



Gastrointestinal well-being

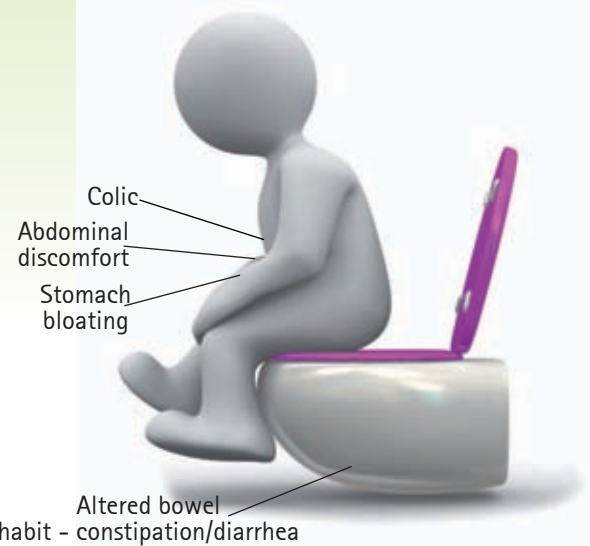
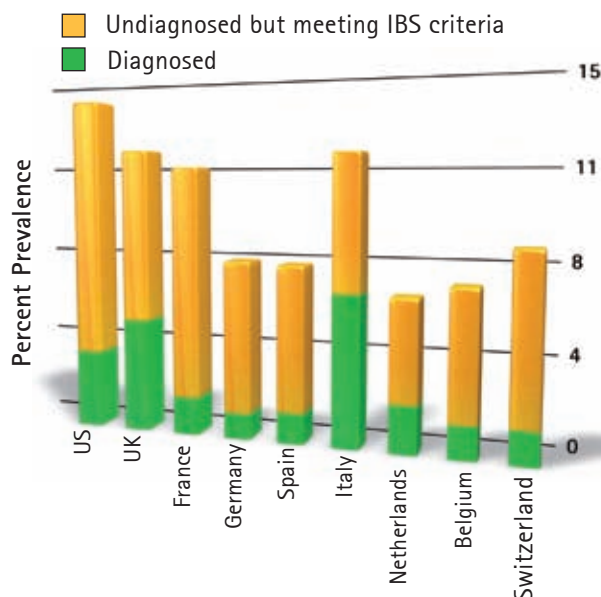


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THE IRRITABLE BOWEL SYNDROME

WHAT IS IBS?

- Irritable Bowel Syndrome (IBS) is a condition characterized by abdominal pain, discomfort, bloating and altered intestinal motility and transit, where symptoms are not explained by structural abnormalities.
- IBS is considered to be the most common gastrointestinal disorder. Prevalence in western world is estimated to be 10-15% of the adolescent and adult population (Dapoigny, et al., 2004; Hungin, et al., 2005; Hungin, et al., 2003).



- IBS is the second leading cause of absenteeism after the common cold, with patients with IBS showing up to 21% reduction in work productivity, equivalent to working just 4 days in a 5-day workweek (Dean, et al., 2005).
- Overall, the sum of direct and indirect costs of IBS is similar to that of other common long-term medical disorders, such as hypertension, and congestive heart failure (Cash, et al., 2005).



Diagnosis

IBS is considered a functional disorder in that diagnostic testing does not show any visible disease process, yet the symptoms can be from mild to disabling. To determine whether a person is just having temporary digestive distress or if he does have IBS, doctors use a standard called the Rome III criteria to make a confident diagnosis of IBS:

"Symptoms must be present for at least six months and have been experienced on at least three days of at least three months. Specifically, symptoms must consist of recurrent abdominal pain or discomfort with two or more of the following:

- Pain is relieved by a bowel movement
- Onset of pain is related to a change in frequency of stool
- Onset of pain is related to a change in the appearance of stool"

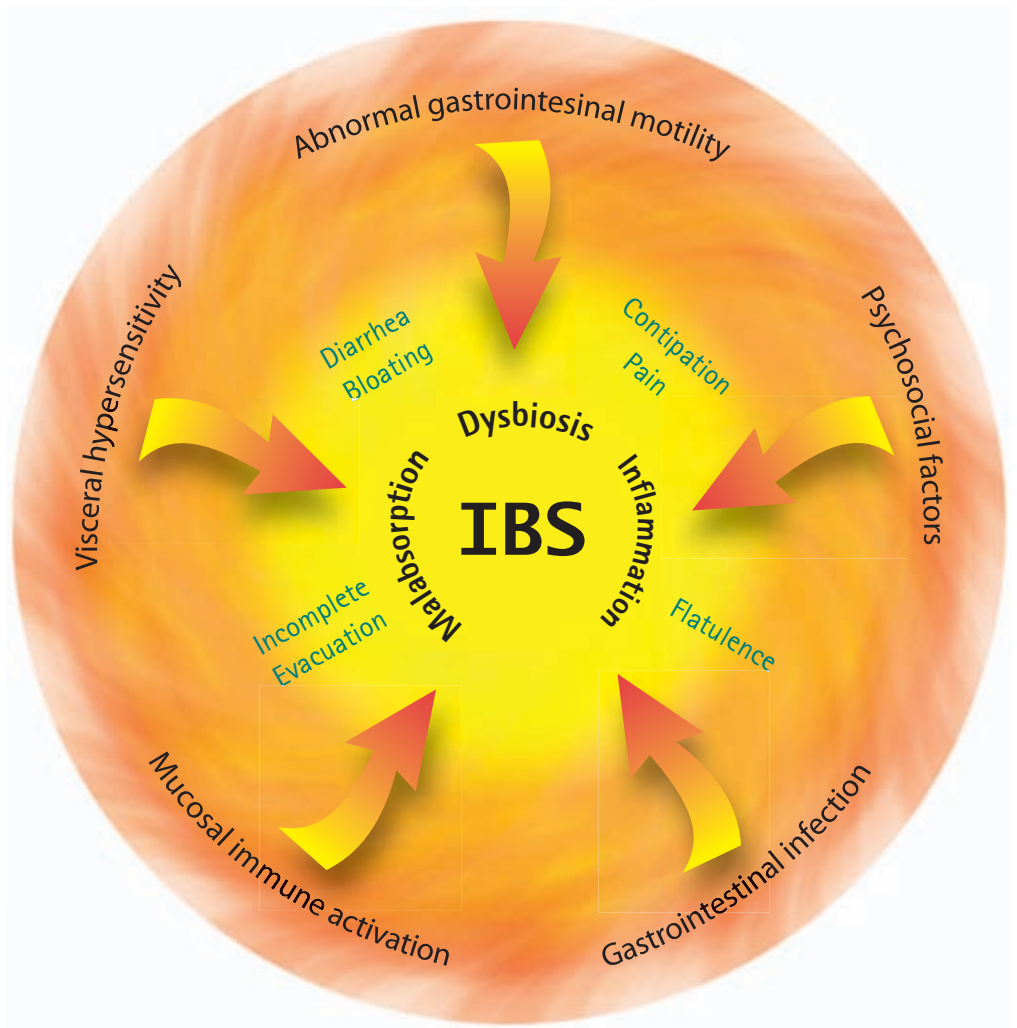
Ref: Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders, The Rome Foundation, 2006 (<http://www.romecriteria.org/criteria/>)

Sub-types of IBS

- Constipation-predominant (C-IBS)
- Diarrhea-predominant (D-IBS)
- Alternating or Mixed (A-IBS)

CAUSES

The pathophysiology of irritable bowel syndrome is multifactorial: abnormal gastrointestinal motor functions, visceral hypersensitivity, psychosocial factors, autonomic dysfunction, and mucosal immune activation leading to microscopic inflammation of the mucosa (Camilleri and Andresen, 2009; Spiller, 2007). Also, altered gastrointestinal flora is being recognised as a common trait in IBS patients. For instance, lower counts of Lactobacilli have been reported as a common trait in patients suffering from diarrhea-predominant IBS (Malinen, et al., 2005).



SHORTCOMINGS OF PRESENT TREATMENTS

Current approaches to management of IBS consist of identification of symptoms consistent with the syndrome and the exclusion of organic disease with a similar presentation. Pharmacological therapeutic options are limited and the effectiveness of many is poorly documented (CPMP/EWP/785/97, 2003). Some serotonergic agents have demonstrated efficacy on the global symptoms of IBS; however, concerns about safety have severely limited their use (Camilleri and Andresen, 2009).

On the other hand, there are several non-pharmacological approaches, but their efficacy is limited. For instance, current approaches include behavioral management therapies such as dietary intake changes and stress reduction cognitive restructuring (Heitkemper and Jarrett, 2008). However, studies suggest only one quarter of patients may have their symptoms caused or exacerbated by dietary components. Also, soluble fiber is marginally beneficial, despite a long history of enthusiastic use, and insoluble fiber may actually worsen symptoms (Heizer, et al., 2009).

I31

THE PRODUCT

Probiotics are defined as "living microorganisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition" (Araya, et al., 2002; Guarner and Schaafsma, 1998). The current evidence points that the beneficial effects of probiotics are strain specific. According to the World

Gastroenterology Organisation (WGO) Practice Guidelines on Probiotics and Prebiotics, the potential probiotic health benefits "can only be attributed to the strain or strains tested, and not to the species or the whole group of lactic acid bacteria or other probiotics." (Guarner, et al., 2008).

OVERCOMING THE DIGESTIVE TRACT

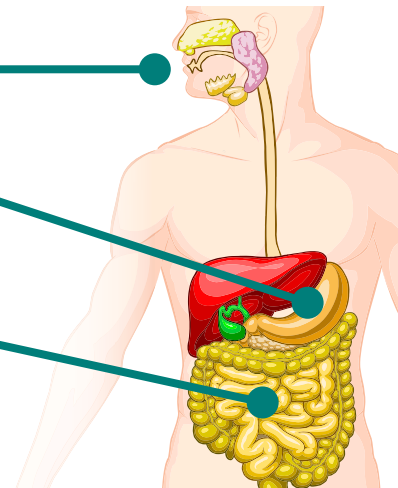
Since the active principle of the I31 formula is composed of probiotic strains, the first step in the process of strain selection was to ensure that they would be able to overcome the physicochemical barriers found in the digestive tract in order to safely reach the small intestine, where they will exert their effect.

Strains in I31 have demonstrated more than 40% survival when sequentially exposed to the different physiological conditions found in the digestive tract, before reaching to the small intestine:

Mouth: Lysozyme and Hydrogen peroxide

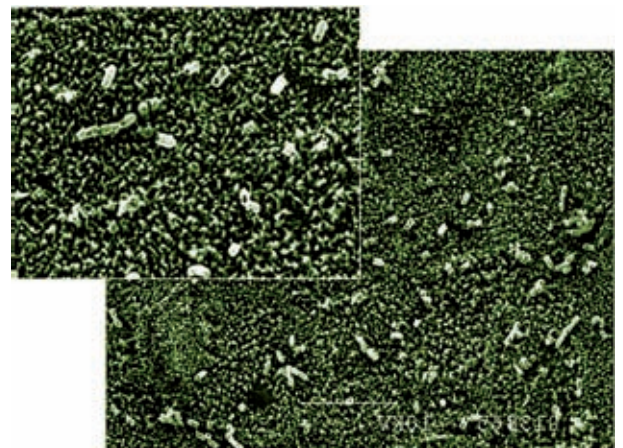
Stomach: Extremely acidic pH (2-3) combined with pepsin

Small intestine: Combination of low pH and bile salts plus pancreatin at the duodenum



ADHESION AND COLONIZATION OF THE DIGESTIVE TRACT

Once in the intestine, the bacteria included in I31 attach to the intestinal mucosa and colonize the small intestine, which is essential for their probiotic effect (Collado, et al., 2007; Li, et al., 2008)



CHARACTERIZATION OF THE STRAINS

I31 consists of a cocktail of three lactic acid bacteria strains of the Lactobacillales group. Taxonomic identification was obtained by sequencing the 16S gene. To ensure the exclusiveness of the product, the Pulsed Field Gel Electrophoresis (PFGE) technique was used to further characterise the strains at the genomic level.

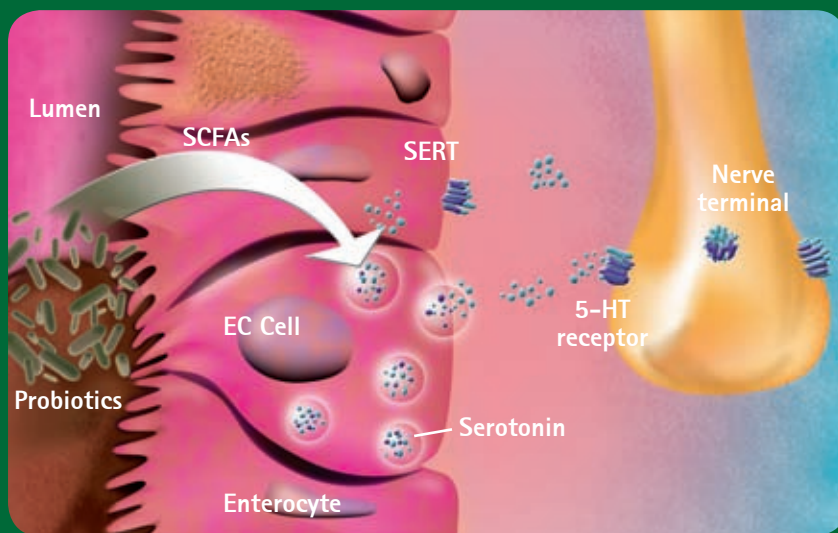
Therefore, the identity of the strains can be readily established, and strains can be distinguished from other commercially available strains belonging to the same species. PFGE is considered the Gold Standard technique for strain typing, recommended by the FAO and WHO in their guidelines for probiotics (Araya, et al., 2002).

PROBIOTIC BIOFUNCTIONALITIES

A. PRODUCTION OF SCFAs

- Strains in the I31 formula are able to ferment the fibers normally found in the diet to produce high amounts of short chain fatty acids (SCFAs), such as acetic, propionic and especially, butyric acid.
- SCFAs, especially butyric acid, are readily absorbed by intestinal mucosa and stimulate sodium and water absorption in the colon (D'Argenio and Mazzacca, 1999; Tedelind, et al., 2007). Fecal SCFA profile of patients with D-IBS is characterized by lower concentrations of total SCFAs (Treem, et al., 1996)
- Serotonin release modulates both gut motility and sensation. SCFAs are known to stimulate serotonin (5-HT) release in rat colon (Fukumoto, et al., 2003), and butyric acid has been shown to decrease visceral sensitivity of the intestine in human volunteers (Vanhoutvin, et al., 2009).
- Therefore, in situ production of SCFAs by strains of the I31 formula can be useful to modulate gut motility and reduce visceral pain.

SEROTONIN IS A PRIMARY NEUROTRANSMITTER REGULATING GUT MOTILITY AND SENSATION



Enterochromaffin (EC) Cells:
Secrete the neurotransmitter serotonin, into the intestinal wall.

Serotonin: Binds to receptors on nerves to modulate motility, secretion and sensation.

SERT (serotonin reuptake transporter):
Mediates the uptake of serotonin into epithelial cells to inactivate it.

Probiotic bacteria: Produce Short chain Fatty Acids (SCFAs) that stimulate serotonin release and thus may improve gut motility and sensation

B. GROWTH INHIBITION OF PATHOGENIC MICROORGANISMS

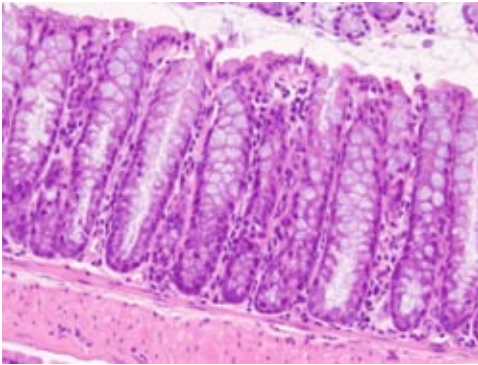
I31 displays a significant inhibitory activity against many pathogenic bacterial strains, such as *Escherichia coli*, *Salmonella enterica* cholerasuis, *Salmonella enterica* typhimurium, *Campylobacter jejuni*, *Klebsiella oxytoca*, *Clostridium perfringens* and *Candida albicans*.

On the other hand, I31 allows the growth of common commensal strains of the human gastrointestinal flora, such as *Bacteroides vulgatus* and *Bacteroides thetaiotaomicron*.

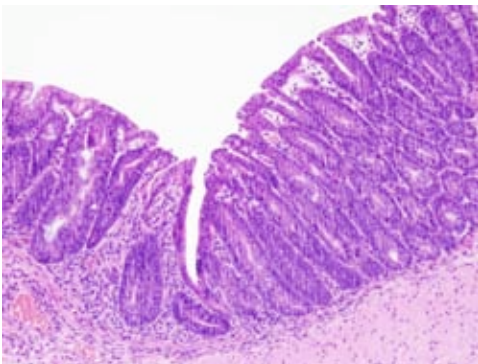
C. EFFECT ON MODELS OF GUT INFLAMMATION

In two different animal models displaying mild gut inflammation, probiotic formula I31 is able to:

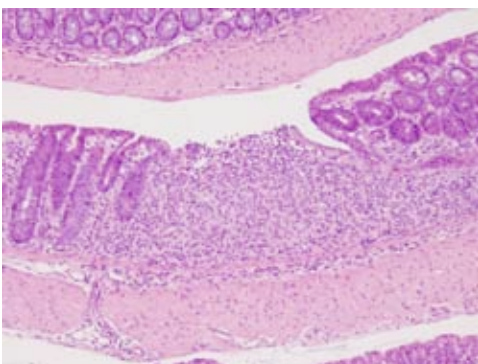
- ✓ Significantly reduce external symptoms (weight loss and diarrhoea)
- ✓ Significantly reduce molecular markers of inflammation in the gut mucosa (pro-inflammatory cytokines Interleukin-6 and gamma-Interferon).



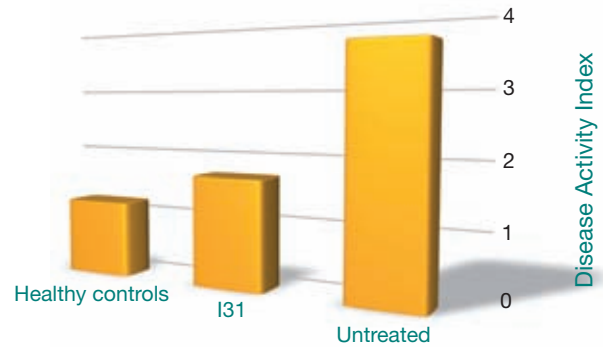
Healthy intestinal mucosa



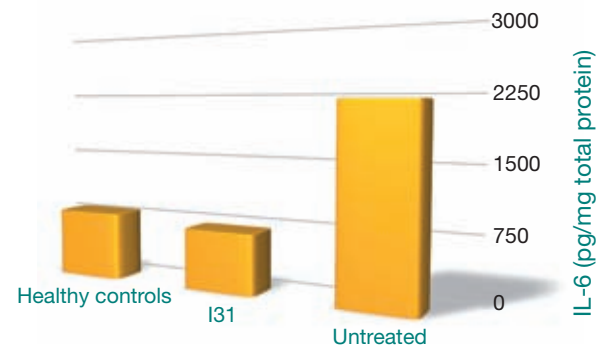
Inflamed mucosa treated with I31 (regenerating villi)



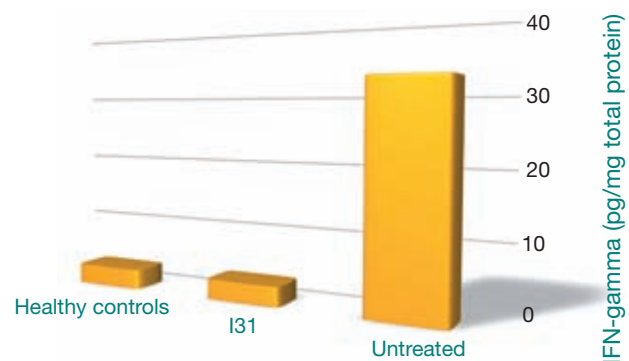
Inflamed mucosa (eroded villi and leukocyte infiltration)



External symptoms (Disease Activity Index = weight loss + diarrhoea + stool blood)



Pro-inflammatory cytokine Interleukin-6 (acute inflammation marker)



Pro-inflammatory cytokine gamma-Interferon (chronic inflammation marker)

CLINICAL TRIAL

A randomised placebo-controlled clinical trial is currently running in order to further demonstrate the efficacy of once a day I31 on the global symptoms of IBS. Also, the trial has been designed to study dose-related effects as a secondary endpoint.

Technical Sheet

SAFETY OF THE PRODUCT

- All three strains have EFSA Qualified Presumption of Safety Status (Andreoleti, et al., 2008).
- Strains from I31 formula do not display acquired antibiotic resistances, and thus cannot transfer antibiotic resistance genes to pathogenic bacteria (Bories, et al., 2008).
- Strains from I31 formula do not display any undesirable effect after 10 weeks of administration in mice of a human equivalent dose (HED) of 1.5×10^{11} cfu/day (CDER/CBER, 2002)

In summary, I31 is a probiotic formula able to colonize the small intestine without any health risk.

SPECIFICATIONS:

- I31 formula is produced and manufactured under GMP conditions.
- The product is available in freeze-dried form.
- Certifications:
 - Products are produced under the EU standards 178/2002 (Basic food requirements incl. traceability) and 852/2004 (Hygiene, HACCP concept)
 - Allergen Free Statement
 - BSE Free Statement
 - Identity Preserved Status Statement.

APPLICATIONS

Pharma

- I31 can be dosed both in capsules, tablets and in sachets, with a product stability of at least 2 years.
- Final product distribution can be done at room temperature.
- Dosage has a safety margin to ensure that a sufficient dose is always delivered



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