Manufacturing, distribution and marketing challenges for probiotic products for the Developing World


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Probiotic Bacteria are needed in Emerging Economies

Example: Possible Prevention of Rotavirus-induced Diarrhea
Major causes of death among children under 5 years of age and neonates in the world, 2000-2003 (WHO)

Undernutrition is an underlying cause of 53% of deaths

Parashar U.D. et al., EID, 2003, 9, 565-71, and other sources

Industrialized countries
Winter epidemics
Moderate severity

Developing countries
Endemic infections
High mortality by dehydration

1 : 293  
1 : 65  
1 : 5  
1 : 1  

Deaths 440,000 – 610,000
2 million inpatient visits
25 million outpatient visits
111 – 130 million domiciliary episodes

Parashar U.D. et al., EID, 2003, 9, 565-71, and other sources
If we could prevent rotavirus diarrhea:

We would save the lives of 1500 children a day.

**History**

Discovered in 1973, in the intestine of diarrheic children (*Bishop, 1973*)

The morphology looks like a wheel (rota means wheel) (*Flewett, 1974*)

In 1979: genus Rotavirus, in the family of Reoviridae
Rotaviruses multiply in, and kill mature enterocytes at the top of intestinal villi.

Mature enterocytes are replaced by immature cells from the crypts. Digestion functions are altered.

A toxin is produced by the virus.

Objectives

- Select a probiotic bacteria inhibiting rotavirus
- Develop *in vitro* and *in vivo* models for this screening.
- Purify and identify the bacterial factor displaying antiviral properties in order to create new products.
- Elucidate the molecular mechanism of the antiviral effect.

- Develop a (tasty) product able to prevent rotavirus diarrhea in young children...
A simple *in vitro* model

Increasing concentrations of virus

Days before infection | Days after infection
---|---

Uninfected cells


*Strain identification by phenotypic and genotypic methods*

- *Genus, species, strain*
- Deposit strain in international culture collection

**Strain(s) in specific product formulation**

**Safety assessment**

- *In vitro* and/or animal
- Phase 1 human study

Double blind, randomized, placebo-controlled (DBPC) phase 2 human trial with sample size and primary outcome appropriate to determine if strain/product is efficacious

Second independent study to confirm results

True Probiotic
Example of an optimized process for the probiotic production (freeze dried)

<table>
<thead>
<tr>
<th>Fermentation</th>
<th>Concentration</th>
<th>Cryoprotectants</th>
<th>Freeze drying</th>
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Counts: $10^9$ CFU/ml  
$10^{10}$ CFU/ml  
$10^{11}$ CFU/g

Optimun for cells:
- Medium GeM
- Time 15 h – 22 h
- Temperature 37°C
- pH 5.8

Adjustement of pH
- pH5 – pH7

Protection with
- milk based
- milk free cryoprotectant

Optimal conditions
- survival
- storage stability at 37°C, 10°C, -20°C

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TECHNOLOGICALLY INDUCED STRESS RESPONSE

Various stresses during production of LAB culture concentrates

**Nutritional Stress**
- Limitation of:
  - Carbohydrate
  - Amino Acids
  - Nucleotides
  - Phosphate

**Temperature Stress**
- Heat Shock
- Chaperones
- Proteases
- Isomerases
- Cold Shock
- CspA

**Oxidative Stress**
- SOD
- Catalase
- Non-HEME Catalase

**pH Stress**
- H+ ATPase
- Glutamate/GABA Antiporter
- Histidine Decarboxylase
- Stringent response

**Osmotic Stress**
- Cysteine, Proline
- Glycine Betaine
- Rile salt hydrolases
- Porin synthesis
- Potassium Transport
- Trehalose Transport
- Compatible Solutes
Survival in powder at 20° C

- **Bifidobacterium lactis**: down from $6 \times 10^9$ to $4 \times 10^8$ cfu/g
- **L. casei**: down from $2 \times 10^7$ to $5 \times 10^5$ cfu/g
- **S. thermophilus**: stable around $2 \times 10^9$ cfu/g

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**Fig. 1**: Survival of strain only (♦) and gum acacia-treated (■) Lactobacillus paracasei NFBC 338 in reconstituted skim milk, during 3 environmental stresses:

- **(a)** Oxidative: 1 mol L$^{-1}$ H$2$O$_2$ for 30 min
- **(b)** Temperature: heat at 60°C for 30 min
- **(c)** Osmotic: 10% (w/v) bile for 30 min

**Fig. 2**: Scanning electron micrographs of (top) strain only and (bottom) strain + gum acacia-containing powders

Source: Danone Research (Pierre Aymard and Laurent Marchal)

Source C. Desmond et al., Journal of Applied Microbiology 2002, 93, 1003-1011
A unique “community” model
Bangladesh

- 144 million inhabitants
- Dhaka (12m) : fastest growing city in the world
- 41% below the age of 14, family size : 4.9
- 56% can’t read & write
- GDP / cap : 600$ a year (Indon.: 1,200$)
- 60% live below 2$ / day income level
- 5% have tap water access
- 80% rural – 62,000 villages
- very high density : above 1,000 p / km2
- industries: cotton, textiles, jute, garments, tea processing, paper newsprint, cement, chemical fertilizer, light engineering, sugar

Grameen

- Muhammad Yunus, a teacher in economy
- 27$ lent to 42 people in one village in 1976
- 9 million direct customers (families of 5 persons)
- 98% women, half crossed the poverty threshold
- They are now mutual owners of GB
- 5 bn$ loans -world class credit statistic
- Largest micro-credit bank in the world
- Social bz model into textile, mobile phones, etc.
- 200,000 Grameen Ladies in 62,000 villages
Grameen Danone:

the mission

Reduce poverty by a unique proximity business model, which brings daily healthy nutrition to the poor

The Product

• 30% of the RDA in minerals and vitamins
• Supplemented in vitamin A (eyesight), iron (prevents anaemia), iodine (physical and mental growth), and zinc (required for growth)
• Rich in calcium and milk protein
• Contains yogurt cultures
The Bogra Factory

- 3-5 t/year
- 50 employees
- delivers 25km radius
- renewable energies
- water treatment
- PLA packaging
- factory linked to 100 adjacent households
Uniqueness lies in the blend of 3 objectives in one single, hybrid, « integrated business model »

- Financial / economic
- Health / nutrition
- Community impact

10-year end game

- Pay-back on capex on a 3-5 year period, requiring constant model reengineering (pack, size, prices)
- Replicated in most rural areas (co-owned basis)
- 50 plants covering half of the 62,000 villages
- 25,000 farm jobs created
- 100,000 sales and distribution jobs created
- Benchmark is Grameen Telecom: 200,000 phones in 62,000 villages in 5 yrs
Actions in other countries

South Africa - Danimals
One out of two children take in less than 50% of iron, zinc and Vitamine A

North Africa - Middle East
• Several products developed to suit local needs
• Ensure product affordability
• Free placement of fridges and transfer of Danone know-how and best practices

Indonesia - Milkuat
• Launched in September 2004
• Dairy beverage with Vitacal to help children grow

Conclusion
While we continue our research efforts to overcome technical hurdles related to the relative fragility of probiotic food...

our approach in proposing valuable nutritional solution in developing countries is based on pragmatism

and on stimulating an active involvement of the local population to allow a sustainable development
Thank you for your attention