Protocols & Products for a Healthy Life

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EXECUTIVE SUMMARY

CLINICAL STUDY

PERSISTENCE OF S. CEREVISIAE SUBSPECIES BOULARDII IN HEALTHY VOLUNTEERS GUT

Background

Saccharomyces cerevisiae subspecies boulardii is a variety of the yeast Saccharomyces cerevisiae, it differs from S. cerevisiae in several taxonomic, metabolic and genetic properties. S. boulardii is resistant to acidity, to proteases and to all antibacterial antibiotics.

S. boulardii is a non-pathogenic yeast, it is used in France for diarrhoea clinical treatment and its clinical employment is currently extended worldwide. Many clinical studies evidenced the efficacy of S. boulardii in prevention or treatment of various intestinal disorders, including antibiotic-associated diarrhoea, Clostridium difficile related enterocolopathies and traveller's diarrhoea. Efficacy of S. boulardii is also demonstrated in Crohn's disease and ulcerative colitis. Furthermore, an antiinflammatory, antimicrobial and anti-toxinic activity has been associated to S. boulardii.

CitriSafe Probiotic™ S. boulardii is a proprietary yeast strain showing a strong genetic homology with the known S. boulardii strain Hansen CBS 5926. CitriSafe Probiotic™ is produced with a patented process including an innovative drying step.

Objectives

The ability of CitriSafe Probiotic™ strain to persist in digestive apparatus and colonize the colon of healthy adult volunteers was evaluated in this trial in comparison to a commercial lyophilized strain of S. boulardii (Codex).

Experimental Design

Twenty healthy volunteers (10 males and 10 females) were enrolled in the study, all presenting normal conditions to a physical examination. All the participants were asked to sign a written informed consent. Women enrolled had a negative pregnancy test and were not breast-feeding. Volunteers were divided in two

arms of ten subjects each (5 males and 5 females). One group of volunteers received a daily oral dose of CitriSafe Probiotic™ S. boulardii strain for 7 consecutive days, and the other gropup received lyophilized Codex S. boulardii with the same treatment scheme. All volunteers received about 5.0 x 10^9 Colony Forming Units (CFU, viable yeast cells) in each daily yeast dose. Detailed living yeast cell counts for both products are reported in Table 1. Participants to the trial had to disperse S. boulardii powder in a glass of drinking water and drink the suspension.

Faeces were collected at three different time stations, namely at 0 (pre-treatment), 7 and 10 days from the start of treatment; thus including in the observation period, pre-treatment, the entire S. boulardii intake period, and a 3 days follow-up after treatment completion.

Table 1 – viable counts contained in S. boulardii based products

S. boulardii strain	Microbial counts (CFU/g)
CitriSafe Probiotic™	4.8 x 10^9
Codex	5.1 x 10^9

End Points of the study

Faecal samples were plated on Sabouraud agar selective medium with the addition of ampicillin and chloramphenicol to promote exclusively the growth of yeasts. Plates were incubated at 37°C for 48 hours in aerobic environment. Thus, colonies were counted in order to determine S. boulardii viability and its degree of intestinal colonization. In order to obtain information concerning the wellbeing related to the product intake, a questionnaire was given to volunteers. **Results**

The increase in viable yeast cell counts in faeces after 7 days of treatment was similar for both products and it was consistent with the expected colonization based on previous trials. S. boulardii Colony Forming Units after 7 days of treatment, in the colon of the volunteers, increased of 3.2 and 3.4 Logs for CitriSafe Probiotic™ and Codex respectively (Fig. 1, Table 2). The persistence of the two yeasts in the gut after treatment completion, resulted to be similar as well. At the 10^{th} day from the beginning of treatment, in fact, i.e. three days after the suspension of the daily intake of both CitriSafe Probiotic™ and Codex S. boulardii specialties, CFU in faeces samples regressed to a value that was 0.7 Log and 1.0 Log more than pre-treatment background, respectively (Fig. 1, Table 2), thus decreasing respectively of 2.5 Logs and 2.4 Logs.

Concerning the questionnaire on the physiological impact of CitriSafe Probiotic™ and Codex, volunteers answered about symptoms relieve and gut health improvement. Fig. 2 shows the main physiological effects claimed by the 20 volunteers, answers are reported as a percentage ratio to the total volunteers answering in the specific group, multiple answers were allowed and for this reason total value is >100%. CitriSafe Probiotic™ treatment was claimed to result in a better intestinal regulation for a significantly higher percentage, while improved faecal consistency was noted with slight higher percentages in the group of Codex consumers. One volunteer over 10, claimed an improved ease of evacuation in both arms.

Table 2 – Individual data of S. boulardii intestinal colonization after CitriSafe Probiotic™ or Codex oral administration. Values are expressed as Delta Log (Log CFU_t – Log CFU_0) / g of faeces.

CitriSafe Probiotic™			Codex		
Subject	7 days	10 days	Subject	7 days	10 days
1	5.1	3.1	11	5.7	2.1
2	3.2	1.3	12	5.9	1.2
3	0.7	-0.3	13	0.0	0.0
4	4.2	0.1	14	2.5	1.0
5	3.6	1.5	15	4.1	1.4
6	1.5	0.3	16	3.6	2.2
7	1.6	-0.3	17	0.0	0.0
8	2.0	-1.2	18	4.5	1.8
9	5.3	0.0	19	4.7	0.0
10	5.2	2.6	20	2.9	0.0
Average	3.2	0.7	Average	3.4	1.0
Std. Dev.	1.7	1.4	Std. Dev.	2.1	0.9

Figure 1

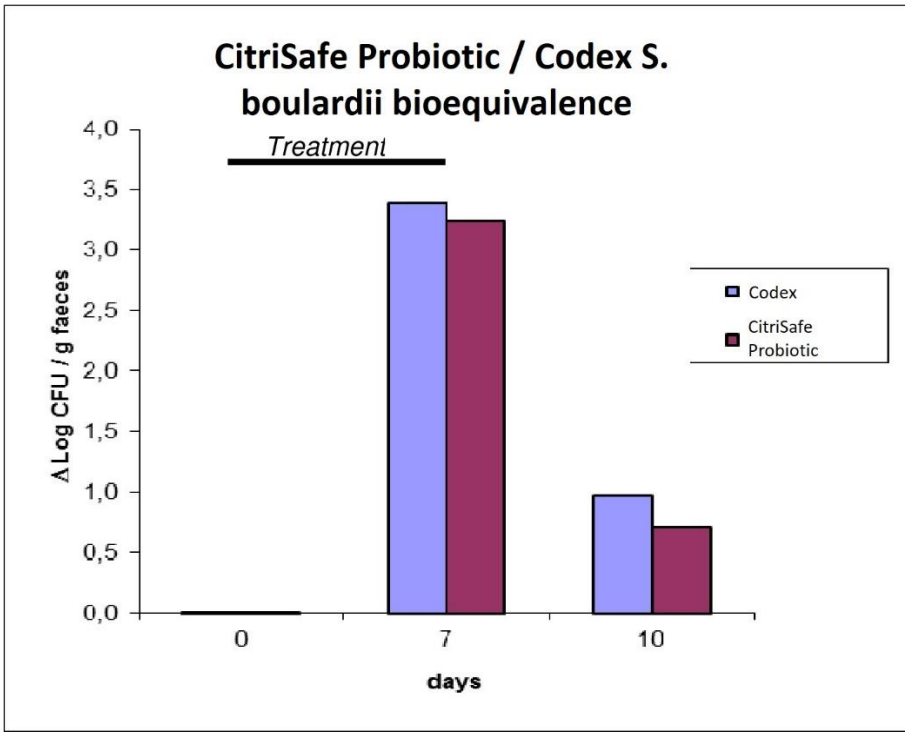
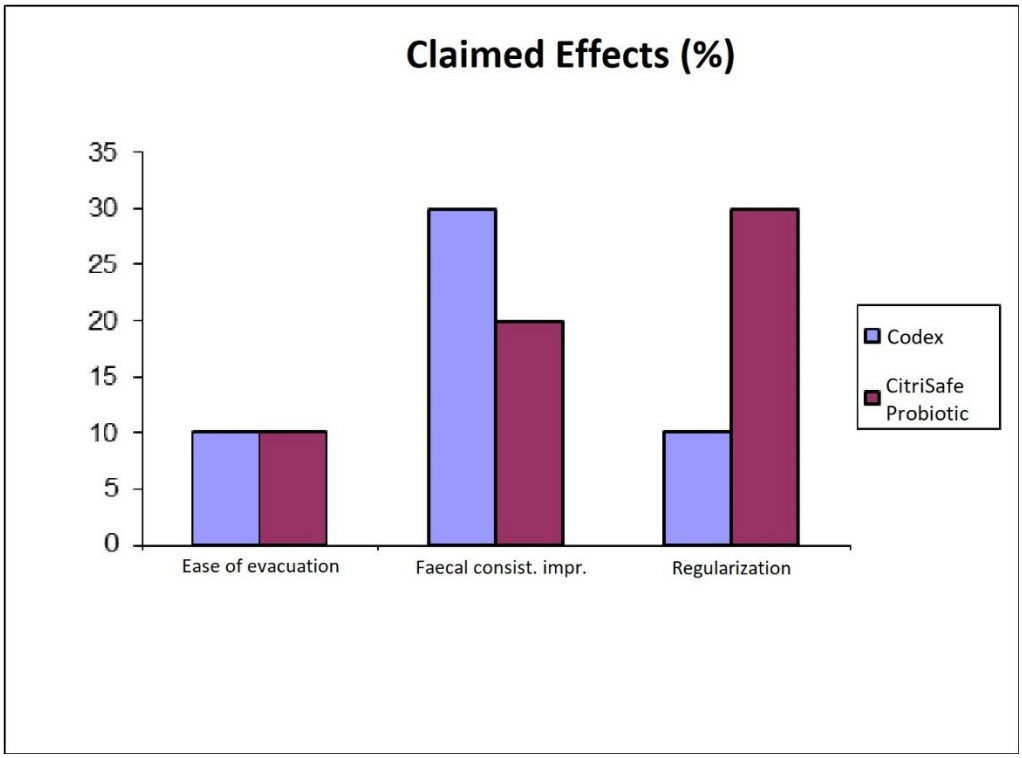


Figure 2



Conclusion

CitriSafe Probiotic™ showed a temporary colonization of the gut in healthy volunteers, showing a peak in CFU at the end of the administration period, the presence of S. boulardii living colonies was still appreciable 48 hours after the end of treatment at a level still significantly higher than prior to treatment. CitriSafe Probiotic™ colony numbers raised and decreased in a comparable way with the reference product Codex. A significant improvement in promoting intestinal regularization vs. Codex was claimed by volunteers.

Publication

Methodologies and procedures utilized in the trial are reported in a report describing volunteer enrolment, inclusion and exclusion criteria, microbiological methods for S. boulardii determination in faeces, also reporting questions and answers of the questionaire.

Note about Scientific Collaboration References

The study was conducted by AAT (Advanced Analytical Technologies), a CRO which is a spin-off company of Università Cattolica del Sacro Cuore, located in Piacenza, Italy, operating to offer bioequivalence studies. AAT also conducts microbiological analysis on the biological samples generated during clinical trials.