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**Trial 125: Evaluation the effects of the lactic yeast *Kluyveromyces marxianus fragilis B0399* added to the fermented milk containing *Bifidobacterium lactis BB12, Streptococcus thermophilus and Lactobacillus bulgaricus* , in subjects with intestinal dysfunctional symptomologies not tied to organic pathologies.**
**A controlled, randomized monocentric double blind clinic study.**

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List of abbreviations: IBS -- irritable bowel syndrome, RCT—randomized controlled trial, QOL – QOL, SCFA – Short-Chain Fatty Acid, CFU –colony forming units, FM: fermented milk

**Introduction:**

Dysfunctional symptomology is a common gastrointestinal disorder, characterized by coexisting abdominal pain/discomfort and alteration in the frequency and composition of the feces. This

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frequently leads to a worsening of the quality of life which have a repercussion in the social sphere and personal functions of those afflicted, equivalent to organic, chronic diseases.
It is estimated that about 10 – 15% of the world's population suffers from functional gastrointestinal disorders (IBS); this figure is quite probably an underestimation since only a small percentage of these subjects report their symptomology to their doctor, while the greater percentage use self-medication.
As of today, there is no unanimous agreement regarding pharmaceutical therapy—we are basically limited to reducing the symptomology; a therapeutic target has not yet been found which can alter the natural history of the subject. .[7,8,9]
Physiopathological studies have not yet explained with precision the genesis of this symptomology, but theories which imply alterations of gastrointestinal motility and of visceral perception are today the most "popular" [14,15,16].
It has been recently demonstrated that gastrointestinal infections have a role, although it has not yet been completely defined, as well as a state of mucosal chronic microinflammation in the genesis of IBS.
Retrospective and prospective studies have documented the onset of IBS after gastrointestinal bacterial infections, a state of low-degree mucosal inflammation and of immunoreactivity.
Alterations in the intestinal bacterial flora also seem to play a role in the genesis and in physiopathological maintenance. Today, however, the major part of bacteria which colonize the intestine (>80%) are still unknown to us and there are no existing microbiological techniques capable of growing them in a culture. [32].
Studies in microbiomics and metabolomics estimate that the quantity of DNA present in the intestinal lumen, and consequently the metabolic possibilities che ne derivano, which derive, are even greater than those in human DNA. It is estimated that the DNA of the intestinal microbiota (largely made up of bacteria, fungi and bacteriophage) can encode more than 300.000 genes which, when added to the 30.000 structural genes of the human genetic patrimony, allow infinitely greater metabolic capabilities for the human "superorganism"[36].
These hypotheses rewrite the history of biology by discovering, inside the intestinal lumen, a microbiote with infinite metabolic and physiological capabilities and show the human being to a symbiont form of life with its own mutualist flora [31].
From the capacity to determine the bacterical DNA of the human gut microbiota both qualitatively and quantitatively, and from the possibility of differentiating the bacterial flora of a healthy individual from one who is sick, we have witnessed a renewed interest in probiotic therapy, in both functional and organic pathologies (f.e. *Saccharomyces boulardii* in poliposis and in intestinal carcenogenesis; therapies with probiotics in ulcerative rectocolitis and in Crohn's disease).
Although much has been stressed and described regarding bacterial diversity, information on the eucariotica of the gut microbiota (yeasts) in scarse, and there are no omnicomprehensive studies of a culture-independent nature based on sequential methods available in literature.
Recently, a study was conducted on a small group of healthy volunteers; the results found in this work show how eucariotc diversity of the human intestinal ecosystem is low and largely temporally unstable. Specific analyses of the fungi population have indicated that an evident disparity exists between the fraction that can be cultivated, referable at this time to the well-known *Candida* species, and the fraction that cannot be cultivated. The latter is principally populated by members of the *Gloeotinia*/*Paecilomyces* and *Galactomyces* geni. The results focus attention on the presence of an intestinal eucariotic population not previously studied which could have a functional role which is still unknown in both healthy subjects and those suffering from intestinal pathologies [35].

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In certain circumstances such as diarrhea and antibiotic treatment, but also change of diet, stress and physiological changes in the intestine, the intestinal microbiota can undergo substantial alterations in terms of microbic classes represented and relative abundance. These dismicrobisms can have general negative effects on the health of the "host" and induce an increase of the susceptibility of the infection on the part of enteropathogenic bacteria.
There are various theoretic reasons, which indicate that probiotics can represent an effective preventative treatment and reduce symptoms of abdominal discomfort; most importantly, various strains of probiotics contain an antibacterial and antivirial capacity which can prevent or modify the course of post-infective modifications that determine the onset of these symptoms [33].
It has been demonstrated that certain probiotics have an intrinsic anti-inflammatory activity on the intestinal mucosa and by reducing the mucosal inflammation; they can modulate the immuno-mediated activation of the neurons and therefore modifying neural traffic between the intestine and the central nervous system [34].
Recently, there has been major interest for the properties of use of lactic yeasts on the gastrointestinal apparatus. The most studied at this moment is *S. boulardii.* Systematic studies have shown a high infinity between the gene from the families of *S. boulardii* and *Kluyveromyces* [37].
It is well-know, in clinical practice, that the administration of strains of lactic yeast is more effective in cases of diarrhea associated with the use of antibiotics. *Kluyveromyces marxianus B0399* has a profile of antibiotic resistance which guarantees vitality even during antibiotic therapy [25,26,27,28,29].
It has been demonstrated that strains of yeasts have immuno-modulating, anti-inflammatory and anti-oncogenic capabilities. For a more detailed account, see recent overviews found in literature [38,39].

Nonetheless, the number of scientifically acceptable studies which have evaluated the effect of probiotics on the gastrointestinal symptomology is relatively small. Some studies have implied the efficacy of the lactobacilli and bifidobacteria species, but just a few have directly set up comparisons between various formulations [1,3,4,5,18,19,20,21,22,23,24].
A number of studies which evaluated the effects of the administration of *Kluyveromyces marxianus fragilis B0399* in humans has recently been presented to the Italian Ministry of Health [41,42,43].

**Objective of the study**
The objective of the clinical study is to evaluate the effects of fermented milk containing *Bifidobacterium BB12* supplemented with the lactic yeast *Kluyveromyces marxianus fragilis B0399*, in subjects with intestinal dysfunctional symptomologies not tied to organic pathologies.

The results were subsequently evaluated as modifications of the symptomology scores of the basal state of the subject and of the "control" group, in order to avoid interpersonal differences in the perception of the symptoms.

End-point :

- To evaluate the modifications of the basal state and of the "control" group, after the administration of fermented milk supplemented with *Kluyveromyces marxianus fragilis B0399, of* the symptomatological scores utilized in the daily clinical diary, relative to abdominal pain, bloating, number of daily evacuations and characteristics of the evacuations.

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- To evaluate the perception of the subject regarding the global assessment of the treatment: a personal judgment on the efficacy and on the personal satisfaction at the end of the period of treatment and at the end of the period of wash-out.
-

**Materials and methods**:

Layout of the study. The protocol of the study together with the calculation of a sufficient number of subjects were preliminarily approved by the Independent Ethic Committee of the University Hospital Institute of the Sant'Orsola Polyclinic of Bologna–Malpighi (Italy). It is a controlled, randomized monocentric double blind study on the comparison between fermented milk with *Streptococcus thermophilus, Lattobacillus bulgaricus and Bifidobacterium lactis BB12* as a base, and fermented milk with the same base but with the addition of *Kluyveromyces marxianus fragilis B0399*, in parallel groups, in subjects without organic pathologies of the gastrointestinal tract with intestinal dysfunctional symptomoligies.
The study was articulated in a phase of collection of basal (preliminary) data, a phase of treatment and one of wash-out, for a total duration of 8 weeks for each subject.

Calculation of the number of subjects to be enrolled in study

The estimate of the number of subjects to enroll in the study was made according to the procedure described by Dupont e Plummer WD [1, 2] using the software "PS Power and Sample Size Calculations - Version 2.1.30, February 2003" - Department of Statistics - Vanderbilt University - Nashville, TN, USA.
The size in number was estimated to identify at least a 40% difference in the response between the group in study and the group with placebo, considering that the data in literature shows that 30% of the patients with placebo have a response.
For this calculation the following level of significance was considered: 0.05 (alfa:error of first kind) with a power of 0.90 (1 – beta – error of second kind).
The calculation of the number of subjects resulted in a value of 36 subjects to be treated in each group.
To identify the specific effect of the active treatment, a protocol evaluation was also conducted on the subjects who had actually been administered the treatment. It was theorized that there would be a possible frequency in interruption of treatment and/or drop-outs of about 10%. Therefore, it was agreed to enroll 40 subjects per group in order to obtain about 36 subjects per group, which would be useful for this evaluation.

Treatments in study. The subjects were randomly divided into two groups of treatment.

The "control" group (code AB778) was administered a basal fermented milk containing *S. thermophilus, Lb. bulgaricus and Bifidobacterium lactis* BB12 ($4 \times 10^9$ CFU/dose) (placebo)

The "active"group (code AB 262) was administered the same basal fermented milk containing *S. thermophilus, Lb. bulgaricus and Bifidobacterium lactis* BB12 ($4 \times 10^9$ CFU/dose), but supplemented with *Kluyveromyces marxianus var. fragilis B0399* ($10^7$ UFC/dose), produced by Turval Laboratories of the Scientific Park of the University of Udine (product under exam.).

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The two products were peach flavored and were undistinguishable, one from the other, in appearance, aroma, texture and flavor

The subjects ate one jar of fermented milk a day, at any time during the day, for the entire period of treatment (4 weeks).

Assignment of treatment. At the end of the basal period, the subjects were randomly divided into one or the other of the two groups of treatment in study.

Phases of study. The study was divided into 3 periods, as follows:
- Basal (2 weeks)
- Treatment (4 weeks)
- Wash-out (2 weeks)

| *t0* | *t0+2 weeks* | *4 weeks* | *2 weeks* |
|---|---|---|---|
| Signature of consent for release of information | Basal period | Period of treatment | Wash Out |

From the moment of enrollment and of the signature of consent for the release of information, clinical evaluations were initiated— information on the symptomology of the subjects at the end of the basal period, the end of the 4 weeks of treatment and after 2 weeks of wash-out, was collected.

Subjects. From February 2009 to June 2009, 92 subjects of both sexes were enrolled, under the supervision of the gastroenterology ward of the S'Orsola-Malpighi Hospital of Bologna. All the subjects met the criteria of inclusion and exclusion; in particular, all suffered from irritable bowel syndrome, according to the diagnostic criteria of Roma III [2].
All enrolled subjects over 40 years-old underwent at least one invasive or radiological exam, which would exclude organic pathologies of the colon.
Subjects with serious clinical conditions were not enrolled—those with chronic organic pathologies and those mentally incapacitated and therefore unable to sign a consent for the release of information.
Parallel therapies. None of the subjects in study at the moment of enrollment and during the study took neither drugs related to the gastrointestinal system, nor supplements or foods with lactic ferments or added fiber. The subjects during the course of treatment did not take antibiotics or corticosteroids.

Variables in study. Each subject was asked to fill out a daily clinical diary, in which to write down the following daily symptoms:
- bloating and/or meteorism (scale of 0 to 10)
- abdominal pain or discomfort (scale of 0 to 10)
- number of daily evacuations (number)
- characteristics of the evacuation (scale of 0 to 10)

Every two weeks the solidity of the feces was evaluated, according to the Bristol Stool Scale [48].
At the end of the treated period and at the end of the wash-out period, the experimenter personally asked the subjects to give a global assessment of the efficacy of the treatment.

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Daily diary. Each subject, for the entire period of study, was asked to gather clinical information every day and to write it down on a daily diary (see sample 1).

The diary contained information in 4 areas, three of which were on symptoms, and the absolute value on the number of daily evacuations. The symptoms under observation were bloating, abdominal pain and the characteristics of the evacuations.
The three symptoms were written down according to a scale of 0 – 10 (Visual Analogue Scale VAS) in which a low number represented a symptom which was rarely present and a high number if a symptom got worse (ex.- 0= absence of symptom. 10= maximum symptomology).
The patients were directly instructed by the clinical experimenters in how to fill out the daily journal. The value attributed to bloating also includes meteorism.
The pain symptom also includes the sensation of abdominal discomfort.
The characteristics of the evacuations is a score (a value of 1 – 10) in which the patient indicated the difficulty in bowel movements [5]. The unification in a single variable of the symptomology of difficulty in evacuation, of urgency and incomplete evacuation, enables the evaluation of the effects of a treatment in patients who tend to be constipated as well as those who suffer from diarrhea or alternating states, thus eliminating the difficulty of a statistical analysis of the data. The daily journal followed the indications of an International Committee coordinated by Jan Irvine [50] [51].

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Statistical analysis.
For the end-point statistical analysis the "intention-to-treat "(ITT) approach was applied.
The cases of interruption of treatment were therefore considered as a failure of treatment.
However, a "per-protocol" (PP) evaluation was also conducted on subjects who were actually treated in order to quantify the specific effect of the active treatment. The effects of the treatments were evaluated in both groups in both the end of the Treatment (T1) and the end of the Study (T2), considering as a base value the evaluation at the end of the registration phase (T0). Average, standard deviation, confidence interval at 95%, range and frequencies were utilized as descriptive statistics.
For the comparison of the end-point variables between the two treatments, the exact test by Fisher was used. The variance analysis (ANOVA) was employed to analyze the ordinal variables. The McNemar test and ANOVA to measure repetitions were used to evaluate the significance of the modifications observed within each group in treatment regarding dychotomic and ordinal variables, respectively.
In regards to the symptomatological scores, the Bristol Stool Scale and the number of evacuations, the Mann-Whitney test was utilized for the comparison between groups and the Wilcoxon test for the comparison of modifications within groups.
The overall impression of efficacy was compared between groups through the chi-square test for linear trend.
Probability values at two tails $P<0,05$ were considered statistically significant.
For the analysis of the data, the program "Statistical Package for the Social Sciences (SPSS) was used.
We evaluated the layered results for weeks, in both the treatment phase and wash-out phase.

**Results**

| **Group** | **Code** | **Kluyveromyces m.f. B0399** |
|---|---|---|
| *Active* | AB262 | + |
| *Control* | AB778 | - |

Demographic characteristics of the subjects. 92 consecutive subjects, of both sexes, resulted eligible, ranging in age from 18 to 65. The demographic data of the eligible subjects, expressed as standard average deviation +/-, is summarized in Tab. 1. No statistically significant differences between the two groups in study were found, as can be seen in the charts.

Tab.1 – Demographic characteristics of the eligible subjects

| | **Totals** | **AB262** | **AB778** | **P** |
|---|---|---|---|---|
| *N°* | 92 | 46 | 46 | |
| *Age* | 38.5±13.6 | 37.3±14.3 | 39.7±13.0 | *0.244* |
| *Sex (M/F)* | 26/66 (M 28.3%) | 15/31 (M 32.6%) | 11/35 (M 23.9%) | *0.257* |

The data is expressed as standard average deviation +/-

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Of the 92 subjects, 7 declined to take part in the study and were therefore not considered in the statistical analysis. The characteristics of the subjects, which took part in the study, expressed as an average +/- DS, are summarized in Tab. 2.

Tab.2 – Demographic characteristics of the subjects who took part in the study (ITT)

| | **Totals** | **AB262** | **AB778** | **P** |
|---|---|---|---|---|
| *N°* | 85 | 42 | 43 | |
| *Age* | 37.6±13.1 | 35.3±12.5 | 39.8±13.5 | *0.106* |
| *Sex (M/F)* | 23/62 (M 27.1%) | 12/30 (M 28.6%) | 11/32 (M 25.6%) | *0.474* |

The data was expressed as average +/- standard deviation

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## Symptoms

### Bloating

In regards to the effects of the two products ("active" and "control") a significant reduction of the bloating score compared to the basal evaluation was observed, which was consistently significant from the second week of treatment until the end of the study in both groups (See results in Tab. 3.). The comparison between the two treatments did not show significant differences.

*Tab.3 Bloating – course in time and significance*

| | **AB262** | *active* | | **AB778** | *control* | |
|---|---|---|---|---|---|---|
| **week** | Mean | DS | P | Mean | DS | P |
| 1°basal | 5,161905 | 2,220999 | | 5,175703 | 2,020646 | |
| 2°basal | 5,183333 | 2,206359 | | 5,342857 | 2,050736 | |
| 1°treatment | 4,766234 | 2,317079 | 0,318 | 5,068615 | 1,975238 | 0,163 |
| 2°treatment | 4,531926 | 2,450845 | 0,037 | 4,518272 | 2,107191 | 0,001 |
| 3°treatment | 4,476757 | 2,498905 | 0,002 | 4,378073 | 2,050394 | 0 |
| 4°treatment | 4,121315 | 2,435302 | 0 | 4,495903 | 2,076222 | 0,005 |
| 1°wash-out | 4,462018 | 2,564456 | 0,013 | 4,129252 | 2,062684 | 0,001 |
| 2 wash-out | 4,428281 | 2,595818 | 0,041 | 4,45102 | 1,994183 | 0,014 |

*P in the central column ( P vs Basal)*
Result statistically significant: P<0.05

### Pain

The score for pain shows a significant reduction in the "control" group at the second week of treatment (P=0.012) and at the first week of wash-out (P *=0.003*). In the "active" group a significant result is reached at the last week of treatment (P=0.007), which is confirmed in the first week of wash-out (P=0.041) and confirms a trend in reduction compared to the basal score, even if insignificant (P=0.064) in the second week of wash-out (Results shown in Tab. 4).
The comparison between the two groups did not show significant differences.

*Tab.4 Pain – course in time and significance*

| | **AB262** | *active* | | **AB778** | *control* | |
|---|---|---|---|---|---|---|
| **settimana** | Mean | DS | P | Mean | DS | P |
| 1°basal | 4,104762 | 2,078113 | | 3,907143 | 2,065516 | |
| 2°basal | 3,97619 | 2,091927 | | 4,160173 | 2,185109 | |
| 1°treatment | 3,967532 | 2,149792 | 0,9 | 3,912338 | 2,208457 | 0,279 |
| 2°treatment | 3,617965 | 2,073187 | 0,156 | 3,594156 | 2,17505 | 0,012 |
| 3°treatment | 3,693878 | 2,266815 | 0,254 | 3,613511 | 1,854793 | 0,058 |
| 4°treatment | 3,349773 | 2,171116 | 0,007 | 3,559302 | 1,984838 | 0,124 |
| 1°wash-out | 3,552721 | 2,349452 | 0,041 | 3,375283 | 2,062279 | 0,003 |
| 2 wash-out | 3,478513 | 2,359353 | 0,064 | 3,863531 | 2,098381 | 0,501 |

Result statistically significant: P<0.05

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**Characteristics of evacuation**

The characteristics of evacuation do not show significant results in the "control" group in any of the periods in study. In the "active" group the course of the characteristics of evacuation shows a trend towards a decrease during the period of treatment, which becomes significant at the fourth week (P=0.043); in the wash-out period the reduction is confirmed and is statistically significant compared to the basal period in both weeks (P *=0.046 e P=0.007)*. (Results shown in Tab. 5.
The comparison between the two treatments shows a significant difference (P=0.007) in the second week of wash-out, in favor of the "active" treatment. AB262/

*Tab.5 Characteristics of evacuation – course in time and significance*

| | **AB262** | *active* | | **AB778** | *control* | |
|---|---|---|---|---|---|---|
| **week** | Mean | DS | P | Mean | DS | P |
| 1°basal | 4,725952 | 2,054239 | | 4,06806 | 1,807058 | |
| 2°basal | 4,572798 | 2,122179 | | 3,949821 | 1,795573 | |
| 1°treatment | 4,325763 | 2,06761 | 0,944 | 3,794173 | 1,689649 | 0,678 |
| 2°treatment | 4,075763 | 2,08295 | 0,153 | 3,761647 | 1,807657 | 0,555 |
| 3°treatment | 4,294595 | 2,078501 | 0,203 | 3,820635 | 1,85107 | 0,778 |
| 4°treatment | 4,009127 | 1,836198 | 0,043 | 3,641331 | 2,110442 | 0,598 |
| 1°wash-out | 4,072993 | 1,922917 | 0,046 | 3,96241 | 1,828453 | 0,581 |
| 2 wash-out | 3,994538 | 2,093749 | 0,007 | 4,122024 | 1,722304 | 0,173 |

Result statistically significant: P<0.05

**Number of daily evacuations**

The number of evacuations during the period of treatment shows a trend towards a decrease (regularization) in both groups; the control group shows a significant result at the second week of treatment (P=0.038). In the first week of wash-out, both groups show significant results (P*=0.027* and P*=0.022*) which in the control group is also confirmed in the second week of wash-out (P=0.047) (Results shown in Tab. 6).
No significant differences between the two groups are observed.

*Tab.6 Number of evacuations – course in time and significance*

| | **AB262** | *active* | | **AB778** | *control* | |
|---|---|---|---|---|---|---|
| **week** | Mean | DS | P | Mean | DS | P |
| 1°basal | 1,555873 | 0,827916 | | 1,248677 | 0,661147 | |
| 2°basal | 1,472063 | 0,762922 | | 1,400423 | 0,740331 | |
| 1°treatment | 1,33746 | 0,653896 | 0,166 | 1,339153 | 0,625325 | 0,281 |
| 2°treatment | 1,341534 | 0,524054 | 0,337 | 1,254497 | 0,645214 | 0,038 |
| 3°treatment | 1,377409 | 0,661335 | 0,421 | 1,275132 | 0,656119 | 0,113 |
| 4°treatment | 1,301938 | 0,673217 | 0,088 | 1,237831 | 0,824194 | 0,117 |
| 1°wash-out | 1,256589 | 0,688476 | 0,027 | 1,210931 | 0,68 | 0,022 |
| 2 wash-out | 1,323413 | 0,675515 | 0,178 | 1,193245 | 0,645564 | 0,047 |

Result statistically significant: P<0.05

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**Success of the treatment on the modification of each symptom**

No significant differences between the two groups were found in regards to the success of the treatment (number of subjects who obtained a reduction of score): symptoms of bloating, pain and number of evacuations.

In regards to the characteristics of evacuation (see Tab.7), the second week of wash-out shows a significantly major success of treatment (P *=0.026*) in the group which took the "active" fermented milk compared to the group which had the placebo or "control" fermented milk (67.6% vs. 38.7%) (Results and significance shown in Tab. 7 and 8).

Tab.7 – Reduction of the score vs. basal – Comparison between treatments (significance)

| **Week** | **Pain** | **Bloating** | **N° Evacuations** | **Characteristics** |
|---|---|---|---|---|
| *1st treatment* | 1.000 | 0.382 | 0.827 | 1.000 |
| *2nd treatment* | 0.261 | 0.369 | 1.000 | 0.815 |
| *3rd treatment* | 0.511 | 0.479 | 1.000 | 0.642 |
| *4th treatment* | 0.511 | 1.000 | 0.661 | 0.632 |
| | | | | |
| *1st wash-out* | 0.822 | 0.178 | 1.000 | 0.460 |
| *2nd wash-out* | 0.269 | 1.000 | 0.824 | 0.026 |

Results statistically significant: P<0.05.

Tab.8- Success relative to the characteristics of evacuation at the 2nd week of wash-out

| | ***Successes*** | ***Rate*** | ***Significance*** |
|---|---|---|---|
| *AB262* | 23/34 | 67.6% | *P= 0.026* |
| *AB778* | 12/31 | 38.7% | |

Results statistically significant: P<0.05.

**Solidity of the feces**

Nothing of statistical significance was observed regarding either the modifications of the Bristol Stool Scale induced by the two treatments, or in the comparison between the two groups.

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**Global impression of efficacy**

As of protocol, the subjects were asked to give a personal evaluation on the efficacy and on the personal satisfaction of the treatment at the 6th week of the study (end of the period of treatment) and at the end of the study (end of wash-out period).

The evaluations expressed by the subjects which were assigned to the group of "active" treatment were significantly better in both periods (respectively P *=0.002* e P *=0.001*) (See Tab. 9 and 10 and see Figure 1 and 2).

Tab. 9 – Global impression of efficacy 6th week (end of period of treatment)

| | | *Worsened* | *Stationary* | *Partial Improvement* | *Major Improvement* | **Significance** |
|---|---|---|---|---|---|---|
| ***AB262*** | N° | 0 | 8 | 18 | 16 | *P=0.002* |
| | Rate | 0% | 19.0% | 42.9% | 38.1% | |
| **AB778** | N° | 3 | 10 | 28 | 2 | |
| | Rate | 7.0% | 23.3% | 65.1% | 4.7% | |
| ***Total*** | N° | 3 | 18 | 46 | 18 | |
| | Rate | 3.5% | 21.2% | 54.1% | 21.2% | |

Tab. 10 – Global impression of efficacy 8th week (end of wash-out period)

| | | *Worsened* | *Stationary* | *Partial Improvement* | *Major Improvement* | **Significance** |
|---|---|---|---|---|---|---|
| ***AB262*** | N° | 1 | 18 | 17 | 6 | *P=0.001* |
| | Rate | 2.4% | 42.9% | 40.5% | 14.3% | |
| **AB778** | N° | 2 | 31 | 10 | 0 | |
| | Rate | 4.7% | 72.1% | 23.3% | 0% | |
| ***Total*** | N° | 3 | 49 | 27 | 6 | |
| | Rate | 3.5% | 57.6% | 31.8% | 7.1% | |

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**Discussion**

By definition, probiotics, on a commercial level, are preparations containing live microorganisms, which have the capacity to benefit health by preventing or treating specific pathological conditions.
Probiotic preparations have been used in both clinical practice and in scientific studies to improve abdominal symptomologies; the scientific substratum for which a clinical observation is observed has been investigated, and there is evidence in literature regarding certain pathological conditions, such as intestinal chronic inflammatory diseases, pouchitis and irritable bowel syndrome. Despite the wide scientific interest, the mechanism through which the clinical benefit is verified is still not completely clear.
The physiological mechanisms which seem to be the therapeutic targets of probiotics in IBS are the immune modulation, the intestinal motility and the modification of the intraluminal milieu.
The subjects suffering from IBS seem to have a persistent state of micro-inflammation which in time leads to alterations in the mucosal functions, with an increase in permeability, structural alterations and infiltration of the mucosa by inflammatory cells.
Furthermore, there is evidence that suggests that alterations of the colic bacterial flora are directly related to alterations of intestinal functions. In particular, there is an average decrease of the colonization of Lactobacilli in fecal samples in subjects with IBS with predominant diarrhotic symptoms.
The colic bacteria physiologically metabolize nutrients and substrate, generating a level of colic gas and short-chain fatty acids—an important nutritive substrate for the intestinal cells.
The bacteria of the intestinal microbiota also intervene in the entero-epathic circulation of bile acids. If the biliary acids, which are normally reabsorbed in the intestinal mucosa of the small intestine, come in contact with the colic mucosa, they can induce a secretion of anionic electrolytes and water, causing the clinical condition called bile acid diarrhea. Some bacteria, mainly Bifidobacteria and Lactobacilli, are capable of metabolizing and deconjugating bile acids that reach the colon, reducing the direct catharsis from bile acids; therefore, by eliminating the irritating and secretory stimulus there is a symptomological improvement of the subject.

From our data on the case histories a trend seems to emerge which confirms some of the hypotheses proposed; both products were effective in significantly reducing the dysfunctional symptomology in the subjects studied; in analyzing the data we see a significant reduction of abdominal bloating and meteorism, together with a regularization of the number of evacuations and an improvement in symptoms of pain in both groups in study.
It has already been hypothesized and partially demonstrated that the administration of strains of eukaryotes is effective and physiological in certain clinical situations, like, for example, "traveler's diarrhea" and diarrhea associated with the use of antibiotics [38,39]. These considerations derive from the resistance of eukaryote strains (lactic-fermenting yeasts) to the common antibiotics used in gastroenterological clinical practice. Also, it seems that the colonization of certain yeasts has a modulating effect on the composition of the intestinal microbiota [40].

In this randomized, double blind controlled study, the data on the effect of the lactic yeast *Kluyveromyces marxianus fragilis B0399* on the gastrointestinal symptomology in humans, is reported.

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*Kluyveromyces marxianus* is a fungus commonly present is fermented dairy products (cheeses and kefir). In particular, *Kluyveromyces marxianus fragilis B0399* was selected and has already been introduced in the zootechnical diet after studies evaluated by the EFSA and approved by the European Community (44, 45), the US-FDA (46, 47), and by Canada CFIA (52), as well as the human diet, on the basis of specific studies (41, 42, 43) which included the evaluation of the Ministry of Health.
As of yet, there is no literature on a controlled randomized study which evaluates the effects on subjects with intestinal dysfunctional symptomology or those suffering from IBS.
Studies *in vitro*, conducted to evaluate the possible implications of dietary technology, took *K. marxianus* B0399 into consideration as a possible fungus with probiotic effects [25,27]. In these models, the capacity of the fungus to both adhere to the enterocite and be able to grow in a culture even in the presence of bile (colic acid 0.1%) and remain vital after passing an acidic environment (gastric) was demonstrated.

In the rat, the diet supplemented with *K. marxianus* increases the fecal concentration of sterols and the cecal concentration of SCFA (Short-Chain Fatty Acid) [29]. This result derives (in the murine model) from the suppression of the dietary absorption of cholesterol, from the action on the entero-epathic circuit and from the modulation of the synthesis of SCFA.

The results of this clinical trial reveal, in first analysis, a profile of tolerance and effectiveness not inferior to other probiotic strains in study.

In regards to the basal data, the end-point of the study was fully reached by both treated groups ("controlled" and "active") registering a significant reduction of the bloating symptom as early as the second week of treatment (P=0.04) and confirmed during the entire period of study, a, reduction of abdominal pain and an improvement in the characteristics of the evacuation (i.e. of straining and urgency).

In regards to the comparison between groups, significant differences were registered to the advantage of the "active" treated group, for the effects on the characteristics of evacuation and for the satisfaction in the global assessment.

Specifically, in regards to the characteristics of the evacuations, in the second week of wash-out, a significantly major success in treatment (P *=0.026*) was obtained in the group which had taken the "active" fermented milk supplemented with *K. marxianus fragislis B0399* compared to the control group.

These results, as with any therapy with probiotics, are consolidated during the course of treatment, reaching statistics of significance after about three weeks of treatment, and confirmed during the wash-out period.
The result, which stands out the most, is the reduction (significant in regards to both the basal period and the comparison with the "control" group) of the score relative to the characteristics of evacuation. This, as protocol, is a parameter which is closely correlated to the problems derived from gastrointestinal dysfunctional pathologies. In fact, the reduction in the urgency to evacuate, mainly in subjects affected by diarrhea, and of the difficulty, in subjects affected by constipation,

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represents an important outcome, especially if not secondary to a modification of the compactness of the faeces.

This result, a significant one at the end of the treatment period and during the entire phase of wash-out, reflects the presupposed effect of modulation on the gastrointestinal mechanism on the part of a probiotic strain [10, 12, 24].
This clinical data represents the element, which determines the subjective perception of improvement on the quality of life and on the control of the gastrointestinal activity and symptomology.

Consequently, the result was a significant personal satisfaction in the subjects in study (see charts 9 and 10).
At the end of the study, the patients were asked to give not only a report on their daily evolvement of single symptoms but their personal global impressions as well. The trend of the two groups in study shows, without a doubt, a subjective perception of well-being in the "active" group (with *Kluyveromyces marxianus fragilis B0399*) compared to the control group, both at the end of the 4 weeks of treatment (P=0.002) and at the end of the period of wash-out (P=0.001).

In the set-up of this clinical trial, a control group which was administered fermented milk containing probiotic strains was selected, through which the efficacy of the strain of *Kluyveromyces marxianus fragilis B0399* in the control of intestinal dysfunctional symptomology was deducted; in particular, at least by the data in our possession, the lactic yeast in study guarantees the subject a sense of subjective well-being, reducing the sense of bloating, reducing personal discomfort and the difficulty in the control of intestinal functions.

**Concluding considerations**
The scientific works published in the past 10 years on the biological effects of probiotics in humans suggest that probiotics improve the sensation of intestinal wellbeing.

- Competitively excluding pathogenic bacteria
- Reducing the permeability of the intestinal barrier, increasing the production of mucous (Lactobacilli)
- Stimulating the production of anti-inflammatory cytokine (Bifidobacteria)
- Decreasing the motoria activity in the colon without altering the form of the feces
- Modulating abdominal pain
- Antagonizing gas producing bacteria
-

It has been amply demonstrated that every strain of probiotics has specific functions in the parameters mentionted aboved and/or other functions; the new frontiers in research are oriented towards these points in identifying new therapeutic strategies in the affections where the intestinal microbiote can be altered, like irritable bowel syndrome, diverticulitis, ulcerous colitis, Chron's disease, certain hepatopathies, and certain precancerous lesions.
This work aims to acquire ulterior information based on a controlled double blind clinical study.
This study was set up to evaluate if the administration of fermented milk containing *Kluyveromyces marxianus fragilis B0399* is able, in the brief time allotted, to improve certain symptoms which are particularly frequent in human pathology.

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The product examined proved to be effective in significantly reducing bloating and abdominal pain as well as improving the characteristics of evacuation.
Compared to the control product (not containing the yeast), the milk supplemented with yeast *Kluyveromyces marxianus fragilis B0399* resulted superior in its effect on the characteristics of evacuation, which represents one of the most difficult aspects to manage of this condition, as well as in the global assessment of the patient.

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Figure 1

**SYMPTOMS: AVERAGE PROGRESSION IN TIME OF MODIFICATIONS OF SYMPTOMS vs BASAL SYMPTOMS**

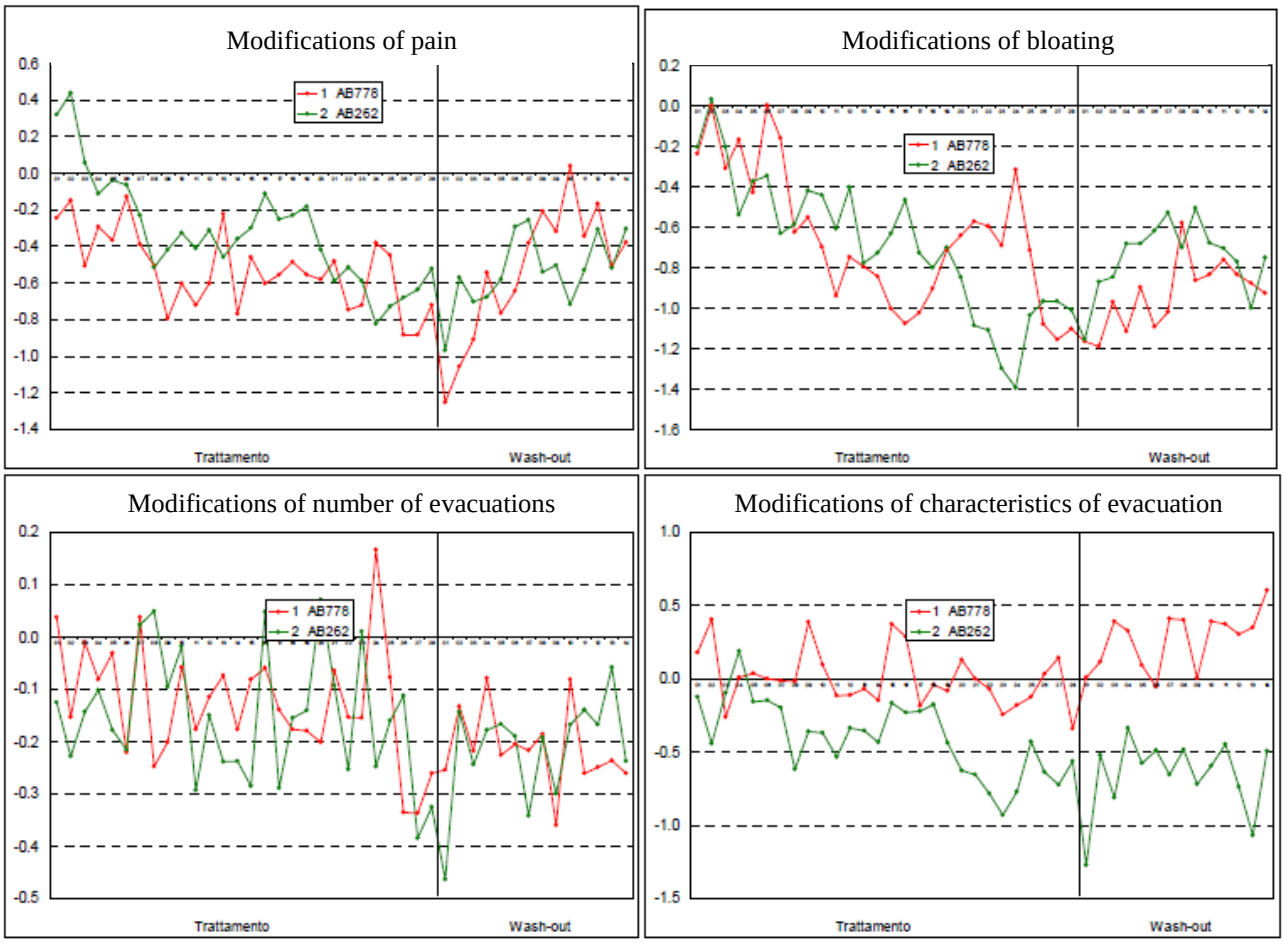

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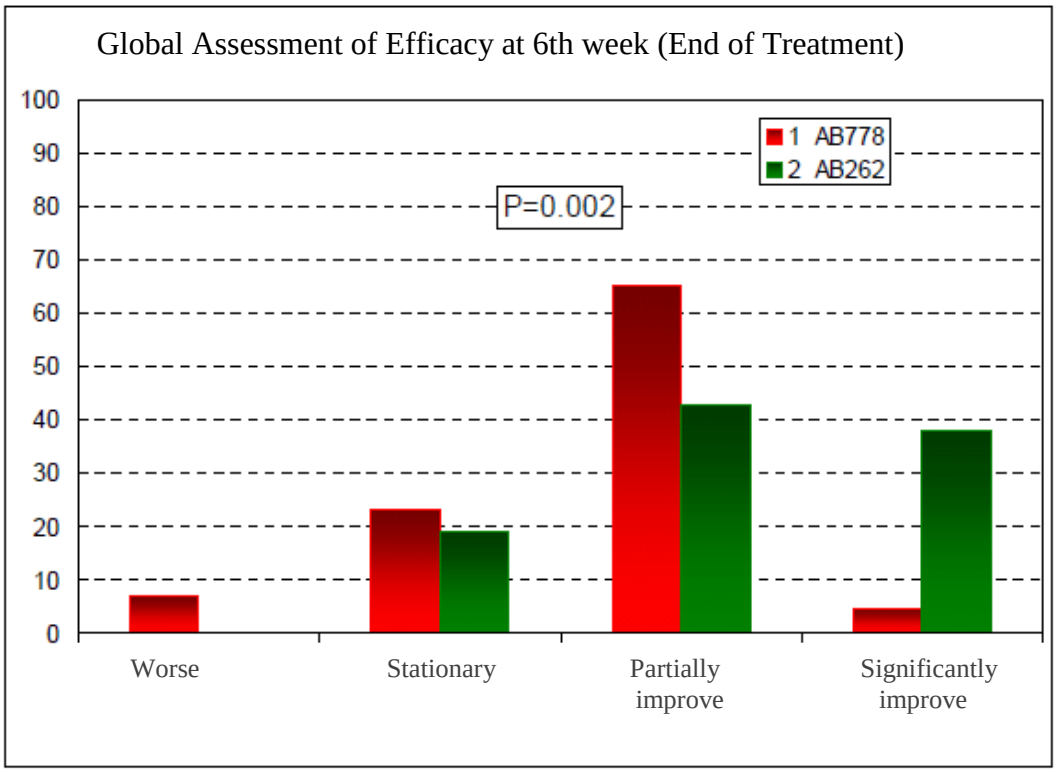
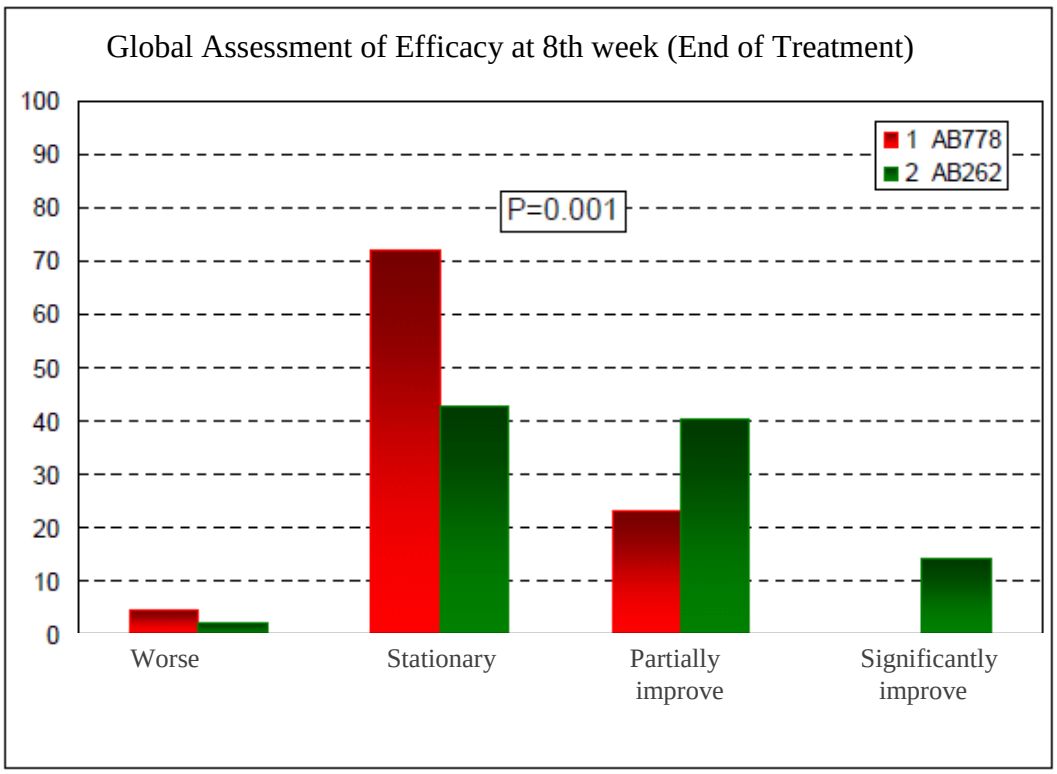
Figure 2

**GLOBAL ASSESSMENT OF EFFICACY**
Distribution of the frequency of answers in the two groups (percentages)



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| **SINTOMI PRESENTATI *** | | | | | | | | | | | | | | |
| **DOLORE ADDOMINALE** o malessere addominale | | | | | | | | | | | | | | |
| **GONFIORE** e/o meteorismo | | | | | | | | | | | | | | |
| **Numero ^ evacuazioni** nelle 24 ore | | | | | | | | | | | | | | |
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*** usare una scala da 1 (minimo) a 10 (massimo)**

**^ Segnare il numero**

**Spazio per note:**