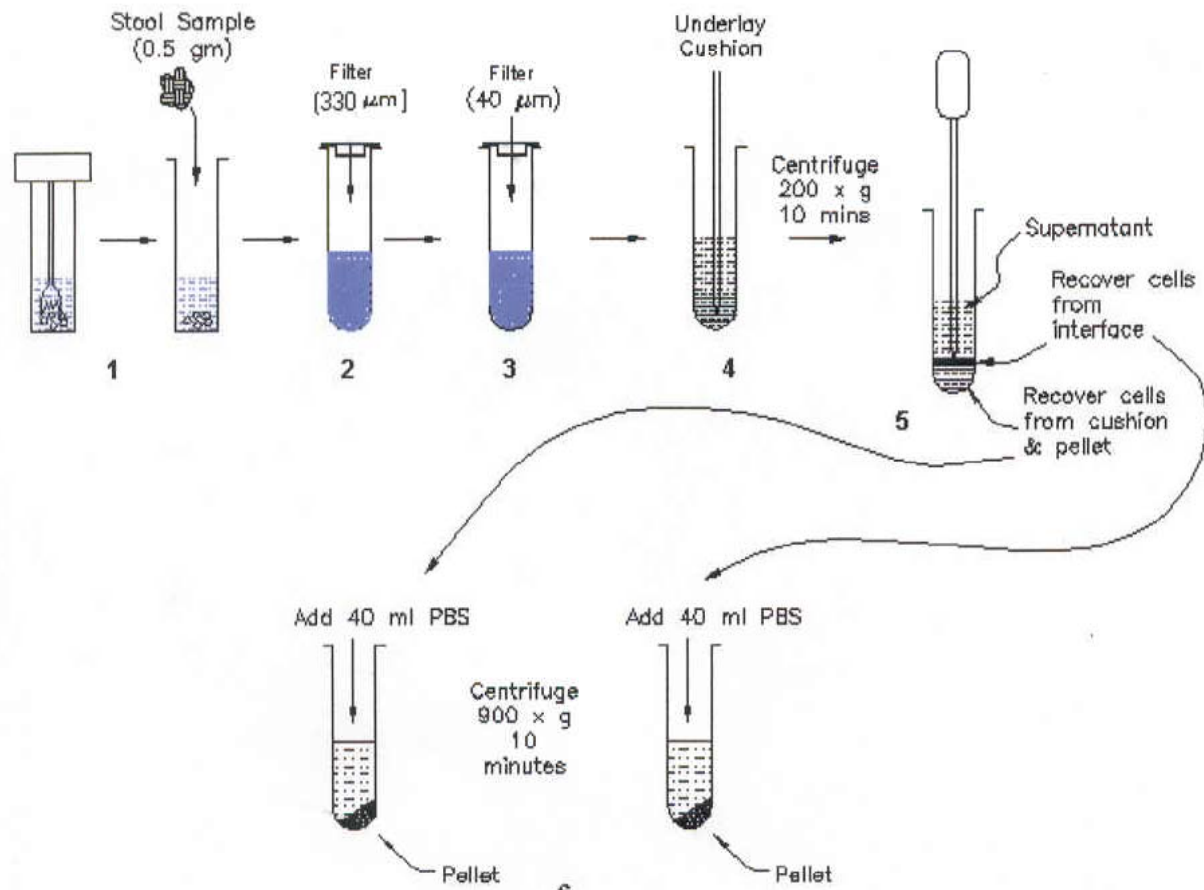
- ✓ **To recover viable cells from stool samples in significant numbers.**
- ✓ **Detect immunoglobulin from cell surface to asses the mucosal immunity by using specific monoclonal antibody (IgA & IgG)**

DBT PROJECT, AIIMS Chaudhry R et.al.2008





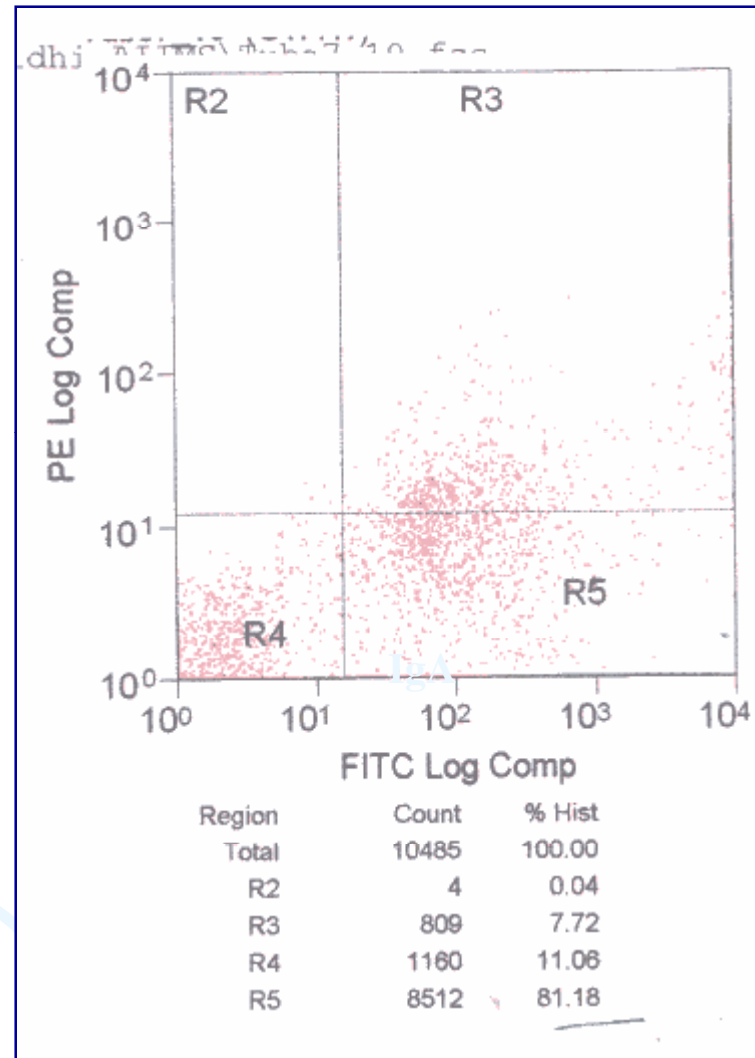
# Isolation of colonocytes from stool



DBT PROJECT, AIIMS<sup>6</sup> Chaudhry R et.al.2008

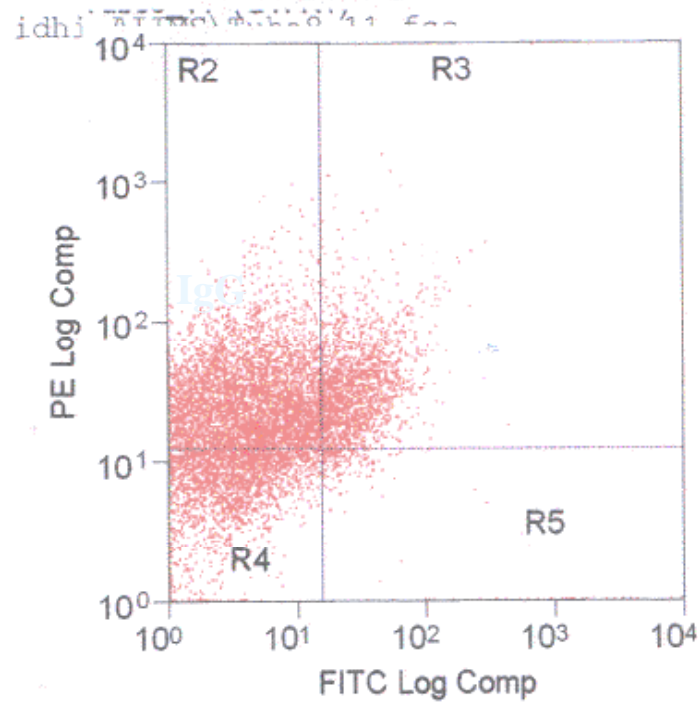


# IgA FITC



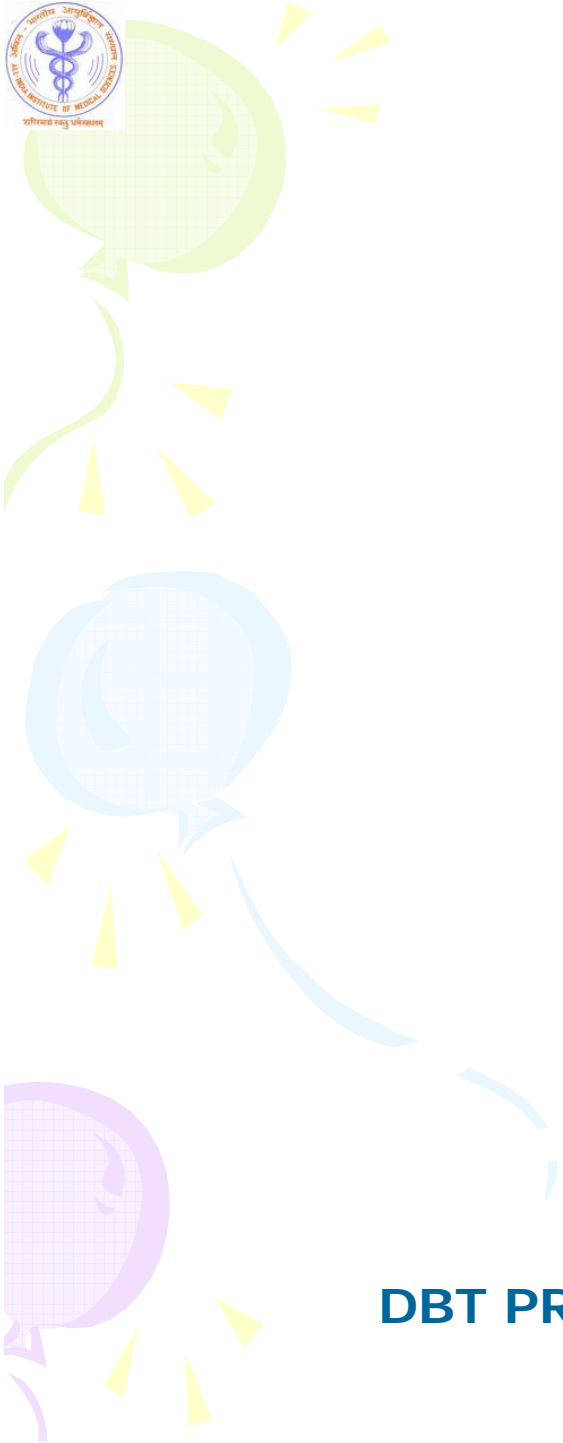
DBT PROJECT, AIIMS Chaudhry R et.al.2008

# IgG PE



Region	Count	% Hist
Total	10346	100.00
R2	5054	<u>48.85</u>
R3	1721	16.63
R4	3384	32.71
R5	187	1.81

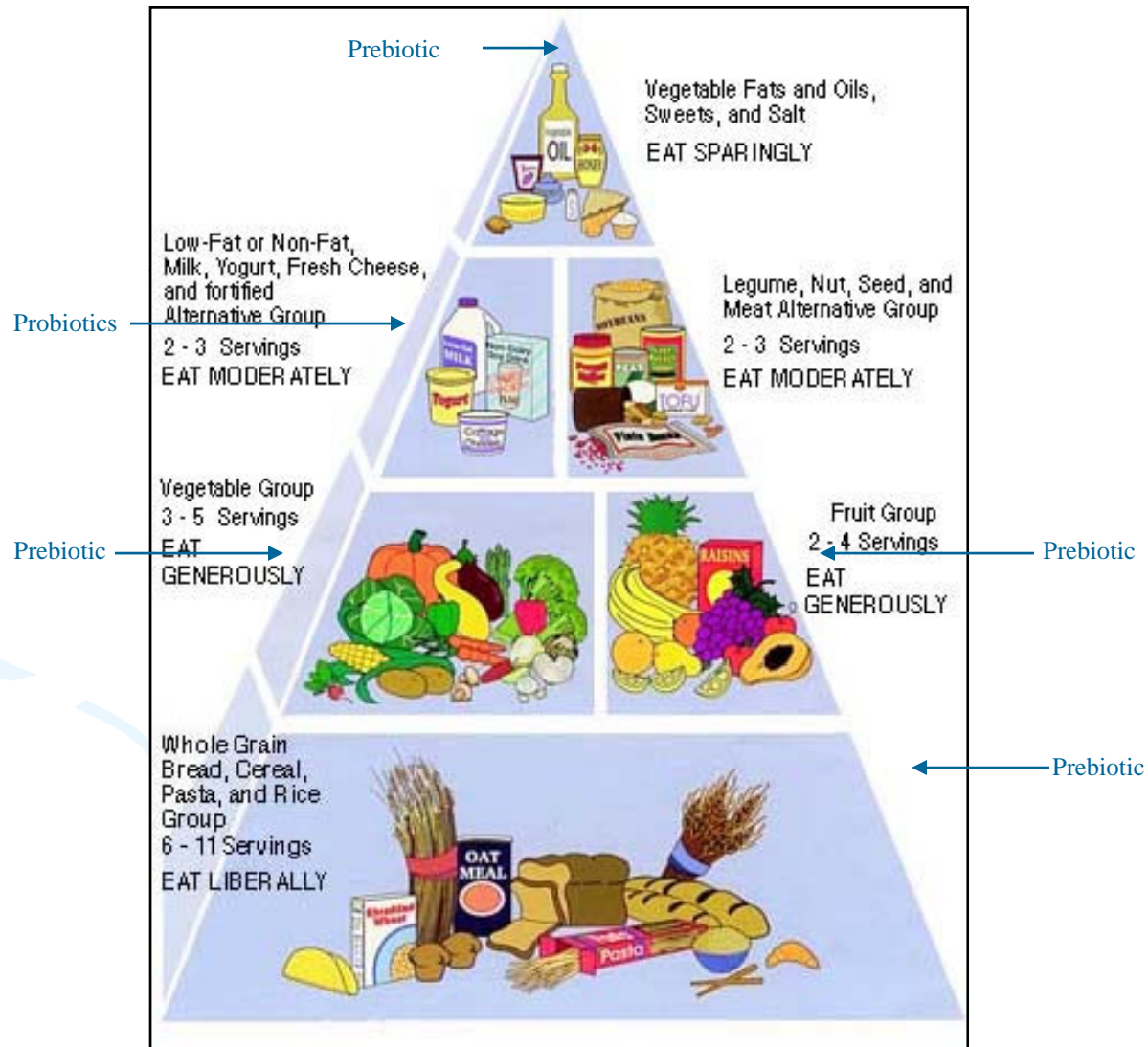
DBT PROJECT, AIIMS Chaudhry R et.al.2008





# PREBIOTICS

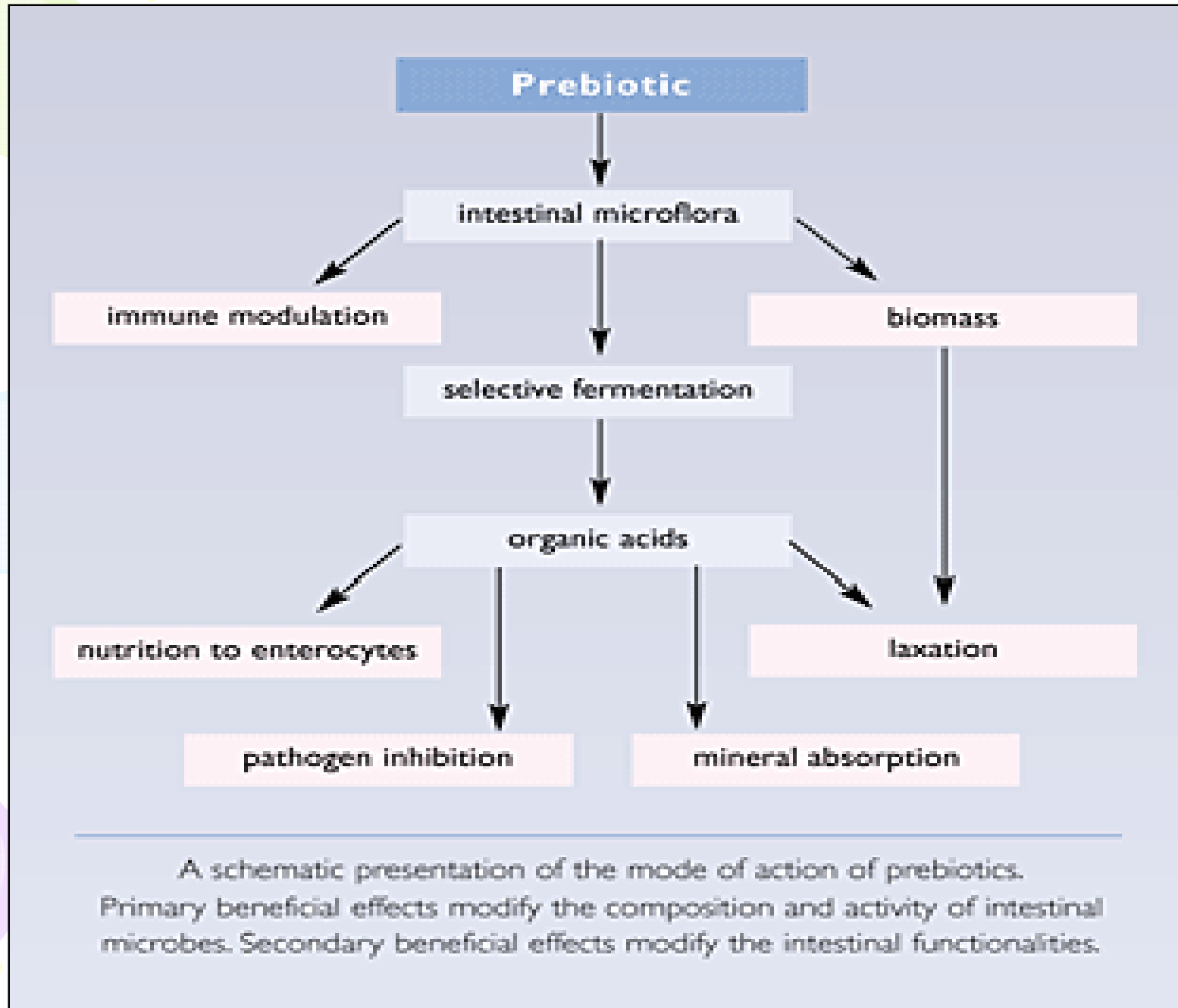
## Important sources of Pro- & pre-biotics





# Prebiotics

- Substrates for production of nutrients.
- Consist of ingested fibers & complex proteins, mucus, GI secretions.
- Glucose/fructose oligosaccharides.





# Digestion of prebiotic In Gut

- Prebiotics escape enzymatic digestion in the upper gastrointestinal tract
- Enter in caecum without change to their structure.
- Not excreted in the stools – indicating fermented by colonic flora.
- Produce- a mixture of short-chain fatty acids (acetate, propionate butyrate), L-lactate, carbon dioxide and hydrogen.
- stimulates *Bifidobacteria*,



# Pathogenic microflora suppression

Inulin specifically supports growth of healthy bacteria

**Bifidobacteria sp.**  
**Lactobacilli sp.**

produce antimicrobial cmpds.  
acetic, lactic & benzoic acids,  
 $H_2O_2$ , & bacteriocins-type cmpds.

Inhibits growth of  
**Pathogenic *E. coli***

Inhibits growth of  
***Clostridium difficile***

Suppresses growth of  
***Candida albicans***





# Implications of Prebiotic for health

## By stimulating Bifidobacteria

- potential protective effects against colorectal cancer and infectious bowel diseases.
- inhibiting putrefactive bacteria (*Clostridium perfringens*) and pathogen bacteria (*Escherichia coli*, *Salmonella*, *Listeria* and *Shigella*).
- improvement of lipid metabolism.
- fibre-like properties by decreasing the renal nitrogen excretion
- improvement in the bioavailability of essential minerals; and low cariogenic factor.

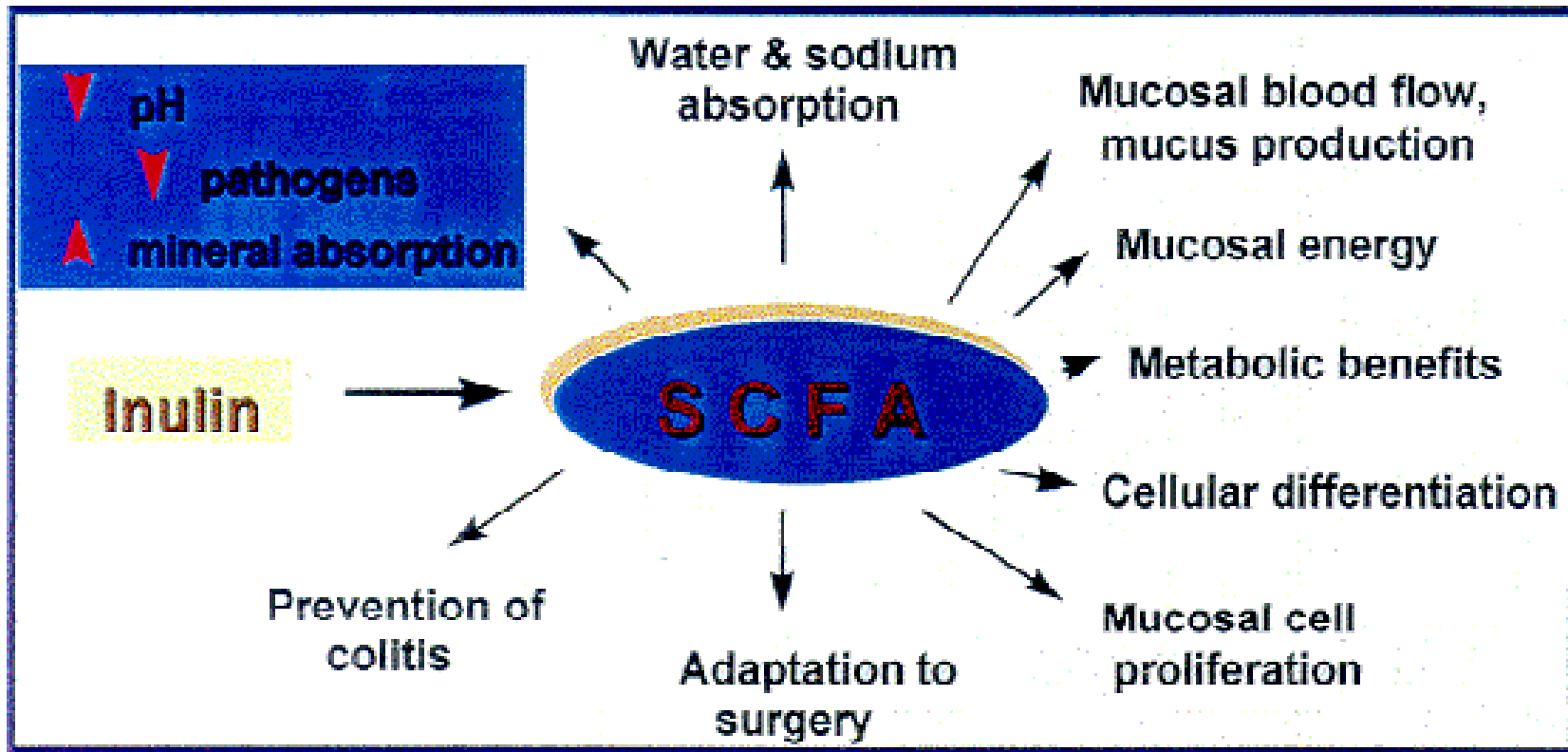


# Health aspects of prebiotics

- **Colonic food**
- **Mineral absorption**
- **Metabolism of lipids**
- **Inhibition of infectious Pathogens**

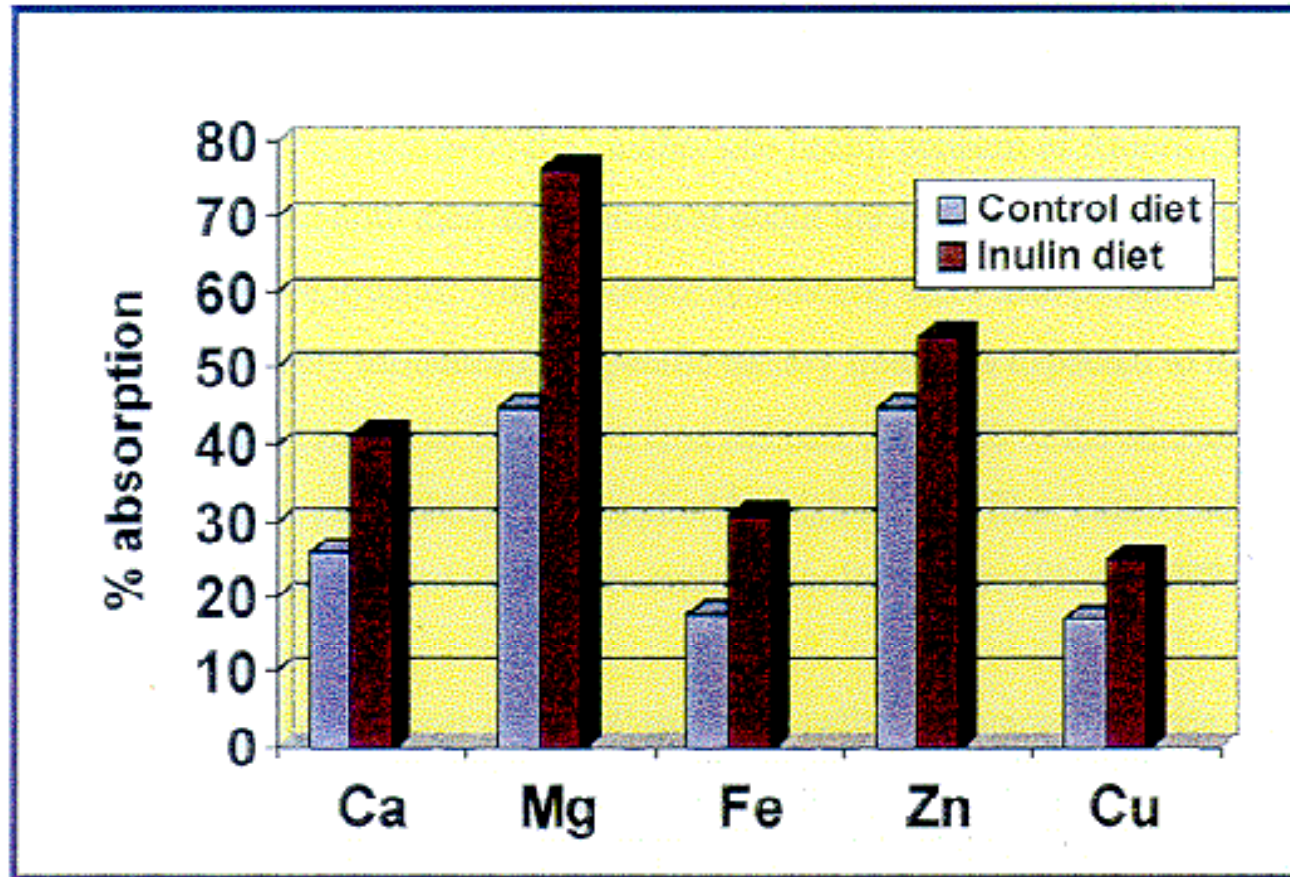


# Factual and Hypothetical Effects of Short Chain Fatty Acids (SCFAS) on Colonic Morphology and Function



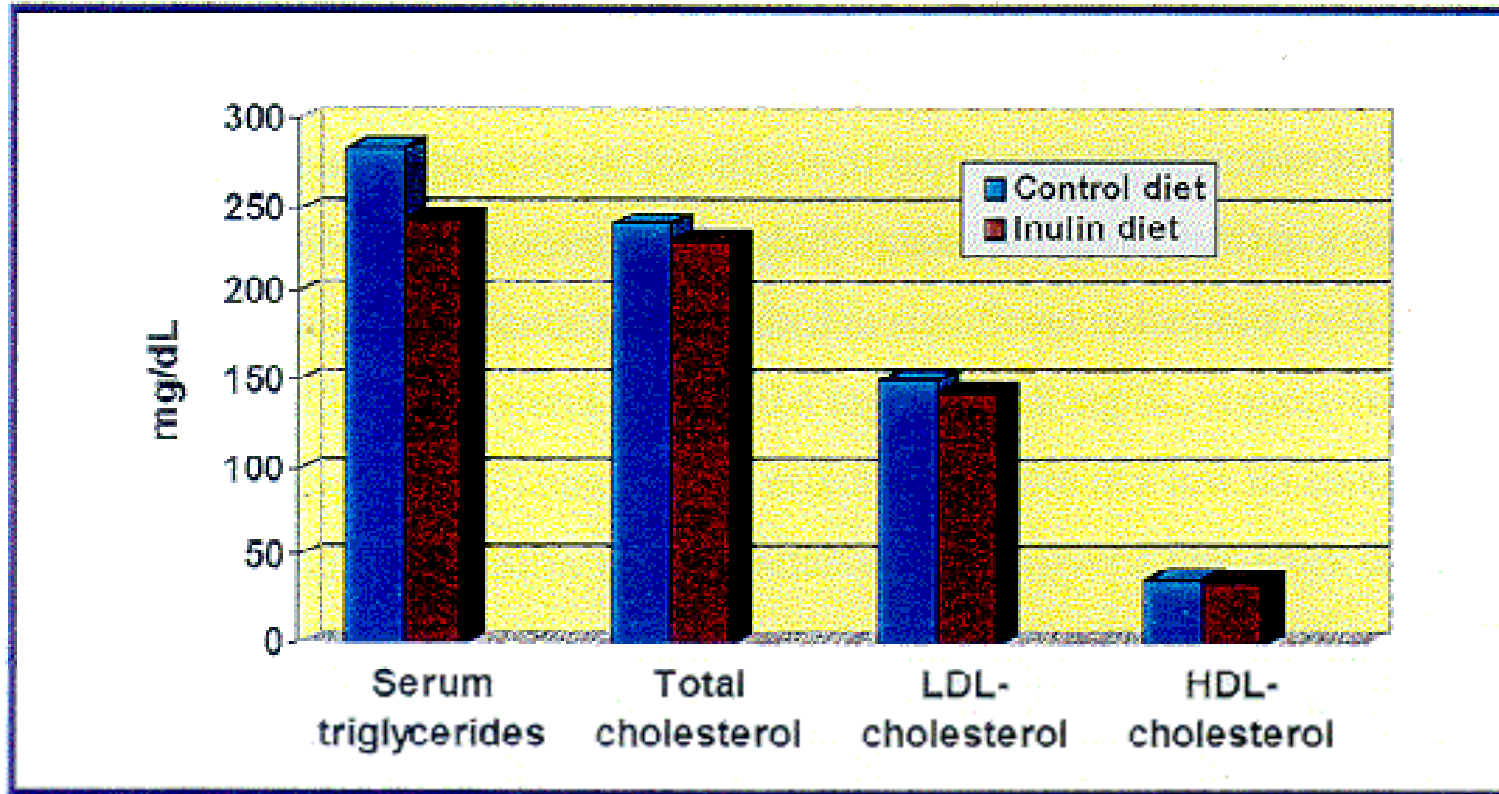


# Effect on Mineral Absorption adapted from Delzene et al 1995.





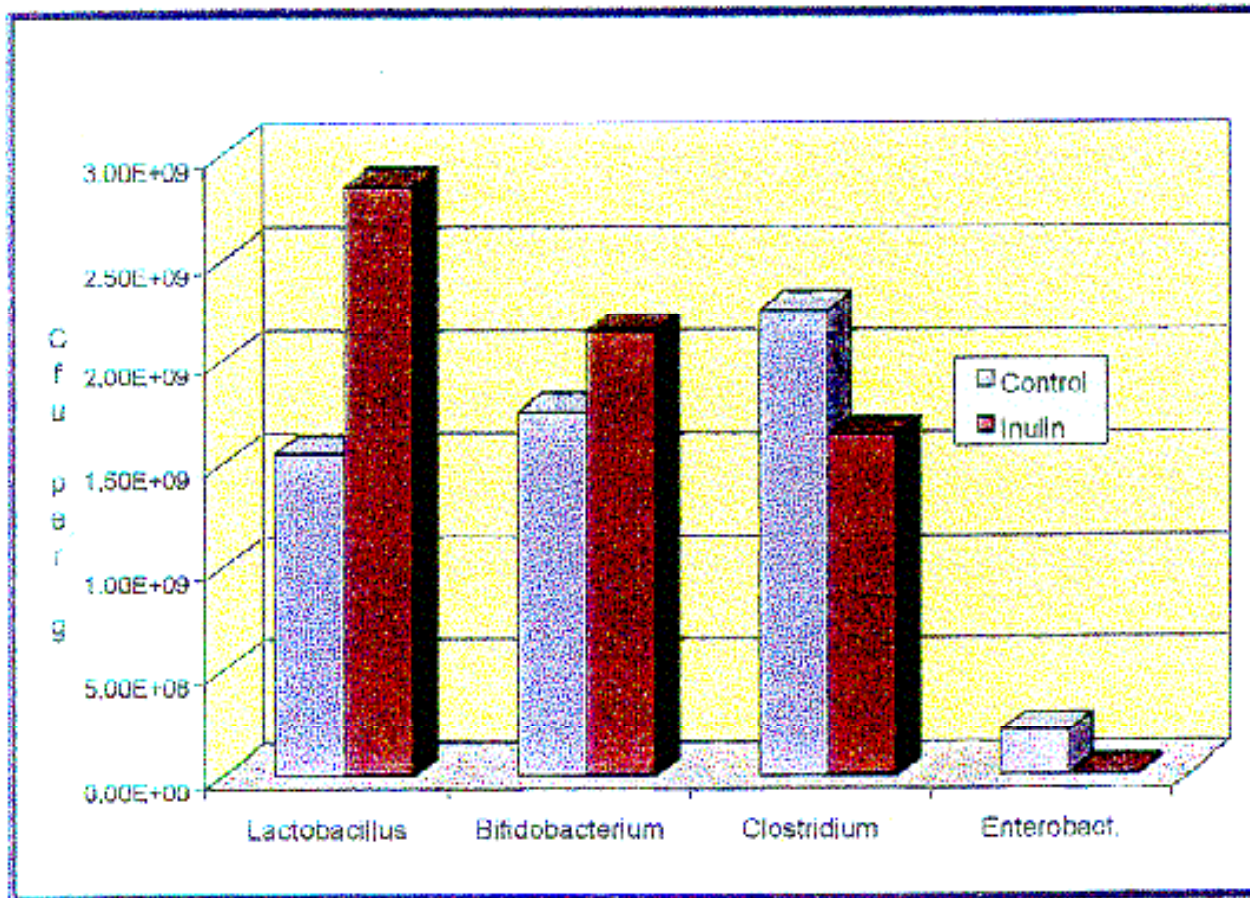
# Effect of prebiotic (Inulin) Ingestion on Lipid Metabolism.



from Causey et al. 1998



## *Effect of 20 g/d full consumption on Enterobacteriaceae*





# CONCLUSION

Well-established probiotic effects are:

- Prevention and/or reduction of duration and complaints of rotavirus-induced or antibiotic-associated diarrhea and in lactose intolerance.
- Reduction of the concentration of cancer-promoting enzymes and/or putrefactive (bacterial) metabolites in the gut.
- Prevention and alleviation of nonspecific and irregular complaints of the gastrointestinal tracts in healthy people
- Beneficial effects on inflammatory diseases of the gastrointestinal tract, *Helicobacter pylori* infection or bacterial overgrowth.
- Normalization of passing stool and stool consistency in subjects suffering from an irritable colon.
- Prevention or alleviation of allergies and atopic diseases in infants.
- Prevention of respiratory tract infections (common cold, influenza) and other infectious diseases as well as treatment of urogenital infections

Contd--



Insufficient or at most preliminary evidence exists with respect to

- cancer prevention
- hypocholesterolemic effect
- improvement of the mouth flora and caries prevention
- prevention or therapy of ischemic heart diseases
- amelioration of autoimmune diseases (e.g. arthritis)





THANK YOU