Saccharomyces boulardii

The ultimate yeast Probiotic
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The gut ecology is a complex system based on the equilibrium of different bacterial species. Disturbance of this equilibrium by infectious diseases and very often by antibiotic treatments leads to clinical symptoms of diarrhoea. Severe antibiotic-associated diarrhea can give rise to *Clostridium difficile* diarrhoea having a high rate of relapse and being difficult to cure by conventional treatments. The use of probiotics as alternatives to antibiotics is therefore more and more attractive.

The approach of keeping a healthy gut flora by the consumption of live microorganisms was first proposed by Metchnikoff in 1907. Following the same philosophy, Dr Boulard isolated a very special natural yeast known now as *Saccharomyces boulardii* which has proven its efficiency in the prevention and treatment of antibiotic-associated diarrheas and in *Clostridium difficile*-recurrent infections. Modern science has now elucidated most of the mechanisms of action of *Saccharomyces boulardii* ranging from: inactivation of *Clostridium difficile* toxins competitive exclusion of pathogens like *E. coli* specific immune stimulation of the gut restoration of a functional lactic acid flora

Institut Rosell and its mother company Lallemand Inc. have for more than a decade now developed specific research programs to increase the use of *Saccharomyces boulardii* in both animal and human health. The present document is a comprehensive review of current knowledge on *Saccharomyces boulardii*.
THE GUT ECOSYSTEM

The human gut can be compared to a fermentor working in a continuous manner. Food comes in and is processed by the stomach digestive fluid into short molecules that will be absorbed by the small intestine. The unprocessed foods, like fibres, that are more resistant to the digestive fluids pass to the large bowel where anaerobic bacterial fermentations will transform them into fatty acids and gas. Indigestible and unabsorbed material will be excreted together with bacteria, forming the faeces.

To perform this complex set of biochemical reactions the gut is colonized by a huge quantity (around $10^{14}$) and a large variety of bacteria (more than 400 different species). The distribution of bacteria in the gut is not even. The stomach contains very low amounts of bacteria ($10^2$/ml) as acidity can drop to pH 2, impairing their survival. In the first part of the small intestine (duodenum, jejunum) the concentration of bacteria is still low ($10^4$ to $10^5$) due to the inhibitory effect of biliary salts on bacterial growth and to the strong peristalsism which prevents the bacteria from multiplying and colonizing (transit time in the small intestine is only 4 to 6 hours). At the distal part of the small intestine the bacterial population increases, together with the diversity of species. At this level (ileum) enterobacteria and lactobacilli are most common and can reach $10^7$ to $10^9$ cfu/g. It is however only after the passage of the ileocaecum valve that the concentration and the diversity of the bacterial population become maximal. The transit time in the colon is a long process (60 to 70 hours) that gives enough time to the bacteria to multiply. Also, as the transit proceeds the population switches from facultative anaerobes to strictly anaerobes like bacterioides and bifidobacteria and many other hydrogen and methane producers. Concentrations as high as $10^{11}$ cfu/g are currently observed.

How does this complex flora develop?

At birth the gastrointestinal tract of the newborn is sterile. By contact with the environment a microbial flora starts to develop. During the first 3 days, whatever the type of feeding (breast or infant formula), E. coli and Streptococci are dominant. After 3 days a strictly anaerobic flora appears, mostly composed of bifidobacteria in breast-fed infants and of a mixed flora containing bacterioides and clostridium with inconstant bifidobacteria populations in infants fed with replacement milk. It is now recognised that breast milk contains bifidogenic factors that help the bifidobacteria to grow and colonize.

With contact with environmental bacteria and the intake of different foods the gut flora matures and becomes more complex. The mechanisms by which certain species or strains become dominant in a given individual are not well understood but probably have something to do with genetic and immune factors. For instance, only 30 to 40% of the western population produce methane which is formed by bacteria able to combine hydrogen and carbon dioxide. It seems that this ability is transferred from parents to children. It is also interesting to note that the intestinal flora is quite stable and returns to normal even after antibiotic treatment or change in food habits. This means that complex relationships do exist between these commensal bacteria and the host.

FACTORS AFFECTING THE MICROBIAL FLORA BALANCE

Like all ecosystems, the intestinal ecosystem is an equilibrium between dominant species and under-dominant, transitory or even pathogenic species which are kept under control. This system works well as long as the equilibrium is not modified by endogenous or exogenous factors. Among the endogenous factors inflam-
Information has been shown to modify the gut flora, especially in patients with rheumatoid arthritis and allergic diseases. Inflammatory bowel disease, which is a chronic intestinal inflammation mediated by the “normal” gut flora, also affects the equilibrium by constantly switching between episodes of diarrhea and constipation.

Exogenous factors which modify the gut flora are more widespread and related to either infectious diseases (bacterial, fungal or viral) or antibiotic treatments.

It is not our purpose to discuss infectious diseases in detail as toxigenic and pathogenic factors are quite complex and specific but one example, Clostridium difficile, will be treated later. We will therefore concentrate on the effect of antibiotics on the gastrointestinal flora, as they are the main cause of disturbance of this flora.

**Antibiotics and modification of the gut flora**

Most antibiotics (AB) affect the gastrointestinal flora so it is unrealistic to think that antibiotics are only targeted to specific pathogens. How antibiotics modify the gut flora will depend on:

- The concentration of AB in the lumen. This is related to the permeability of the intestinal epithelium to the AB when taken orally and/or to the part of the AB which is re-excreted with the bile salts in the lumen when taken by any route.
- The activity of the AB on the gut flora.

The result of an antibiotherapy is therefore complex and difficult to predict but generally follows a series of steps:

- Elimination of sensitive bacteria or at least decreasing of their initial population (goal)
- Selection and proliferation of resistant bacteria from the existing flora (side effect)
- Possible colonization by exogenous resistant bacteria as space is available (side effect)
- Selection of resistant bacteria which can find new conditions for colonization (side effect)

Therefore antibiotic therapy, although efficient at targeting pathogens, relies on the host to quickly restore a normal flora that will be protective against the side effects.

It is important to mention the individual variation in gut flora as a result of antibiotherapy. Léonard et al. have illustrated this phenomenon by treating healthy volunteers with cephalosporin. They observed that the population could be separated into two groups: Group 1 for which cephalosporin was excreted in high amounts in the faeces and where they noticed a strong decrease in the anaerobes and further a colonization by Candida albicans; Group 2 for which no detectable cephalosporin was measured in the faeces and no noticeable variation in the flora occurred. In particular they didn’t notice secondary colonization by Candida albicans. They were able to show that Group 2 had a dominant flora of bacterioides which strongly expressed β-lactamase activity and therefore inactivated the cephalosporin in the lumen of the GI track. Group 1 didn’t have this dominant flora and therefore the concentration of cephalosporin in the lumen was high and killed most of the anaerobes, leaving room for Candida albicans to colonize.

By acting not only on pathogens but also on the dominant flora antibiotics are a major source of flora imbalance. This imbalance quite often has clinical symptoms in the form of diarrhea.

**Antibiotic-associated diarrhea**

AAD is frequent both in children and elderly people. In children the main reason seems to be an over-prescription of antibiotics for upper respiratory tract infections to prevent secondary infections. In the elderly, nutritional status, polymedication and the diminution of the intestinal physiological functions are the main reasons.

A few epidemiological studies are available showing the incidence of antibiotics on diarrhea. In one study done in ambulatory paediatric practice on 650 children aged one month...
to 15 years receiving antibiotics for a likely bacterial infection, the following results were observed:

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>AAD(*)</th>
<th>One day diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G and V</td>
<td>3%</td>
<td>8%</td>
</tr>
<tr>
<td>Penicillin A and M</td>
<td>11%</td>
<td>21%</td>
</tr>
<tr>
<td>Amoxicillin + B-lact Inhibitor</td>
<td>23%</td>
<td>43%</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>9%</td>
<td>17%</td>
</tr>
<tr>
<td>Macrolides</td>
<td>8%</td>
<td>15%</td>
</tr>
<tr>
<td>Trimethoprim + Sulfamethoxazol</td>
<td>6%</td>
<td>25%</td>
</tr>
<tr>
<td>Erythromycin + Sulfafurazol</td>
<td>16%</td>
<td>24%</td>
</tr>
</tbody>
</table>

(*) defined as >= 3 liquid stools per day for at least 2 consecutive days

It is interesting to note that new generations of antibiotics like amoxicillin associated with B-lactam inhibitors are likely to increase the incidence of AAD, probably because they are more effective.

The clinical aspects of AAD are very diverse, from mild diarrhea to very severe diarrhea. Although most AADs are moderate and stop after the antibiotic treatment is finished and are not life-threatening (except for newborns and infants if rehydration is not properly done), some and especially *Clostridium difficile*-associated diarrhoes are more difficult to treat and can give rise to pseudo-membranous colitis.

**CLOSTRIDIUM DIFFICILE AND PSEUDO-MEMBRANOUS COLITIS**

Pseudo-membranous colitis (PMC) is the most severe form of AAD. The microorganism responsible is *Clostridium difficile*, a gram positive anaerobic bacteria forming spores resistant to antibiotics. *Clostridium difficile* also produces two toxins, A and B. These toxins bind to specific glycoproteins in the intestinal mucous membrane. This binding triggers the lesion process, with disintegration of the actin filaments of the enterocytes, and increases the intracellular permeability. Diarrhea is the most clinically-observed sign. The final consequence is an infiltration of the mucous membrane by polynuclear neutrophils and an edema of the submucosal membrane.

PMC is a problem for all age groups although more of a concern for the elderly. The association of PMC with antibiotic treatments is well established but a weak immune system, malnutrition and aging are increasing risk factors. Nosocomial transmission is also well established as only 3% of the general adult population is harbouring *C. difficile* while it is present in 10% to 20% of hospitalized patients. Interestingly, newborns and infants up to 2 years old are asymptomatic carriers of *C. difficile*, with frequency as high as 60% in newborns and 5% to 10% in infants while PMC in this age group is very rare. It seems that either the intestine does not have receptors for the toxins or that breast feeding protects the newborn through specific antibodies.

The treatment of PMC requires vancomycin or metronidazole at high dosages. However relapse is very frequent (greater than 20%) and can reach 60% after the first relapse.

The use of the yeast Saccharomyces boulardii has been shown particularly effective to prevent relapse of *C. difficile* and more generally in the prevention and treatment of AAD. Modern scientific research on this important yeast has revealed its possible modes of action.
**ORIGIN AND CHARACTERIZATION**

The Saccharomyces boulardii history started around 1920, when a French microbiologist, Dr. Boulard, made a unique discovery in Vietnam. He noticed that consuming a particular local drink could alleviate symptoms of diarrhea in villagers afflicted by an epidemic of cholera. This drink was made from tropical fruits such as lichee and mango. Dr. Boulard isolated an active agent from this drink, which proved to be a live yeast of natural origin, and which is now known as *Saccharomyces boulardii*.

For many years taxonomists have discussed whether *S. boulardii* was a new species of *Saccharomyces* or a specific strain or variant of *Saccharomyces cerevisiae*. Mitochondrial DNA analysis (Mallié, 2001) and microsatellite typing techniques (Hennequin, 2001) have recently shown that *S. boulardii* is a unique strain or variant of *S. cerevisiae* but not a new species of the genus *Saccharomyces*.

The proper taxonomic name is therefore *Saccharomyces cerevisiae boulardii* (Mallié, 2001). The original strain is currently described as *Saccharomyces cerevisiae* Hansen CBS5926 from which most of the active pharmaceutical ingredients on the market are produced.

**MECHANISMS OF ACTION**

Some physiological characteristics have been found associated with *Saccharomyces boulardii* that may explain its action mechanisms. The outer membrane is richer in mannose, a sugar allowing the binding of pathogenic bacteria that contain Type 1 fimbria, than it is in other yeasts of the same species (Gedek, 1999; Ofek, 1984). Also, numerous publications have shown the production of a specific 54-kilodalton protease active against the bacterial toxins produced by *Clostridium difficile* (Castagliuolo, 1999, 1996). Today our understanding of the probiotic properties of *S. boulardii* is the following.

**a) Binding of enterohaemorrhagic E. coli and Salmonella**

*S. boulardii* through its mannose-dominant outer membrane has the ability to bind *E. coli* and *Salmonella*, bacteria responsible for diarrhea, especially traveller’s diarrhea. The large cell surface of the yeast allows the binding of many bacterial cells limiting their capacity to bind to the intestinal epithelium. In this way the bacteria are likely to be eliminated in the stools.

**b) Protection of the digestive mucosa**

In microbial ecology it is generally admitted that 3 mechanisms can be described for interactions...
between bacteria for a single attachment site. These are:
• Protection
• Competition
• Displacement

Protection means that a given strain administered first will occupy the attachment site and therefore leave no room for the second one to bind later.
Competition means that when administered together one strain will bind to the site while the other will not.
Finally, displacement means that one strain is able to replace another one that was bound to the attachment site first.

**Figures 4**

In an experiment done on mice (Castex et al.) *Clostridium difficile*-infected mice were challenged by *S. boulardii*. As shown below, *S. boulardii*-treated mice were protected from the infection. According to the authors, *S. boulardii* inactivated the *Clostridium* toxin and therefore induced protection.

c) **Favour growth of lactic acid bacteria in the gut**

One of the positive aspects of *S. boulardii* is its ability to favour the growth of the resident lactic acid flora. Although the mechanism is not completely understood it probably has something to do with the ability of *S. boulardii* to bind enterobacteria like *E. coli*. By doing so it leaves more room for the lactobacilli to develop, especially in the jejunum where enterobacteria and lactobacilli are both present. A study done by Gedek et al. in 1993 on piglets has shown the following results:

d) **Protection against Clostridium difficile toxins**

As mentioned earlier, *Clostridium difficile* produces two toxins, Toxin A (enterotoxin) and Toxin B (cytotoxin), responsible for the necrotic effect on the intestinal mucosa. In 1990 Castex found that *S. boulardii* does not alter the popu-
lation of *Clostridium difficile* in the gut but actually reduces the amount of toxin found in the faeces. The mechanism of action was proposed by Castagliuolo (in 1996 and 1999), who isolated a 54 KD serine protease from *S. boulardii* able to inactivate the toxins. In 2001 Qamar demonstrated that an administration of *C. difficile* toxin A to *S. boulardii*-treated mice gave a fourfold increase of specific IgA compared to non-treated mice.

In VEROS cells, *S. boulardii* has been shown to limit the binding of *C. difficile* toxins to the mucous membrane by steric hindrance thereby preventing the liberation of toxins (Tasteyre, 2002).

The proposed mechanism for the protective effect of *S. boulardii* against *Clostridium difficile* is likely to include the following steps:

- Inactivation of the toxins by the proteolytic action of P54 Protease
- Limitation of toxins’ mucosal binding by steric hindrance
- Increase in specific IgA anti-*Clostridium* toxins

**e) Stimulating effect on the intestinal mucosa**

*S. boulardii* has been shown to increase enzymatic activities (sucrase and maltase) at the level of the mucosa leading to better sugar assimilation (Buts, 1986). More recently (Goals, 1994, 1999; Kollman, 2001) it was demonstrated that *S. boulardii* increases the level of spermine and spermidine, two polyamines involved in cell proliferation. It seems that this ability can explain the effect of *S. boulardii* on brush border (increase of crypt depth and villi height) which has been shown in piglets (Milan, 2002, 2003).

**CLINICAL BENEFITS**

*Saccharomyces boulardii* has a long history of use as an adjunctive treatment for prevention and treatment of antibiotic-associated diarrhea. More specifically, its effectiveness has been demonstrated in *Clostridium difficile*-associated diarrhea.

In a placebo-controlled study (Surawicz *et al.*, 1989) on patients under antibiotic treatment the following results were obtained:

<table>
<thead>
<tr>
<th></th>
<th>Placebo group</th>
<th><em>S. boulardii</em> group</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of patients with diarrhea</td>
<td>21.8 %</td>
<td>9.5 %</td>
</tr>
</tbody>
</table>

Although *S. boulardii* does not suppress all antibiotic-associated diarrhea, the fact that it reduces the risk by half is significant (Marteau, 2000).
In a study done by McFarland (1994) on a subgroup of diarrhea linked to the presence of *Clostridium difficile*, although there was no difference between the placebo group and the *S. boulardii* group for the first episode of diarrhea, relapse was reduced by half in the *S. boulardii* group of patients.

<table>
<thead>
<tr>
<th></th>
<th>Antibiotic group + placebo</th>
<th>Antibiotic group + <em>S. boulardii</em> probiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st episode of diarrhea</td>
<td>24.2 %</td>
<td>19.3 %</td>
</tr>
<tr>
<td>Recurring infection</td>
<td>64.7 %</td>
<td>34.6 %</td>
</tr>
</tbody>
</table>

**GENETIC IDENTIFICATION**

Institut Rosell’s *Saccharomyces boulardii* (ATCC 74012) has been compared by genetic typing to the original type *Saccharomyces cerevisiae var boulardii* Hansen CBS 5926 and as shown below proven to be genetically identical.

**Analysis Report**

*Report asked by Lallemand to Sigmo on the 6th of December 2001.*

**Object**: Comparison between strains ATCC 74012 and CBS 5926

**Technical**: PCR

**Samples analysed**: Ultralevure (lot 7331, Biocodex Laboratories) SB (#087)

**Results**: Ultralevure strains present the same PCR profile.

**SPECIFICATIONS OF THE PHARMACEUTICAL INGREDIENT**

Institut Rosell supplies a freeze-dried pharmaceutical grade *Saccharomyces boulardii* without lactose with the following specifications:

**Specifications**

- **Product**: SACCHAROMYCES BOULARDII (ATCC 7412) (genetically identical to Saccharomyces cerevisiae CBS5926)
- Freeze-dried
- Without lactose
- Pharmaceutical grade

- **Living cells/g**: $\geq 20 \times 10^9$
- **Tot. bact./g**: $\leq 10^4$
- **Coliforms**: $\leq 10^2$
- **E. Coli**: Negative in 1 g
- **Salmonella**: Negative in 10 g
- **Staphylococcus aureus**: Negative in 1 g
- **Molds/g**: $\leq 10^2$
- **Wild yeast/g**: $\leq 10^3$
- **% Solids**: 96-98
- **Colour**: beige
SELECTION OF ARTICLES


Elmer G.W., G. Corthier. 1990. Modulation of *Clostridium difficile* induced mortality as a function of the dose and the viability of the *Saccharomyces boulardii* used as a preventative agent in gnotobiotic mice, Can J. Microbiol. 37. 315-317.


Méd Mal Inf; 18 (n° hors série): 14-20


Vidon, N., B. Huchet, and J.C. Rambaud. 1986. Influence de Saccharomyces boulardii sur


