SECTION I

USAGE OF *PROBIOTICS* IN FOOD PRODUCTS

CHAPTER 1

INTRODUCTION TO PROBIOTICS

1. INTRODUCTION

The term '***Probiotic***' was originally derived from the **Greek**, meaning '*for life*'. Consequently, *two* world renowned noteworthy organizations, namely:

- **The Food and Agriculture Organization (FAO)** *of the United Nations*, and
- **The World Health Organization (WHO).**

They have vehemently affirmed that there prevails ample solid scientific evidences to indicate that undoubtedly there exists enough potential for the so-called '***Probiotic* Foods'** in these ***two* major aspects**, such as:

- **to provide definite '*Health Benefits*'**, and
- **certain '*Specific Strains*' prove to be absolutely safe for humans*.**

McFarland and Elmer (1995)*, based upon an expert panel delibration commissioned jointly by **FAO** and **WHO** put forward the following *definition* of *Probiotics* as-

"**Live microorganisms which on being administered in adequate amounts confer a definite health benefit on the host".**

It is, however, pertinent to state here that the above cited definition must always be used and followed ***ad verbatim;*** and, therefore, ***Probiotics*** should never be regarded or referred to as the '**Biotherapeutic Agents'****.

The most important and critical characteristic features of the ***FAO-WHO* definition** are that the '***organisms'*** require to be certainly **alive**, and should have been proven explicitly in a controlled, human investigative study to ascertain a definite health benefit; otherwise they must not be called as '***Probiotics***'.

Points to Ponder: Let us look into the following ***two* aspects:**

1. Quite a few research studies have also reported significant *health effects* particularly with the *cellular components of the microorganisms;* however, these are not regarded '***Probiotics***', since they are ***not alive****.

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* Food and Agriculture Organization of the United Nations and World Health Organization, 2001 (**Regulatory and Clinical Aspects of Dairy Probiotics**).

** McFarland LV and Elmer GW: **Biotherapeutic Agents: Past, Present and Future**, *Microeol Ther., **23:*** 46-73, 1995.

*** **That is, with the exception of the animal and plant viruses.**

2. Likewise, not all '***live cultures'*** (or '**live organisms**') are considered '***Probiotics***' since not all of them actually have been demonstrated to have a **health benefit**.
3. Should not we consider the '***Probiotics***' as an altogether ***diverse group of microorganisms***?

Interestingly, the term '***Probiotics***' was first ever coined by **Lilley** and **Stillwell (1965)** in order to describe vividly the substances duly *secreted* by one **particular microorganism** that eventually *stimulated* the **growth of another microorganism,** but **McFarland and Elmer's (1995)** definition seems to be most befitting and appropriate definition of '***Probiotics***'.

Highlights: In short, one may simply conclude that the foretold definitions of '***Probiotics***'; do relate to the various divergent microorganisms; and may include:

- **Bacteria** • **Bacteriophages** • **Fungi** • **Viruses** and • **Yeasts.**

Importantly, all these groups of organisms* have been proven to possess *several useful effects* when administered to animals.

- **Microorganisms**- included as '***Probiotics***' are invariably regarded to be the so-called *non-pathogenic components* of the *normal microflora e.g.,* **Lactic Acid Bacteria.**

[A] **Similarity Between '*Probiotics*' and 'Live Cultures'**- In true sense, the '**live cultures**' do represent the microbes which invariably find their usage in the preparation of an array of ***fermented food products***, for instance:

- **Beer** • **Bread** • **Cheese** • **Cultured Cottage Cheese** • **Fermented Milk**
- **Fermented Meats** • **Kefir**** • **Vegetables** • **Wine** and • **Yoghurt.**

***Salient Features*:** Following are the three important points to take cognizance of certain aspects, namely:

(i) In some instances, the usual **culture** still remain alive in food products just prior to their consumption, specifically in the *dairy-products*; and perhaps, this could be the valid reason for these foods to serve as a genuine and dependable source of '**live cultures**'.

(ii) It is worthwhile to observe that **not all '*live cultures'*** are '***Probiotics***', since *not all livecultures* have virtually undergone the critical evaluation to prove that they do impart significant '**health effects**'.

(iii) Even though these **cultures** do possess **health effects** but while making a strong positive recommendation, on the sheer basis- that just because the substance has the *live cultures*, does not really mean it should clearly have a specific physiological effect in the humans.

[B] Importance for *Probiotics'* Dose- There is another equally important careful thought pertaining to the emerging *field of Probiotics* is the **dosage regimen**[***]. Obviously, it may not be

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* Lilley DM and Stillwell RJ: **Probiotics Growth Promoting Factors Produced by Microrganisms,** *Science***, 147:** 747-748, 1965.

** **Kefir:** ***Kefir*** is made of Ewes', Goats', or Cows' milk. During the, fermentation, *lactic acid* and *alcohol* are produced. Originally, the '**Milk Drink** was made in Russia and South western Asia. It is now being made in several countries on an industrial scale by using **Cow's Milk**.

*** **Dosage Regimen: It** refers to the strictly regulated amount of *medicament* (or *drug*) and schedule for administration to a patient.

quite possible to assign '*one member*' as the '**minimum dose'** well across the board for all the '***Probiotics***' known as-to-date. It is perhaps based on the fact that the so-called ***effective dose'*** could most probably vary depending on such factors as:

- **strain,**
- **consumer (end-user),** and
- **health end point being examined.**

Besides, quite often the observed **'*dose dependency'*** effects for the ***Probiotics*** has not yet been fully established; and hence, if the overall results are found to be promising and useful at ***one particular tested level*****, at *another lower level*** the products may not exhibit the **same effect**.

NOTE

Therefore, one may conclude that the *bare minimum* level of an *individual 'Probiotics'* invariably used in commercial products [*viz.,* Yakult$^{(R)}$] should always be based upon the actual levels that are found to be efficacious in human studies.

Examples: The actual observed broad range of the **effective doses** for *two* duly evaluated '***Probiotics'*** are stated as under:

(i) ***Lactobacillus reuteri*** **[ATCC 55730]** and ***Bifidobacterium infants*** [**35264**]: Both these '***Probiotics***' have duly proven in individual ***clinical investigative studies*** to be reasonably effective at **1×10^8 (100 million/day);** and

(ii) **VSL-3:** It is a '***Probiotic***' usually employed in the *frequent recurrence* of '***pouchitits'***- is invariably used at **1.8×10^{12} (1.8 trillion/day).**

[C] Colonizing Microbiota*: Its Role in Human Health- In a broader perspective, the *human bodies* are largely colonized *i.e.,* divided into various septums. Therefore, there are almost **10^{14} critical microbial cells** which are found to be intimately associated with the *human* body that are located strategically in various organs, namely:

- **Intestine** • **Mouth** • **Vagina and** • **Skin Surface.**

In fact, these **microbial cells** are found to be at least ***ten times*** higher *vis-à-vis* the corresponding **human cells** which essentially comprise the human body.

The ***combined*** **microbiota** has the metabolic capacity almost ***100 folds*** **higher in comparison to the quantum what human genes encode normally**. In the *intestine*, the microorganisms present in the intestinal flora are indeed of a quite divergent nature loaded with 1000's of altogether different microbial species**.

Need For Revision of the Definition '*Probiotic'*:

During the period ***1996-2001***, *three* researchers put forward the following **definitions** of '***Probiotics***':

Salminen (1996)***-** proposed that '***Probiotic***' is- " *a live microbial culture dairy product that beneficially influences the health and nutrition of the host*".

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* **Microbiota**: The **microscopic organisms** of an area.

** That is, they have been duly **identified in the intestine**.

*** Salminen S: **Uniquness of Probiotic Strains, IDF**: *Nutr News Lett,* **5**: 16-18., 1996.

Schaafsma (1996)*- suggested that the **oral *Probiotics*** are- "*the living microorganisms which on ingestion in certain specified numbers, do exert health effects much beyond the usual inherent basic nutrition*".

Molin (2001)-** put forward the logical explanation that the matrix of a product may critically affect the overall *activity profile of the microorganisms*; and, therefore, may tantamount to:

- **crucial survival,** and
- **effect of the microorganisms,**

which could obviously deserve an admirable consideration. It is, however, pertinent to state at this point in time that since the ***non-dairy products***, for instance:

- **Fermented cereals and other plant-based food products,**
- **Sauerkraut** (*i.e.,* shredded cabbage fermented in brine), and
- **Salami** (*i.e.,* highly sliced sausage, invariably comprise an array of **viable *probiotic* microorganisms,** such as: ***Lactobacillu splantarum***.

Schrezenmeir and Vrese (2001)*** promulgated the following *definition* which eventually proved to be the *closest* to the *definition* of the term '***Probiotic*'** suggested by **Havenaar and Veld (1992)******-**

"*A preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a component of the host and by that exert beneficial health effects in the host".*

Following are the ***four* valid and logical reasons for the revision of Havenaar and Veld definition** stated earlier:

1. There is a dire need to include all such products in addition to:
   - **Microorganisms,** and/or
   - **Preparation of the microorganisms.**
2. There is always a genuine prime requirement of the '**sufficient quantum of microbial numbers'** in order to exert ***significant health effects***.
3. To upheld the preference for the phrase- '**Alteration of the Microflora'**-over the- '**Improving the Properties of the Microflora'**- which is due to the fact the ensuing optimal characteristic features of the so-called '***indigenous microflora'*** were neither established nor defined until recently. Thus, the glaring '**evidence of benefit'** may be demonstrated only due to the ***health effects.***
4. The actual definition of the terminology- '***Indigenous Microflora'*** relates to-'**the usually complex mixture of the ensuing microbial population that actually helps to colonize a given area in the host which has not been duly affected by:**

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* Schaafsma G: **State of Arts Concerning Probiotic Strains in Milk Products**, *ibid*, **5**: 23-24, 1996.

** Molin G: *Arn J Clin Nutr,* **73** (Suppl.): 380 S-385 S, 2001.

*** Schrezenmeir J and Vrese M: **Probiotics, Prebiotics, and Symbiotics- Approaching a Definition, Am J** *Clin Nutr,* **73:** 361 S-364 S, 2001.

**** Havenaar R and Vrese MJH: **Probiotics: A General View**: In: *Lactic Acid Bacteria in Health and Disease: Vol.1,* Elesevier Applied Science Publishers, Amsterdam, 1992.

- **medical intervention, or**
- **experimental intervention, or**
- **disease condition(s).**

Nevertheless, the proper usage of the phrase '*to colonize'* is done to describe a specific '*bacterial populations'* which predominantly establishes in size over time without the need for a ***periodic reintroduction*** **of the microorganisms** either:

- **by repeated oral doses,** or
- **other modalities.**

Having seen the definition of the term '***Probiotics***' and its various aspects briefly, let us now examine the following ***two*** **terminologies**, namely:

- ***Prebiotic*****,** and
- **Symbiotic**.

PREBIOTIC

Gibson and Roberfroid (1995)[*] were pioneer in the introduction of the term '**Prebiotic**'- who intelligently exchanged "***pro***" for "***pre***" which means "**before**" or "**for**". Thus, they redefined the term ***Probiotics*** as-

"a non-digestible food component (ingredient) that affects beneficially the host by stimulating selectively either the growth and/or the activity of one or a limited number of bacteria in the colon".

In other words, it relates to a substance that specifically modifies the prevailing composition of the so-called '***intestinal micro-flora'*** in such a fashion that quite a few of the potentially health-promoting microorganisms *viz*.,

- ***Lactobacilli*****,** and
- ***Bifidobacteria*****,**

do become predominant in numbers.

Prebioties: The Short-Chain Carbohydrates- The newly developed '***Prebiotics***' do essentially have the **short chain carbohydrate structures,** such as:

- ***Oligosaccharides***[**]**,**
- ***Fructo-oligosaccharides***[***]**,** and
- ***Galacto-oligosaccharides***[****].

Importantly, the '**Prebiotics'** are found to be digested rather sluggishly by the *human-enzymes*; and hence, are often termed as "**non-digestible *oligosaccharides***" (as already mentioned above). It has been duly established that both of them cause definite stimulation in the specific development of the '***Bifidobacteria***' located strategically in the *colon* (**large intestine**). In addition, the '***human***

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* Gibson GR and Roberfrid MB: **Dietary Modulation of the Human Colonic Microbiota : Introducing the Concept of Prebiotics,** *J Nutr.,* **125**: 1401-12, 1995.

** These are the '**Polymers'** of the *plant origin* exclusively.

*** They are invariably produced from *lactose* by **enzymatic transgalactosylation**.

**** That is, the **directly derived disaccharides**- from *lactose*.

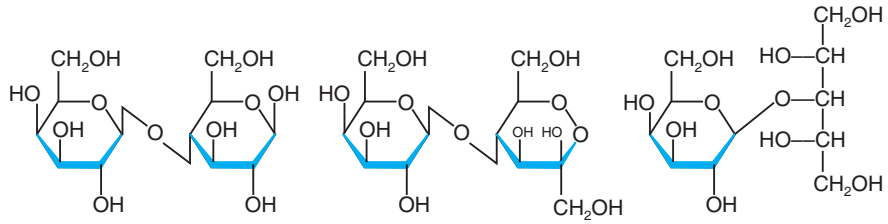
milk' particularly comprises the **lactose-derived *oligosaccharides***, that eventually stimulate the generation of a highly specialized '*bifidus flora'* in the **breast-fed infants**.

NOTE **Besides, the above cited '*oligosaccharides'* do have a pivotal role to play in the *preventive-infection* in the usual course of breast feeling.**

In reality, **lactose, lacutose**, and **lactitol** (as shown below) do exhibit appreciably the **prebiotic effects**, most prevalently on the ***stool habit***. Furthermore, they are either:

- **hydrolyzed-rather slowly,** or
- **least hydrolyzed,**

even in the presence of the so-called ***human digestive enzymes*****;** and thus, may reach the '**colon'**- thereby affecting the ensuing composition of the **microbial flora** noticeably.



Lactose **Lactulose** **Lactitol**

Physiological Effects of Prebiotics- The physiological effects of the ***prebiotics*** have been duly demonstrated in quite a few vital ***human investigative studies*** as enumerated under:

1. They show a marked improvement in the frequency of **waste material of defection** (*faeces*); which is most probably caused due to the proper stabilization of the specific ***bifidobacteria*-intensified colon microflora**.
2. The regular ingestion of some quantum of ***oligosaccharides*** (***prebiotics***) does minimize the so-called ***faecal concentration*** **of the following *two* components:**
   - **ammonia (NH_3),** and
   - **degradation products,**

to a significant degree.

3. It has also been suggested emphatically that the **Prebiotics** do help in the *modulation of the immune system* significantly.

NOTE **Precisely, the *probiotics* fail to influence directly the ensuing immune system, but can endorse a possible change in the intestinal microflora which may boost up the prevailing immune system appreciably.**

SYMBIOTIC

Symbiotic usually refers to a product which essentially comprises various divergent microorganisms, namely:

- ***Probiotics,* and**
- ***Prebiotics***.

Since the term refers indirectly to ***synergism***, this term (**symbiotic**) most preferentially be reversed for such ***specialized products*** in which one may vividly observe that the-'***prebiotic*** **chemical entity selectively favours the** ***probiotic*** **compound'**.

Example: A particular product critically comprising both:

- ***Oligofructose*** and
- ***Probiotic Bifidobacteria***,

should elegantly fulfill the aforesaid definition.

[Unfortunately, the alternate combination containing each of an '*oligofructose'* and a '*probiotic*' (*Lactobacillus casei*) stain will **not** fulfill the above definition at all].

NOTE **Nevertheless, one may obviously be in complete agreement for the underlying statement of fact that- 'the synergism is duly accomplished *in vivo* by the critical ingestion of '*lactobacilli'* on the one hand; whereas, the promotion of *indigestion bifidobacteria* on the other hand.**

2. DEVELOPMENT OF *PROBIOTICS*

Matchnikoff (1907)[*] first and foremost theorized that under normal occurrences the prevailing microflora confined to the lower gut (colon) exhibited distinctly an altogether *adverse effect*-invariably termed as '***Autointoxication***'[**].It was subsequently suggested by him that the *ingestion of saccharolytic microorganisms*, such as:

- ***Saccharomyces cerevisiae***[***] and
- ***Saccharomyces allipsoideus***[****],

present in the form of *fermented milk*, was thought to be reversing the underlying effects of the so-called **proteolytic enzymes** (*viz.,* ***Papin, Trypsin, Pepsin, Thrombin, Chymotrypsin***),-which was very much responsible for the **autointoxication**.

NOTE **The earlier belief that microorganisms are harmful has proved to be completely erroneous, since quite a few of them turned out to be the useful ones, amongst which the *lactic bacilli* do enjoy an honourable status.**

Microorganism to Ferment Milk to Yoghurt- It was indeed ***Metchnikoff*** who first of all isolated the microorganisms from the ***fermented milk*****,** and subsequently, used them in the initial feeding trials on the humans. Amazingly, the so-called ***Bulgarian bacillus*** was one of the *isolates*. Though it was not-so-quite certain; however, it was later known as ***Lactobacillus bulgaricus***, and at present termed as ***Lactobacillus delbrueckii*** subsp. *thermophilus*, being utilized to ferment **milk to yoghurt**.

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* Metchnikoff E: **Essais Optimistes**, A Maloine, Paris, 1907.

** **Autointoxication**: A popular belief prevailed between late 1800s and early 1900s that the retained faeces would be reabsorbed by the body thereby causing serious medical complications.

*** That is, **Baker's Yeast.**

**** That is, **Wine Yeast.**

Soonafter the sad demise of the famous scientist **Metchnikoff in 1916,** the specific center of active research shifted ultimately from **Europe** to **North America**. The researchers in the USA reasoned out logically that as actual on-going effect was manifested perceptively in the gut, it could be far better to really make use of an organism that *eventually originated from that site*. Interestingly, *Lactobacillus acidophilus*, a **known bacterium** for **lactic acid'**, was most abundantly isolated from the gut. However, this particular organism was duly explored intensively and extensively in the ***'human feeding trials'***. It yielded wonderful encouraging results to justify its legitimate use in the **treatment of constipation** effectively.

In 1930s-the treatment of infectious diseases was mostly dominated by **chemotherapy** (*i.e.,* ideally-the *chemical should destroy the pathogen* without harming the host).

1940s-the **Pencillins*** were discovered by **Alexander Fleming,** a bactericidal antibiotic that block the final stage of cell wall biosynthesis in bacteria by critically inhibiting *transpeptidase* (an enzyme).

NOTE

Though such an epoch making discovery in the field of '*Medicinal Chemistry*' did intend to throttle and suppress reasonably in the ever-growing interest in '*probiotics*'. Nevertheless, in an indirect manner it instigated our research interest substantially towards the innumerable benefits which could be obtained from the intestinal (gut) microflora.

In late 1940s, it was established beyond any doubt that the ingestion of very low levels of **Antibiotics** along with the growth thereby suggesting that a good number of microorganisms duly present in the gut might probably affect their development adversely, Thus, such a challenging *food-for-thought* revolutionized these ***two* important aspects**, namely:

- **to know precisely the *modus operandi* of such a function,** and
- **to intensify/stimulate research pertaining to the exact composition of the gut microflora.**

In short, it may be added that an array of different species of the general ***Lactobacillus, Streptococcus***, and ***Bifidobacterium*** gained a miraculous entry into the ever-expanding domain of '***probiotic* products'**.

Key Points in Development of *Probiotics*: Following are some of the key points in **development of *probiotics***, namely:

- ***Essential Determinant Factor***- The essential determinant factor for a ***probiotic microorganism*** relates critically to its inherent capability to gain an access, survive, and persist in the specific environment where it is supposed to exhibit its activity.
- ***Resistance to Low pH in Stomach and Bile Acid in Intestine***- The ***probiotics*** invariably comprise of various species of the **lactic-acid microorganisms** and ***bifidobacteria*** which surprisingly do have an in-built ability to the following *two* naturally occurring environments, such as:
  - **a prevalent low pH in stomach,** and
  - **presence of bile acid in the intestinal passage.**

* They refer to a chemical produced by a microorganism or prepared partially or totally by synthetic means that inhibit growth or kills other microbes at low concentration.

- ***Human Origin***- It is highly desirable that the ***probiotics*** must be of human origin; and hence, be able to adhere firmly to the **intestinal epithelium**.
- ***Ability to Affect its Environment***- It has been duly established that a particular ***probiotic microorganism*** may produce any significant effect to its environment unless its ensuing population attains a certain minimum level *i.e.,* it should range between **10^6-10^8 CFU*.g^{-1}** of the entire intestinal content.
- ***Major Sites of Colonization***- The ***two*** **major sites of colonization** in the human body are:
  - **terminal ileum,** and
  - **colon (large intestine),**

where the *intestinal lactobacilli* and *bifidobacteria* occur respectively.

- ***Efficacy of Probiotic Microorganism***- Importantly, the efficacy of *probiotic microorganism* brings forth a considerable mileage in producing a given health effect soonafter adherence and colourization predominantly needs certain additional pre requisite, such as:
  - **critical generation of antimicrobial substances,**
  - **significant *antagonism* against *pathogens*,** and
  - **active competition for preffered adhesion sites.**

NOTE **In a broader perspective, with the privileged help of such mechanisms, the *probiotic*s may be profusely successful in *balancing the intestinal flora* after the disturbance caused by either:**

- ***viral diarrhoea*,** or
- ***associated diarrhoea*,** or
- ***rotavirus diarrhoea*.**

- ***Stimulation of the Immune System***- This is another outcome of the ultimate effect of ingested ***lactic acid bacteria*** and ***bifidobacteria*** invariably observed in the human subjects. Incidentally, this unique **'*host system*'** seems to be adequately increased due to the following ***two*** **episodes** explicitly:
  - **non-specific activation of *phagocytes***,** and
  - **enhanced activity of enhanced defense cells.**

NOTE **Nevertheless, the *immune effect* is not normally seen amongst the healthy human beings; and, therefore, '*probiotics*' may not modify perceptively the prevailing well-balanced immune response.**

- ***Antineoplastic Characteristic Features***- The various investigative studies carried out in '**animal models**' have demonstrated explicitly that the recommended dietary intake of certain specific ***lypholized bifidobacteria*** may reduce the incidence of

* **CFU**: Colony Forming Units.

** **Phagocytes**: It refers to a cell capable of ingesting and destroying **particulate substances** *e.g., Bacteria, Protozoa, Cells,* and *Cellular Debris*.

carcinogenesis by some antigen. Perhaps this could be due to presence of an array of enzyme belonging to the so-called **indigenous microflora** critically inheriting the ability and competence to generate the ***mutagens***[*] right from the dietary chemical entities (compounds) plus the added from the dietary chemical entities (compounds) plus the added advantage of certain particular strains of '***Probiotics***' which may reduce the importance of these enzyme activities finally.

- ***Cholesterol Lowering Effect-*** A few researches conducted in '***probiotics***' have revealed the critical **cholesterol lowering effect** related to milk fermenting microorganisms to a certain extent; however, much work remains to be done to ascertain whether these organisms may reduce serum-cholesterol or not.
- ***Anecdotal Basis of Health Effects-*** In conclusion, it may be added that a good number of health effects of *selected* '***probiotics microrganisms***' have since been established, recogniozed, and accepted unequivocally.

The Way Forward- Based upon the tremendous growth and wonderful breakthroughs in the domain of '***Probiotic* Research'** over the past *two* decades, the most opportune time is not far away when the wide application of ***Probiotics*** in the *general clinical practice* should be a day-to-day affair. According to **Kullen and Klaenhammer (1999)**[**] the advent of the *molecular tools* would preferentially continue to be fully utilized to *understand*, *manipulate*, and *explore* the **lactic acid microorganism** with a view to producing:

- **newer range of vaccines,** and
- **improved *probiotic* products**.

In addition, the host of critical steps involved in the intrinsic development and wider application would be to evolve such products that not only '**safe'** but also '**proven clinically'**- in a *particular formulations* (**marketed product**) accessible to both '*physicians'* and '*consumers'*. It is the need of the hour to indulge in tremendous sincere efforts to focus the prime thrust and attention to the following areas of paramount interest, namely:

- **thorough scientific knowledge of *probiotics*,**
- **determine their underlying mechanisms of action,** and
- **also to specify explicitly when and why they fail in some crucial instances**.

The development of accurate and highly specialized means and ways to optimize the *delivery* as well as the *survival* of various strains at the site of action are needed most rigorously with respect to **different processing advances**, such as:

- **microencapsulation,**
- **microbial coating,** and
- **addition of prebiotic compounds**[***]**.**

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* **Mutagen**: It relates to an agent capable of producing **genetic mutations** in *cells* or in the body.

** **Kullen MJ and Klaenhammer TR**: **Identification of the pH inducible**, **proton-translocating F1FO-ATPase (atpBEFHAGDC) operon of *L. Acidophilus* by differential display gene structure, cloning, and characterization**, *Mol Microbiol*, **33**: 1152-1161, 1999.

*** Normally used as the '**Growth Factors'** by ***probiotic organisms*.**

3. CONCEPT OF *PROBIOTICS*

The **International Guidelines**[*] with respect to *Probiotics in Food* largely specify the actual stringent tests which may be needed to establish the following ***two*** **cardinal aspects**, namely:

- **safety of *probiotics*,** and
- **health claim of a '*probiotic product*' in food.**

NOTE **It is worthwhile to state that such rigorous tests are solely based upon the current understanding of the subject.**

In order to understand comprehensively the '**concept of *probiotics*'** let us examine briefly the following ***six*** **important pragmatic factual considerations,** such as:

- **Fundamental Concepts of Gut Microflora-** First of all let us explore the gravity of the underlying fact that the so-called overall protective effect of the *gut microflora* is beyond question. However, it has been duly investigated by several groups of researchers across the globe; and has been adequately described as stated under:
  - **Bacterial antagonism (Freter, 1956)**[**],
  - **Bacterial interference (Dubos, 1963)**[***],
  - **Barrier effect (Ducluzeau *et al*., 1970)**[****],
  - **Colourization resistance (van der Waall *et al.*, 1971)**[*****],
  - **Competitive exclusion (L Loyd *et al.*, 1977)**[******].
- **Solid Experimental Evidence-** There prevails a *solid experimental evidence* in support of the concept that some components of the **gut microflora** are involved critically in the protection of the host against an array of *infectious human diseases*. Besides, a good number of experimental investigative studies in the following *two* animal species, namely:
  - **Rats (Wilson and Freter, 1986)**[*******], and
  - **Chickens (Mead and Impey, 1987)**[********],

vehemently suggest that a *huge number of strains* are virtually required for providing the **complete protective effect**.

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[*] Reid G: **Regulatory and Clinical Aspects of Dairy Probiotics** (FAA/WHO Expert Consultation Committee, Cordobc, Argentina, 1-4 October, 2001.)

[**] Freter R: **Experimental Enteric *Shigella* and *Vibrio* Infection in Mice and Guniea Pigs,** *J Exp Med.,* **104**, 411-418, 1956.

[***] Dubos RJ: ***Staphylococci*** **and Infection Immunity**, *Amer J Dis Children*, **105**: 643-45, 156.

[****] Ducluzeau *et al*.: *Zbl Bact.Para Infect Hugg Abt Orig.,* **213**: S:533-548, 1970.

[*****] Van der Waall *et al.:* **Colourization Resistance of Mice During Systemic Antibiotic Treatment**, *J Hyg*., **70**:605-609, 1971.

[******] Lloyd AB *et al.: Aust Vet J.,* **53**:82-87, 1977.

[*******] Wilson KH and Freter R: *Infect Immun*., **54**, 354-358, 1986.

[********] Mead GC and Impey CS: In: **Elimination of Pathogenic Organisms from Meat and Poultry**, [Smulders FJM (Ed.)], Elseveir, Amsterdam., pp:57-77, 1987.

- **Protective Effect of Gut Microflora-** In a situation when the animals are carefully reared in the *absolute microflora free environment* they do have a tendency to become more suspective to various diseases; however, the requisite protection may be restored effectively by '*colonizing'* them with a flush of **gut microflora** derived duly from the ***same animals species***. Individually, the same degree of protective effect has also been studie extensively by the oral administration of '**antibiotics'** so as to comprise squarely:
  - **protective effect,** and
  - **receive certain indications of the kinds of microbes being responsible.**

NOTE

Interestingly, the respective studies performed on the human subjects have been found to be absolutely confusing since the ensuing results have more or less incriminated the so-called:
- ***anaerobes**,**
- ***anaerobes* and *aerobes***, and**
- **non-involvement of *anaerobes****.**

- **Treating Results with Caution- Schoeni and Wong (1994)****** were able to reproduce the protection against the organism *Campylobacter jejumi* by injecting chickens with the following three species of microorganisms, namely:
  - *Klebsiella preunomiae,* • *Citrobacter diversus,* and • *Escherichia coli.*

Obviously, one must regard this sort of result with certain element of caution till such time it has been adequately tested against a few other known strains of *C jejuni*. The earlier reported results **(Barrow and Tucker, 1986*********)** revealed vividly that such a *protection* may also be mustered with *three* other strains of *E.coli*; however, further thorough investigative studies did show that it wall all due to the specific effect that was exclusively active against the strain of ***Salmonella typhimurium*** employed duly in the initial trials.

- **Post Antibiotic Diarrhoea**- The organism *Clostridium difficile* is the main culprit for causing **post antibiotic diarrhoea** in the humans.

A well documented report has duly revealed that such a typical condition in a human, suffering from **post antibiotic diarrhoea** is quite amenable to the specific treatment with an **enema******** suitably prepared from the faeces of a healthy human adult*******.

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* van der Waal D *et al*.: **Colonization Resistance of Mice During Systemic Antibiotic Treatment**, *J Hyg.,* **70**:605-609, 1972.

** Wells CL *et al.:* **Role of Intestinal Anaerobic Bacteria in Colonization Resistance**, *Eur J Clin Microbial Infect Dis.,* **7**: 107-113, 1988.

*** Gorbarch SL *et al*.: **Colonization Resistance of the Human Intestinal Microflora Testing the Hypothesis in Normal Volunteers**, *Eur J Clin Microbial Infect Dis.,* **7**: 98-102,1998.

**** Schoeni JL and Wong ACL: *Appl.Environ Microbial*, **60**: 1191-1197, 1994.

***** Barrow PA and Tucker JF: *J.Hyg.,* **96**: 161-169, 1986.

****** **Enema**: It refers to an aqueous preparation normally intended to be instilled into the rectum used to evacuate the lower bowel, or to treat the lower bowel locally, or to supply medication systematically.

******* Eiseman B *et al*.: **Faecal Enema as an Adjunct in the treatment of *Pseudomembranous Enterocolitis,*** *Surgery*, **44**: 854-858, 1958.

- **Gut Microflora Partially Responsible for Resistance to Intestinal Infection-** The statement holds good since it has been adequately established that there are a good number of factors other than the '***antibiotic theory'***, as described earlier, may also affect substantially the so-called composition of the **gut microflora**.

Examples: Following are ***two*** **befitting examples:**

1. Maintenance of very high standards of hygiene that are absolutely a fundamental necessity and must not be relaxed (in any case) may predominantly inhibit the actual transfer of the '*protective microrganisms'* from another to the off spring.
2. In another extreme instance, such as: babies duly delivered by the *caesarian procedure*-transferred immediately into the **incubators**, there is an ample evidence that the ***gut microflora*** may be quite deficient in ***faecal lactobacilli***[*].

NOTE **Therefore, in such cases the prompt administration of a '*multi-strain probiotic'* dosage might cause an improved condition to their resistance to disease probabilities significantly.**

4. SAFETY CONSIDERATIONS IN *PROBIOTICS*

In the recent past the mankind has encountered quite a few dangerous and severe challenges related to a good number of problems intimately associated with the safety of medicaments. '***Probiotics***' do represent such a sensitive and versatile area of interest that needs an equally thorough and rigorous screening including *clinical trials* on humans before they can be marketed as a beneficial food and drug adjuvants/supplements under the close and strict surveillance (*i.e.,* close supervision) of both **FAO** and **WHO**.

In a broader perspective, one may look into the **safety considerations in '*Probiotics*'** under the following two aspects, namely:

- **Antimicrobial Resistance Profiles of *Probiotics*;** and
- **Safety of *Probiotics* in Humans,**

which would be treated individually in the section that follows:

4.1. Antimicrobial Resistance Profiles of '*Probiotics*'

Salminen *et al.* (1998)[**] reported that very much akin to a host of bacteria the specific and *critical* '***antibiotic resistance*** has a wide spread occurrence amongst several **lactic acid microbes,** including the ***probiotic*** **microorganisms**, such as:

- *Lactobacillus reuteri* [ATCC 55730],
- *Lactobacillus plantarum,*
- *Lactobacillus casei,*
- *Lactobacillus bulgaricus,* and
- *Lactobacillus delbreueckii.*

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[*] Hall MA *et al*: **Factors Influencing the Presence of Foecal Lactobacilli in Early Infancy,** *Arch Dis child,* **65:** 185-188, 1990.

[**] Salminen S *et al*.: **Demonstration of Safety of Probiotics- A Review,** *Int J Food Microbiol,* 44 (1-2): 93-106, 1998.

Later on, the above ***antibiotic resistance*** may be intimately related to either:

- ***chromosomal****, or
- ***plasmid located genes****.

Nevertheless, one comes across insufficient information in such typical instances wherein these available **'genetic elements'** may undergo certain degree of *mobilization***.** Therefore, under such uncertain circumstances a crucial situation may suddenly crop up whereby the entire episode may turn into a '***clinical problem'***.

Over the years of extensive and intensive research being carried out on the development of **newer *probiotics***, there prevails a serious concern pertaining to the use of the '***probiotic bacteria***' in various food products which essentially contain- '**particular drug resistance genes'**. It has been observed that such microorganisms that invariably consist of the so-called ***transmissible drug-resistance genes***, **must not be made use of in food products.**

A survey of literature would reveal that as-to-date, no **standard phenotype**** **methods** are known that could be recognized and accepted internationally for both:

- ***Lactobacilli*****,** and
- ***Bifidobacteria*** (*Non-Pathogens*).

Therefore, there exists a dire need for the development of the '**Standardized Assays'** for the exact and precise determination of either:

- **drug insensitivity,** or
- **resistance profiles,**

in both ***Lactobacilli*** and ***Bifidobacteria***.

NOTE

(1) As a point of 'Caution'- while dealing with the critical selection of the '*Probiotic Strains*', it is invariably recommended that the '*Probiotic Microbes*' must not harbor the undesirable *transmissible drug-resistance genes* thereby encoding the critical observed resistance to the clinically used drug substances.

(2) More intensive research is, therefore, required with regard to the antibiotic resistance of both *Lactobacilli* and *Bifidobacteria*; and hence, the actual potential for transmission of the genetic elements to *microbial* or *food borne* microbes.

4.2. Safety of *Probiotics* in Humans

The **FAO/WHO Expert Consultation** endorsed firmly that the *safety of probiotics in humans* should be guided by certain generalized principles and practical criteria that essentially provided such commendable parameters, namely:

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* That is, the physical structure composed of DNA and some proteins, that contains the genes of an organism.

** These refer to the small structural units of the genetic material containing circular, double stranded DNA that transmit genetic information from one bacterial cell to another *e.g.,* **R-factors in bacteria.**

*** **Phenotype**: The **phenotype** of an organism refers to the entire *physical, biochemical*, and *physiological* make up determined both *genetically* and *environmentally*.

**** Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria, 1-4, October, 2001.

- **definite guidelines**- to test methodically the newly developed *potential probiotic microbes*; to justify as to how one may have a low risk for inducing or indulging with the etiology of disease; and
- **health benefit**- to confer an appreciable advantages when administered to humans.

NOTE **Importantly, such elaborated guidelines must also recognize that certain *probiotic microbial species* may need definitely a more intensive and rigorous assessment than others.**

In short, one may add that the meticulous evaluation of a ***safety profile*** would certainly need at least quite a few exhaustive studies carried out duly in humans; and hence, must intimately deal with various critical aspects for the intended end-use of the desired ***probiotic* strain**.

Between 1993 and 2001 *i.e.*, a short span of less than a decade *four* important sequential break throughs occurred, namely:

- **Aguirre and Collins (1993)*-** reported the total absence of either *pathogenic* or *virulence*** characteristic features have been vividly identified particularly in:
  - ***Lactobacilli*** • ***Bifidobacteria*** or • ***Lactococci*.**
- **Saxelin *et al*.(1996)***, Naidu *et al.* (1996)****-**ascertained that the '***lactobacilli*'**, with a pretty long history of usage as: '***probiotics*'** in humans without any adverse effect, stands as the best evidence or proof of their safety status.

Nevertheless, the **FAO/WHO Consultation Committee** generously acknowledge that under the preview of some parameters, certain ***Lactobacilli strains*** do have a close link with '*adverse effects'*, for instance: occasional incidence of '***bacteremia*'**.

- **Salminen *et al*. (2001)*****-came up with a recent **epidemiological investigative study** with respect to the *case reports* of the ***Lactobacilli bacteremia*** collected systematically in one specific country that exhibited explicitly which ultimately affirmed the following *two* findings without an iota of doubt:
  - **no enhanced incidence of *bacteremia*, and**
  - **no increased frequency of *bacteremia*,**

even with a generous use of the ***Probiotic lactobacilli***.

Important Point: It has also been strongly suggested that certain members of the **lactic acid microorganisms**, for instance: ***Enterococci***- may also exhibit *virulence properties* to a great

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* Aguirre M and Collins MD: **Lactic Acid Bacteria and Humans Clinical Infection,** *J Appl Bacteriol*, **75**:95-107, 1993.

** **Virulence**: It relates to the ability of a microorganism to invade tissues and produce symptoms of disease or death.

*** Saxelin M *et al*." **The Safety of Commercial Products with Viable Lactobacillus Strains,** *In fact Dis Clin Back,* **5(5):** 331-335, 1996.

**** Naidu As *et al,*: **Protriotic Spectra of Lactic Acid Bacteria (LAB), Crit Rews Food Sci & Nutr, 39: 13-126, 1996.**

***** Salminen S *et al.*: **Inereasing Consumption of Lactobacillus GG as a Probiotic and the Incidence of Lctobacilli bacteremi in Finland,** *Clin Microbiol Infect,* **7(1):** 802, 2001.

extent. In the light of the aforesaid factual evidences and findings the **FAO/WHO- Expert Consultation Committee** strongly recommends that:

"*Enterococcus* must not be referred to a '***Probiotic***' for use in the humans particularly".

Thus, the above **rationale** could be based upon the following *two* critical aspects,

1. In fact, certain typical '**strains**' could exhibit clearly a high degree of resistance to '***Vancomycin***' (*i.e., an inhibitor of bacterial cell wall synthesis*)*, or also may acquire a similar kind of resistance. In case, such a resistance appears to be prevalent, transference to other microbes could take place, thereby increasing perceptively the *pathogensis of such recipitents significantly***.
2. Besides, there exist some strains of ***Vancomycin*** resistant *Enterecocci* that are found to be intimately associated with ***nosocomical infections***[***] in the hospital environment particularly[****].

In conclusion it may be added that the **FAO/WHO Expert Consultation Committee** does recognize that certain specific strains of ***Enterococcus*** do exhibit '*probiotic*' characteristic features prominently. Thus, they may not critically show the very presence of the so-called ***Vancomycin Resistance*** just at the point of inclusion. Nevertheless, it all solely depends upon the responsibility of the actual producer to ascertain and prove that any '*given strain*' may not either:

- **acquire/transfer the inherent *vancomycin resistance*,** or
- ***become virulent* and *induce infection*.**

5. GENETIC ENGINEERING

Preamble

Evidence from literature reveals that the humans have been duly engaged in the critical production of new or improved strains of *domestic animals*, *food*, *plants*, and *microorganisms* so as to improve upon the characteristic features in an appreciable manner, for instance:

- **Appearance** • **Disease Resistance**
- **Fermentation Characteristics**
- **Processing Attributes and** • **Yield,**

stretched over a couple of centuries.

- **Domestic Animals**- In this instance, the traditional breeding is usually carried out by mating a male and a female in hopes that the offspring might possess the desired characteristic features.

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* Eaton TI and Gasson MJ: *Appl. Environ Microbiol*, 67: 1628-1635, 2001; Lund B and and Edlund C: *Clin Infee Dis*., **9**: 1384-1385, 2001.

** Noble We *et al*.: *Microbiol Lett*, **72**:195-8, 1992; Leclerq R and Courvalin P: *Clin Infect Dis.,* **24:** 545-54, 1997.

*** **Nosocomical Infections**: That is, infections acquired during **hospitalization**.

**** Leclerq R and Courvalin P: **Resistance to Glycopeptide in Enterococci,** *Clin Infect Dis,* **24**: 545-554, 1997; Woodford N *et al*.: **Current Perspectives on Glycopeptide Resistance,** *Clin Microbiol Rev,* **8**: 585-615, 1995.

- **Plants and Microorganisms-** In this specific case, the so-called multiplication phenomenon is invariably accomplished by means of the direct ***mutation of the genes.*** Obviously, the **genes** do contain practically all the inheritable characteristic features (or *traits*) of the **living organisms**. Therefore, in true sense, such breeding is involved in **selecting** and *directing* the ensuing genetic makeup of the three aforesaid species: ***animals, plants*****,** and ***microorganisms***.

Problem Encountered in Conventional Breeding and Mutation- The host of problems associated with the *conventional method* and *mutation of genes* to serve as the most appropriate methods of selecting for certain **desirable traits** do invariably fall into one of the *three* following categories:

- **not always predictable,**
- **not mostly successful,** and
- **usually time-consuming.**

Besides, in the *conventional breeding process* there exists the remote chances to **cross the species barrier,** for instance: it may not be feasible to induce the ability to produce a higher yield of **Vitamin C (*ascorbic acid*)** being transferred to *apples* (rather than the *oranges*).

GENETIC ENGINEERING

In the recent past, a tremendous discovery of newer techniques have taken place so as to afford a more direct approach in manipulating the prevailing '**genetic properties of organisms'** that are usually referred to as **genetic engineering**, which have been developed duly based upon commendable major breakthroughs in '**molecular biology'**. Thus, obviously the **genetic engineering** *via* the use of such unique *and versatile sophisticated methodologies* as:

- **Recombinant DNA Techniques,**
- **Cell Hybridization,**
- **Protoplast Fusion,** and
- **Miscellaneous Methods.**

Recombinant DNA Techniques: The **Recombinant DNA** relates to the genetic material that has been *cleaned enzymatically at specific sites* and *recombined** meticulously after insertion of a segment of DNA, invariably from another species. It has been used successfully in the development of certain extremely useful pharmaceuticals products, such as:

- **Growth Hormone** • **Insulin** • **Interferon and** • **Vaccines.**

Cell Hybridization: In genetics, the offspring of parents that are of different varities of species. Thus, an altogether **new DNA** comes into being from the insertion of a *foreign segment* (*i.e.,* from another species) of DNA into the genome of an organism by **recombinant DNA methods**.

Protoplast Fusion: The **protoplast fusion** relates to a method by which the recombinants may be duly accomplished in bacteria wherein the transformation with DNA is not normally feasible, but is now possible.

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* **Recombination:** It refers to a process in which **DNA molecules are broken and rejoined in altogether new combinations.**

Example- In ***B. megaterium***, the *recombinants* have been duly obtained by the specialized technique of **protoplast fusion**. Thus, in this particular process, two genetically distinct strains are ***mixed*** and ***protoplasted****[*] together with the use of ***lysozyme,***[***] and finally/finally fused in the presence of **polyethylene glycol (PEG).** The **fused protoplasts** are subsequently allowed to grow inside the vegetative cells. In this way the recombination takes place very much within the *transient diploid protoplasts* which is invariably followed by both **segregation** and **haploidization**. The observed frequency of obtaining **recombinants** by this method in ***B. megaterium*** ranges between **10^{-7} to 10^{-8} per *bacterium*.** Hence, this proves to be a relatively more useful method to bring forth the **recombination** amongst microorganisms wherein other known mechanisms with respect to *genetic exchange* are not yet known.

Miscellaneous Methods: The advent of major advances in the field of **Molecular Biology**, several viable techniques have emerged for indulging in direct manipulation of genetic *characteristic profiles* of microorganisms (**Genetic Engineering**). As-to-date, there are other methods that may be used successfully to:

- **remove genes from cells of one organism,**
- **reinsert them into the cells of organism,** and
- **programme them specifically to do certain desired functions.**

Methods: These essentially include:

1. Most of these methods are almost identical whereby they are able to identify critically:
   - ***specific genes*** **solely responsible for the intended characteristic features (traits) in *one specific or* types of organisms; and**
   - **subsequently transfer these *specific genes* to an altogether *different organism*.**
2. In this manner, the *recipient organism* gains these traits.

Examples: (1) **Insulin*****-** the protein duly produced in the human *pancreatic cells*, does play the most critical, role of controlling the normal blood-sugar in a healthy person. Hence, people having '**pancrea**s' that does not make *insulin* do suffer from **diabetes** and should take insulin regularly.

Explanation- Thus, the *genes from human cells*, which eventually tell the *pancreas to prepare insulin*, have been duly transferred to the respective microorganisms. Now, the microorganisms comprising the *genes for insulin* are freely competent to **produce insulin in culture**. Ultimately, this particular **insulin** (*yielded in culture*) is *collected, purified*, and *used* to treat diabetics.

(2) ***Improving Yields of Traditional Fermentation Products***- In the recent past these *newer techniques* do find their optimized utility with particular reference to:

- **improved yields of *traditional fermentation products* (*e.g.,* beverages and food products);**

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* That is, a substance being formed as the **living contents of a cell.**

** An enzyme found in phagocytes, neutrophils and macrophages that eventually destroys microorganisms by cleavage of their walls.

*** **Insulin**: **A peptide hormone product** obtained from porcine pancreas and on being administered to 'diabeties' to lower the blood-sugar level.

- **logical conversion of *underutilized raw materials into useful substrates*;**
- **production of *improved, modified, and newer enzymes*;**
- **newly improvised *food activities e.g.*, gums, sweetners, and flavouring agents; and**
- **improved performance of cultures under the influence of cost-effective processing parameters.**

Examples: A few **classical examples** are as stated under:

(i) The development of the *virus-resistant strains of certain very important fermentation microorganisms.*

(ii) The selection and identification of such *microorganisms* that critically give rise to the production of certain highly *specific enzymes* required to make *food products*, such as: **cheese**.

(3) **Improved Yeast Strains for Brewing Industry-** The ***brewing industry*** invariably embraces the following categories, namely:

- **Production of Malt Spirit,**
- **Production of Beer and Allied Beverages, and**
- **Production of Wine (*viz.*, Red Wine, White Wine).**

Importantly, the aforesaid divergent **brewing industries** do make use of the phenomenon of ***cell hybridization*** (see '***Genetic Engineering***' under Section 5) to produce much '**improved yeast strains**'.

Fig: 1.1 depicts explicitly the production of improved yeast by means of the **spheroplast fusion**.

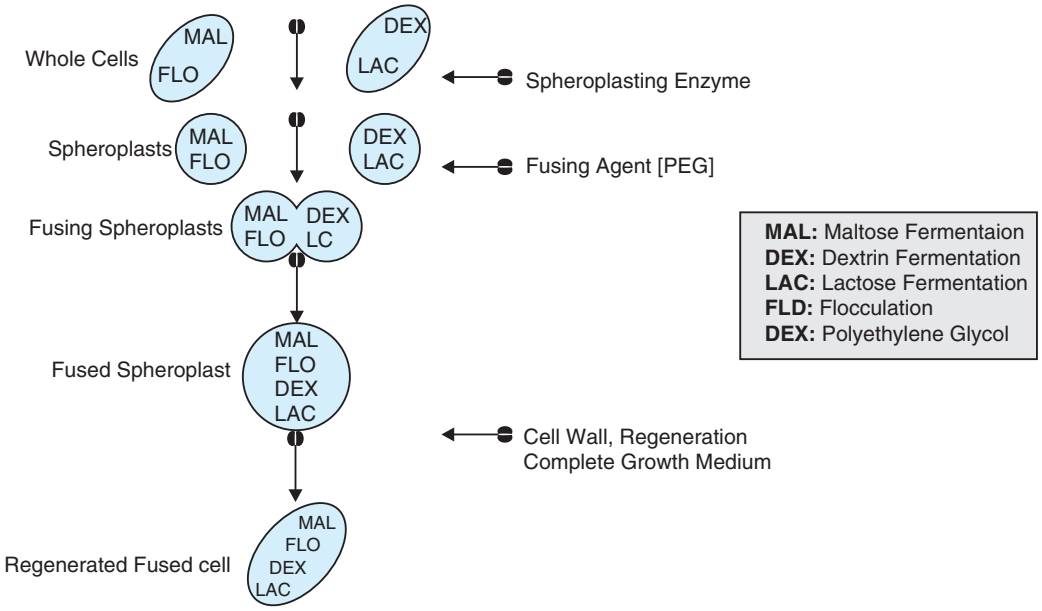

Fig. 1.1: Outline for the Production of Improved Yeast by the Spheroplast Fusion.

Explanation: The various steps involved in the production of an '**improved yeast version'** by the aid of *spheroplast fusion* are as given under:

1. The initial process critically undertakes the removal of the cell walls from ***two*** **yeast strains** having essential desirable attributes.
2. The earlier step promotes diligently the interchange of the **genetic component** *via* **fusion** of their respective *spheroplasts* that eventually provides an *appropriate medium* and *needful conditions* for the meticulous **cell-wall generation.**
3. The '***new yeast'*** thus formed, that being capable of both **division** and **replication phenomena**, may boost up the on-going fermentation of a variety of substances: ***maltose, dextrin***, and ***lactose***.
4. The enhanced fermentation process entails a much wider scope and range of the **utilizable sybstrates** than either of the ***two*** **starting strains** obviously.
5. In addition, the characteristic features of '***flocculation***'; does help in the ultimate removal of the yeast from the respective ***fermentation wort (broth),*** thereby rendering a much better clarification (transperancy) of the *fermented beverages e.g.,* **Beer**; and also more effective reuse of the ***residual yeast***.

NOTE

Some more glaring meritorious characteristics of the *hybridized yeast* as a consequence from the initial priority '*strain selection'* essentially comprise:

- **superb *alcohol tolerance*,**
- **excellent yield of certain *desirable flavour* chemical entities (compounds), and**
- **commendable *genetic stability*.**

Genetic Engineering in Agricultural Sciences: In the last couple of decades **Genetic Engineering** has already made its well- recognized, widely accepted, and more productive advantages in the most versatile field of ***Agricultural Sciences***. Interestingly, the genes derived from **microorganisms** that may kill some typical insects but are found to be **absolutely harmless to mankind** (*humans*) have been transferred into the plants meticulously. The resulting plants subsequently produce the respective '***protein'*** which eventually turns out to be *toxic to the insect*, so that as and when the inset feeds on the plant, it becomes fatal for the insect. It is, however, important to state here that these ***genetically modified proteins*** do have no adverse effect upon the humans since they are promptly rendered; **'inactive'** in the acidic medium in the human stomach.

NOTE

This particular aspect of '*genetic engineering'* may pave the way in a big way to cause a drastic reduction in the use of a vast number of '*synthetic pesticides (insecticides)*' which are not only *detrimental* but also toxic to the humans.

6. VALIDATION OF THE *PROBIOTIC* CONCEPT

6.1. Introduction

Historical Event- It was an epoch-making historical event when the **Nobel Laureate Elie Mechnikoff**[*] duly formulated and pronounced the marvelous ***Probiotic Concept*** almost a century ago. It was indeed he, who for the first time vehemently proposed the following *three* eventful and thoughtful dictums to the scientific world in the interest against disease in **humans** and **animals**, namely:

- **regular consumption of certain *'lactic bacilli'* would enhance one's health profile;**
- **good health by optimizing health- promoting activity profiles of *GI-microbiota*; and**
- **minimizing appreciably their potential harmful-effects.**

Based on an array of statements and counter-statements ethical and antiethical theories, and arguments and retaliatory' arguments put forward by various researchers ultimately enthused **Hentges (1992)**[**] to state that- "**In the restoration of colonization resistance in the *gastrointestinal (GI) trac*t, it is quite improbable that *probiotic* measures designed to alter the ecology of the intestinal luximal contents will be successful".**

Tannock (1992)[***] suggested strongly that an '***Ideal Probiotic***' must possess the following important characteristic features, for instance:

(i) The '***Probiotic***' must continue to stay for a longer duration inside the *GI-tract* and remain active to produce such substances which specifically check and prevent the so-called ***gastrointestinal pathogens*** or **stimulate immunity** in order to enhance the host's in-built resistance to the ***intestinal infections reasonably*.**

(ii) Besides, the '***Probiotics***' must contribute effectively to the host's nutrition profile via the critical *synthesis of the essential nutrients* and/or inquisitive *digestion of dietary components* (*viz.,* ***lactose***) which the host may not be well-equipped (or competent enough) *physiologically* to make use of squarely.

(iii) The '***Probiotic***' must be willing to cooperate fully in the large-scale production of the same; and also duly maintain a perfect harmony towards its ***safety*** and ***original characteristics*** thereby withstanding the goodness of the host' health.

(iv) In general, the newly developed '***Probiotic***' should maintain and sustain ***uncompromised stability profile*** in all the above mentioned characteristic features from (i) through (iii).

Comment: In short, **Tannock (1992)** suggested an alternative cleverly chalked-out-plan would be to lay hand on such a *highly specific strain* by the help of '**genetic manipulation'** rather than an exploratory, futile attempt to isolate such an '***ideal probiotic strain'***.

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* Metchnikoff E: *Essais Optimistes,* A Malonie, Paris, 1907.

** Henteges DH: **Gut Flora and Disease Resistance**: In: Fuller R [Ed.]: *Probiotics: The* Scientific Basis, Chapman and Hall, London (UK), pp: 87-110, 1992.

*** Tannock GW: **Genetic Manipulation of Gut Microorganisms**. In: Fuller R [Ed.] *Probiotics the Scientific Basis*, Champan and Hall, London (UK), 1992.

6.2. SCIENCE OF *PROBIOTICS*: DEVELOPMENT EPISODES

Fuller (1997)* and **Salminen *et al.*: (1998)**** observed critically that their general understanding and overall expectations with, respect to the scientific as well as practical aspects of '***probiotics***' have really undergone a sea change. Under such crucial circumstances perhaps there remains a single consensus to gather facts bit by bit from these related scientific findings and evidences, that the main thrust of '***Probiotics***' has virtually entered magnanimously into the so-called '**Fourth Stage of Development'**.

NOTE **The *first three stages* of development are as stated under:**

- ***First Stage-*** beginning of the 20th century with the revelation of *Metchnikoff's Formulation* pertaining to the *probiotic* concept and prescience of a functional role of diets in the normal human health.
- ***Second Stage-*** between 1920s and 1930s, entrusted upon the findings of Rettger *et al.* (1935)***, who carried out systematic investigative studies and started human clinical trials whereby the intestinal isolates of *L.acidophilus* were tested instead of the '*Bulgarian bacillus*' yoghurt strains- that had earlier demonstrated explicitly to prove fatal while passing via the intestinal canal.
- ***Third Stage-*** in 1950s (soonafter World War II), a resurgent interest specifically in the *gastrointestinal (GI) microbiology* gained a remarkable entry into the 3rd stage of development with great zeal and fervour.

Importantly, ***probiotic* research** designates a sub-discipline invariably termed as ***Gastrointestinal Microbiology***, whose aims and objectives are included in its definition. Nevertheless, **Havenaar and Veld (1992)**** defined the '***probiotics***' as:

"a single-or mixed culture of live microorganisms that, applied to animal or man, beneficially affect the respective host by critically modifying the properties of the indigenous *GI-microbiota*".

Additionally, the above definition does not hold good to all products, but particularly restricted to such products only as stated under:

- Containing '*live microrganisms'* (*viz.,* freeze-dried cells, fresh product, or a fermented product);
- Improving perceptively the health and well-being of humans or animals (including the critical growth profile in animals); and
- May exert their inherent effect on all host mucosal surfaces comprising the *mouth* and *GI-tract* (*viz.,* used in capsule, tablet, food form); *the upper respiratory tract (URT)* (*viz.,* employed as an '*aerosol*'); and the *urogenital tract (UGT)* (*viz.,* as a local application in the form of '*gel'* or '*cream'*).

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* Fuller R [Ed.]: **Probiotics 2: Applications and Practical Aspects,** Chapman and Hall, London (UK), 1997.

** Salminen S and von Wright A [Eds.]: **Lactic Acid Bacteria**, Marcell Dekker, New York, 1998.

*** Rettger L F *et al.*: ***Lactobacillus acidophilus* and its Therapeutic Applications**, New Haven (CT), Yale University Press, 1935.

**** Havenaar R and Veld JHJ: **Probiotics: A General View**: In: Wood BJB [Ed.]: **The Lactic Acid Bacteria in Health and Disease,** Elsevier Applied. Science, New York, pp: 209-224, 1992.

6.3. Lactobacillus Reuteri: AN OBSCURE SPECIES TO A *PROBIOTIC* PROTOTYPE

Based on the scientific evidences until quite recently, ***Reuteri*** prevailed as an obscure species to a ***probiotic prototype*****,** grossly misconstrued as *Lactobacillus fermentum*, which was eventually brought to notice in 1970s. However, the wonderful revaluation by **Kandler *et al.* (1980)*** that the '*lactobacilli*' identified earlier by several researchers in 1965** as, ***Lactobacillus fermentum Biotype II***, were indeed explicitly distinguishable from other respective **'biotypes of this particular species'**- depending solely upon such specific aspects as:

- **many phenotype*** properties, and**
- **genetic characteristic features.**

As a mark of respect and honour, they proposed unanimously that ***Lactobacillus fermentum*** **Biotype II** be given the '*distinct species status'* as the ***Lactobacillus reuteri*** (*i.e.,* named after **Gerhard Reuter**); and also that the ***Reuteri*** **Strain DSM 20016** (duly isolated from **humans**) be designated the **Type Strain**.

NOTE **The aforesaid proposal was accepted equivocally; and since 1980 '*Reuteri'* has been duly classified as an altogether distinct species in the genus *Lactobacillus*.**

Distinguishable Points of *Lactobacillus reuteri*- Since the *Lactobacillus reuteri* could not be distinguished definitely from ***Lactobacillus fermentum*** by the aid of simple **physiological tests** exclusively; therefore, one may have to look for some more authentic, genuine, and reliable distinguishable points as given under:

1. **Determinations of either of these criteria:**
   - **mole % G+C,** or
   - **diamino acid of the *peptidoglycan*, or**
   - **electrophoretic mobility of LDH**** **do separate explicitly the *two* above species.**
2. The ***Reuteri Cells*** are found to be slightly irregular, bent rods with rounded ends, invariably of **0.7-1.0 × 2.0-3.0µm (micrometer) size.**
3. **Reuteri cells** mostly occur singly, in pairs, and also in small clusters having normally a good health profile at **45°C** and yield ammonia gas (NH_3) from the amino acid ***argimine***********.

NOTE **Axelsson (1993)********** has provided a comprehensive account wherein he has reviewed '*newer techniques'* readily available for both identification and classification of *Reuteri*; and an array of other allied species in the complex as well as commercially vital and important genus.**

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* Kandler O *et al.*: ***Lactobacillus Reuterei*** **sp.nov. A New Species of Hetero-fermentative Lactobacilli,** *Zbl Bkt Hyg Abt Orig***,** C1: 264-9, 1980.

** Reuter G: *Zbl Bak Parasit Infeg Hyg I Orig.,* 197S: 468-487, 1965; Lerche et al.: *Zbl Bak Prasit Infact Hyg I Orig.,* 185S: 446-481, 1965.

*** **Phenotype:** The expression of the genes present in an individual microorganism.

**** **LDH**: ***Lactate Dehydrogenase*****.**

***** Kendler O and Weiss N: **Regular Nonspring Gram Positive Rods:** In: Sneath DHA *et al*. (Eds): *Bergeys Manual of Systematic Bacteriology, Vol.2,* Willams and Wilkins, New York, pp: 1208-34, 1986.

****** Axelsson L: **Lactic Acid Bacteria: Classification and Physiology:** In: Salminen S and von Wright A [Eds]: *Lactic Acid Bacteria*, Marcel Dekker, New York, pp: 1-63, 1993.

6.4. *REUTERI* PHYSIOLOGY

The bacteria of the genus *Lactobacillus* (common to milk), fall into one of the ***three*** **recognized groups** that is actually based upon the particular type of ***metabolic pathways*** invariably used to carry out the fermentation of '***carbohydrates'***.

(a) Obligatively Homofermentative Group- Following are three classical examples of bacteria belonging to the genus *lactobacillus*:

- ***L.acidophilus*** • ***L.helveticus*** and • ***L.salivarius,***

that essentially possess a typical ***fructose diphosphate (FDP) aldolase pathway*** thereby directing a *glycolytic conversion of sugars primarily into lactic acid*.

The Homofermentative Organisms* - designates the strains of *lactic acid producing* bacteria invariably yield maximum quantum of **lactic acid** and only trace amounts of other products. In fact, these organisms utilize the *specific metabolic pathway* to produce **pyruvic acid**. It has been duly observed that the overall percent conversion of hexose-sugar to **lactic acid** is nearly equivalent to *two* moles of lactic acid for every mole of hexose-sugar (*theoretical yield*) consumed by the respective organism.

Importantly, there are some other **potential** ***homofermentative organisms*** *e.g.,* ***L.casei, L.leichmanni,*** and ***S.Lactis*** –all of them do possess vital industrial importance and recognition only.

(b) Facultatively Hetero fermentative Group- These essentially comprise ***five*** **typical examples** of bacteria, namely:

- ***L.casei***
- ***L.curvatus***
- ***L.plantarum***
- ***L.sake*** and
- ***L.rhamnosus***

-that could either make use of **FDP aldolase pathway** to ferment certain sugars or they may reduce the ***phosphoketolase pathway*** in order to ferment other sugars present.

Besides, these organisms invariably yield some amount of **lactic acid**; however, due to the ***Pentose-phosphate Metabolic Pathway*** they may give rise to the production of an array of chemical substances *e.g., acetic acid (vinegar), ethanol, carbon dioxide (CO_2),* and *traces of a few other products.*

(c) Obligate Heterofermentative Group- These mostly consist of ***four*** **classical examples of bacteria,** for instance:

- ***L.brevis***
- ***L.buchneri***
- ***L.fermentum*** and
- ***L.reuteri,***

- that has exclusively the ***phosphoketolase*****-based option**.

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* Sambarmurthy K and Kar A: **Pharmaceutical Biotechnology**, 2nd ed., New Age International Publishers, New Delhi, 2016.

6.5. *PROBIOTICS*: FUNDAMENTAL REQUIREMENTS

It is, however, pertinent to state here that prior to reviewing certain investigative studies designed to determine whether ***Reuteri*** does possess the so-called '***probiotic efficacy***' or not, one may have to examine critically the following *two* aspects:

- The criteria that a '***probiotic***' must fulfil in order to be considered both *efficacious* and *safe* with regard to **human and/or animal utility.**
- The various means and ways to adjudge whether the '***Probiotics***' prove to be efficacious even in the presence of a host of ***intrinsic* and *extrinsic* factors** which invariably influence such efforts to measure their usefulness as '***Probiotics***'.

Criteria for Ascertaining '*Probiotics*' Efficacy- Various researchers have recommended vehemently that all futuristic research on '***Probiotics***' should be mostly governed by the same underlying principles pertaining to the *critical appraisal* that falls very much within the scope of other **scientific disciplines**. These ***Probiotic* Studies** may essentially include complete details with respect to the following aspects:

(a) **Culture Identification**[*]: In fact, all *isolated cultures* must be suitably characterized based properly on state-of-the-art classification methods comprising the ***molecular taxonomy*** meticulously:

- **identification of culture source(s);**
- **definition of their nutritional** and **physiological characters**;
- **determination of their genetic stability and relevant activity profiles**; and
- **preferentially the *probiotic* cultures** be ***host-specific* (but need not be *mandatory*).**

(b) **Information on *Colonization***: An in-depth knowledge pertaining to the ecology of the ***Gastrointestinal (GI)* *Tract*** must always be made so as to assess authentically the *inherent ability* of each ***probiotic* culture** individually to:

- **survive in the GI-tract passage;** and
- **help the colonization phenomenon in specific regions.**

(c) **Efficacy Determination *via* Experimental Design**[**]: It is an absolute necessity to design each experiment meticulously with special attention to such essential conditionalities as:

- **adequate control measures;**
- **utilization of reliable scientific techniques;** and
- **authentication that all results be evaluated statistically and interpreted logistically.**

(d) **Field Testing**: Obviously, a fruitful laboratory result may in either way indicate the specific ability of a '***Probiotic***' to exhibit positive results in the '*realistic world*'. However, the actual and authentic efficacy of a '***Probiotic***' could only be ascertained till such time it has been duly subjected to either:

- **exhaustive '*clinical trials*' with humans;** or
- **appropriate '*field tests*' with animals.**

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* Hvensaar R and Veld JHJ: ***Probiotics*: A General View, In: Wood BJB [Ed.]** *The /1992 Lactic Acid Bacteria in Health and Disease,* Elsevier Applied Science, New York, pp: 2/9- 221, A

** Tannock GW: **Genetic Manipulation of Gut Microrganisms,** In: Fuller R [Ed.]: *Probiotics: The Scientific Basis*, Chapman and Hall, London (UK), 1992.

(e) **Declaration of Results in Scientific Journals**[*]: Exclusive inferences obtained from *authentic scientific investigative studies* must be given a serious and due consideration. Besides, such elaborated studies need to be critiqued stringently and only then reported in peer reviewed publications respectively.

(f) **Feasibility and Validity to Large Scale Production:** after all the above cited criteria have been successfully fulfilled, the ear-marked '***Probiotic Strain***' should by all means be feasible to a *large-scale production* maintaining the *Validity-profile* right up to the state of its application.

NOTE

Thus, *Reuteri* has been proved to fulfil all the above requirements from (a) through (f). In addition, an array of '*probiotic strains*' have been duly isolated from a variety of host species which were characterized carefully and evaluated for their colonizing ability profiles. Thus, an avalanche of '*Probiotic Products*' have been formulated- and tested under highly supervised laboratory experimental parameters, with the genuine '*real-world*' field trials have been performed.

6.6. *REUTERI*: AN EFFICACIOUS AND SAFE COLONIZER OF HUMANS AND ANIMALS

Keeping in mind the outcome of various results based on the above mentioned exhaustive studies, one might conclude with great certainty and fervour that the so-called ***host-specific strains of Reuteri*** against the glaring detrimental effects of certain: **chemical, microbiological**, and **physical *stressors***. The results of a good number of ***Clinical Trials*** conducted meticulously by employing a ***human-strain of Reuteri***, duly isolated from the **breast milk of a young healthy mother**, have been recorded upon *three* categories of human subjects, namely:

- **Children as Subjects,**
- **Healthy Adults as Subjects,** and
- **HIV-Positive Adults as Subjects.**

The various results thus obtained are given as under:

Children as Subjects- It was duly concluded that the intelligently produced '***Probiotic Blend***' was tolerated without any sort of adverse reaction. In addition, the *colonization process* came into being based on the presence of relatively **higher** came into being based on the presence of relatively **higher numbers of *Reuteri* found in the faces of the treated children.**

Furthermore, the clinical trials conducted on ***four*** **different groups** elegantly displayed no disparity in the statistical results.

Healthy Adults as Subjects- It has been established that ***Reuteri*** could be fed at a dose of **10^{11} cfu per day** (*cfu* – means colony forming units) without producing any observed:

- **adverse effects in clinical safety profile;** and/or
- **tolerance problem issues.**

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* Barrow PA: **Probiotics for Chickens,** In: Fuller R [Ed.]: *Probiotics: The Scientific Basis,* Chapman and Hall, London (UK), pp: 225-257, 1992.

Importantly, the aforesaid dose level (*i.e.,* **10^{11} cfu per day**) did result in adequate colonization significantly very much within a span of *7 days from consumption*, which was duly maintained for a period of at least *7 days post consumption*, thereby suggesting strongly the indulgence of ***good colonization***.

NOTE **Nevertheless, the *colonization* phenomenon as judged by critical faecal examination revealed that it was almost vanished within a duration of *60 days* after cessation of consumption of *Reuteri*.**

HIV-Positive Adults as Subjects- In general, the investigative studies overwhelmingly indicated that ***Reuteri*** could be administered at a dose of **10^{10} cfu per day** without causing either any kind of *tolerance problems* or *clinically significant safety profile*. Besides, this study registered a rather **low-level of *faecal lactobacilli*** in the ***HIV-positive screened subjects***.

NOTE **The actual and intrinsic reason(s) for the above vital observations have not yet been deciphered; however, it may, in future, offer us with some helpful clue to further our understanding of HIV.**

6.7. *REUTERI PROBIOTICS*: EFFECT ON HUMAN HEALTH

The broad-based investigative researches conducted meticulously on ***Reuteri Probitics*** and its subsequent effect on the overall human health showed enough *clinical evidences* revealing that ***Reuteri*** is significantly effective as a potential therapeutic agent essentially capable of *moderating, controlling*, and *arresting* the severe '***Rotavirus Diarrhoea'*** amongst children. A follow up study mustered sufficient clinical evidences to affirm that it also has marked ***prophylactic effect***.

Example*:** The regular consumption of a *palatable beverage* comprising a unique blend of some selected ***Probiotic Cultures, *e.g.,* ***Reuteri*, *L.acidophilus***, and ***Bifidobacterium animals***, minimized appreciably the overall risk of *young children* ***developing frequent diarrhoea***.

Later on, the effect of ***Reuteri Probiosis*** on *human health* was further extended to the following *two* aspects, namely:

- ***Therapeutic Efficacy* for Rotavirus-Induced Diarrhoea in Children**, and
- ***Prophylactic Efficacy* for Community-Acquired Diarrhoea in Children**,

which shall now be discussed briefly as under:

(a) *Therapeutic Efficacy* for Rotavirus-Induced Diarrhoea in Children:

The **diarrhoea diseases** are often found as one of the most common health-related problems associated during childhood across the globe. **Tazume *et al.* (1993)*** and **Salminen *et al.* (1995)**** observed critically that in the course of an *acute bout of diarrhoea*, one may observe a radical change in the **normal of *GI-microbiota profile*** including the drastic reduction in such microbial species as:

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* Tanzume S *et al.: Clin Infect Dis*, **16**: 77S-82S, 1993.

** Salminen S *et al.*: *Chemotherapy*, **41**: 5-15, 1995.

- ***Lactobacillus***
- ***Bifidobacterium*** and
- ***Bacteroides***.

Besides, ***L.rhamnosus* GG** does help to augment a *quick clinical recovery* from *rotavirus gastroenteritis* in children and **increase intestinal immune responses significantly** (Boundra *et al*. 1990)*.

(b) *Prophylactic Efficacy* for Community-Acquired Diarrhoea in Children: The survey of literature has revealed that several elaborated **Clinical Studies** affirmed that there are certain '***Probiotics*'** which are definitely found to be:

- **adequately safe,** and
- **reasonably effective,**

in the so-called effective biotherapeutic treatments for the **Childhood Diarrhoea**.

Ruiz Palacios *et al.* (1999)** carried out meticulously performed ***two*** **blind-folded-controlled investigative studies** simultaneously so as to determine precisely the overall effect of '***Reuteri*'** in the specific prevention of '***Community-Acquired Diarrhoea in Children'*** (between *12-36 months*) in Mexico:

First Study- In all 243 children (1-3 Yr) were duly given for **14 weeks** either a '***placebo'*** or a '***probiotics mixture'*** consisting of ***Reuteri***.

Observation- The number of children **fed with Reuteri**, were found to be definitely more, having no incidence of *diarrhoea* during the **14 weeks trial** than with those ***placebo*-fed [90/119 Vs 77/120]** respectively.

Second Study- In this instance, ***three*** **individual groups** with a total of *319 children* were studied for a period of **16 weeks**:

Group-I [*Placebo*]- It duly received 2 × 120 mL servings of whole milk daily as a supplement to their normal diet.

Group-II- Received the '*milk-regimen'* duly supplemented with a '***Probiotic Blend*'** developed and marketed by ***Bio Gain Biologics*** **[Stockholm, Sweden]** comprising:

- ***Reuteri*** **(1.5×10^7 cfu.g^{-1})**
- ***L.acidophilus*** **(3.6×10^8 cfu.g^{-1}** and
- ***Bifidobacterium infants*** **(5.1×10^8cfu.g^{-1}).**

Group-III- Received duly a '***Probiotic Blend*,*****' comprising:

- ***L.acidophilus*** **(3.2×10^8 cfu.g^{-1});** and
- ***B.animalis*** **(1.1×10^9 cfu.g^{-1}).**

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* Boundraa G *et al. J Pediatr Gastroenterol Nutr.,* **11**: 509-512, 1990.

** Ruiz Palacios G *et al*.: **Feeding of *Lactobacillus reuteri*- containing Probiotic Drink for Prevention of Infantile Diarrhoea,** *Microbiol Ecol Health Dis,* **11**: 189, 1999.

*** Chr. Hansen's Laboratory (Milkwaukee, WI, USA).

Observation: As compared to **Group-I(*placebo*)-** *62 episodes of diarrhoea*; the ***Reuteri-Fed Group*** (**Group-II**) showed a *significantly lower incidence of diarrhoea* (only **47 episodes of diarrhoea**; **p = 0.04)**; and the ***non-Reuteri-fed group*** **(Group-III)** had almost a *near significance reduction in episodes* (*i.e.,* 47 episodes of diarrhoea; **p = 0.135**).

6.8. *REUTERI* CONTAINING FUNCTIONAL FOODS: *MODUS OPERANDI* TO HUMANS

In a broader perspective, one must take proper cognizance of an array of practical considerations in case a **bacterial strain** has to get on to qualify as a '***Probiotic***'. Therefore, the bare minimum expectations would be that it should by all means- "**concord an extremely high-degree of viability even after being produced on a large-scale in a perfect cost-effective manner**".

It is, however, worthwhile to mention here that the ***Reuteri strain*** being used conspicuously practically in all ***Human Clinical Trial*** is at present available widely as a '**functional food component**'.

NOTE **Importantly, in most 'clinical trials' *Reuteri* was administered gainfully either in:**

- ***Capsule Form*- containing known cfu in each capsule, or**
- ***Suspension Form*- at known concentrations in various dairy products or beverages.**

(a) Prebiotics and *Probiotics*: It has already been proven beyond any reasonable doubt that the so-called '***Probiotic Activity Profile in the GI-Tract***' is not determined exclusively by the *inherent characteristic features of the probiotic*. Besides, a plethora of specific 'host factors' such as: the 'diet' plays a vital role in the accurate determination of the desired '***probiotic efficacy***'.

Examples: **Oyofo *et al.* (1989)*** have demonstrated that the critical incorporation of *sugars* like: **Lactose**, Mannose, diets** of *boiler Chickens* showed little effect on their growth rate, but reduced appreciably the *intestinal colonization* profile by ***S.typhimurium***. Interestingly, on the other hand the very presence of **Glucose, Maltose**, and **Sucrose** (*and some other sugars*), rapidly metabolized by the host itself, never succeeded to increase the **competitive exclusion (CE) effect** significantly.

(b) Perspective on Reuteri as a Functional Food Component: Irrespective of the prevalent mode(s) of action, the typical and characteristic *health-enhancing activity profile* of ***Reuteri*** invariably believe to be focused on *two* cardinal aspects, namely:

- giving added emphasis to the *host's mucosal defense barriers* against the invading *pathogens*; and/or
- such entities that could be held responsible for *inflicting injuries* to the:

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* Oyofo BH *et al*.: *Arian Dis*, **33:** 531-4, 1989.

** That is, **sugars not absorbed;** and therefore, **not metabolized by the avian species.**

- **intestinal tissues,**
- **inciting inflammatory damages,** and
- **attributing ultimately to the loss of intestinal mucosal integrity.**

In this manner, one may obviously come to the conclusion that the presence of a *healthy-gastrointestinal (GI) tract* having **definitely a well-balanced microbiota** shall only permit the passage of '***nutrients'*** right into the **circulating blood stream**; whereas, the existence of a '***leaky gut'*** allows the passage of the '**partially undigested molecules'** (*viz., fats, proteins*) together with *microbes*, *fungi*, and *viruses*. Besides, several other undesirable bacteria and chemical entities (*i.e., compounds*) get duly **Translocated Across the Intestinal Barrier** to the easily accessible *extraintestinal sites*.

Based on several intensive and extensive *clinical investigative studies* revealed that, in fact, a wide range of '**physiological insults'** such as:

- **use of '*antibiotics'*,**
- **radiation therapy in cancer patients,**
- **improper (unhealthy) diets, and**
- **acute gut infections,**

have been collectively induce the '***Leaky Gut*' syndrome**.

Emergence of Serious Health Problems- These incidences may be apparently come into being when the **gut microbiota exhibits an imbalance**; and also when the **gut integrity gets duly compromised whereby the translocation of the respective microorganisms* to the corresponding extraintestinal sites to take place.**

NOTE **The extreme-neglected cases may ultimately lead to a '*septic state'* that would tantamount to *serious immunopathologies* and could even prove fatal in certain instances****

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* That is, primarily but not exclusively ***Gram*-negative endotoxin-bearing microorganisms.**

** Brassant D and Schifrin EJ: **The use of Probiotics to Reinforce Mucosal Defense Mechanisms,** *Trends Food Sci Technol.,* **8**:321-6, 1997; Marston W and Hager M: **Focus on Health Gut Reactions**, *Newsweek*, 1997 (Nov: 1795-6).

SUGGESTED READING

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CONTENTS AT A GLANCE