Which role for Probiotics in Celiac Disease

Ruggiero Francavilla, MD PhD

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Celiac Disease

Celiac disease (CD) is an immune-mediated systemic disorder elicited by **gluten and related prolamines** in **genetically susceptible individuals** and characterised by the presence of a variable combination of **gluten-dependent clinical manifestations**, **CD-specific antibodies**, (HLA-DQ2 or HLA-DQ8 haplotypes) and **enteropathy**.

Fasano A, Catassi C. NEJM 2012;367:2419

Celiac Disease

At this time, the only treatment for CD is lifelong adherence to a **gluten-free diet**, which involves the elimination of grains containing **gluten, wheat, rye, and barley** in addition to food, products and additives derived from them.

Green PH. Lancet. 2003;362:383

Celiac Disease

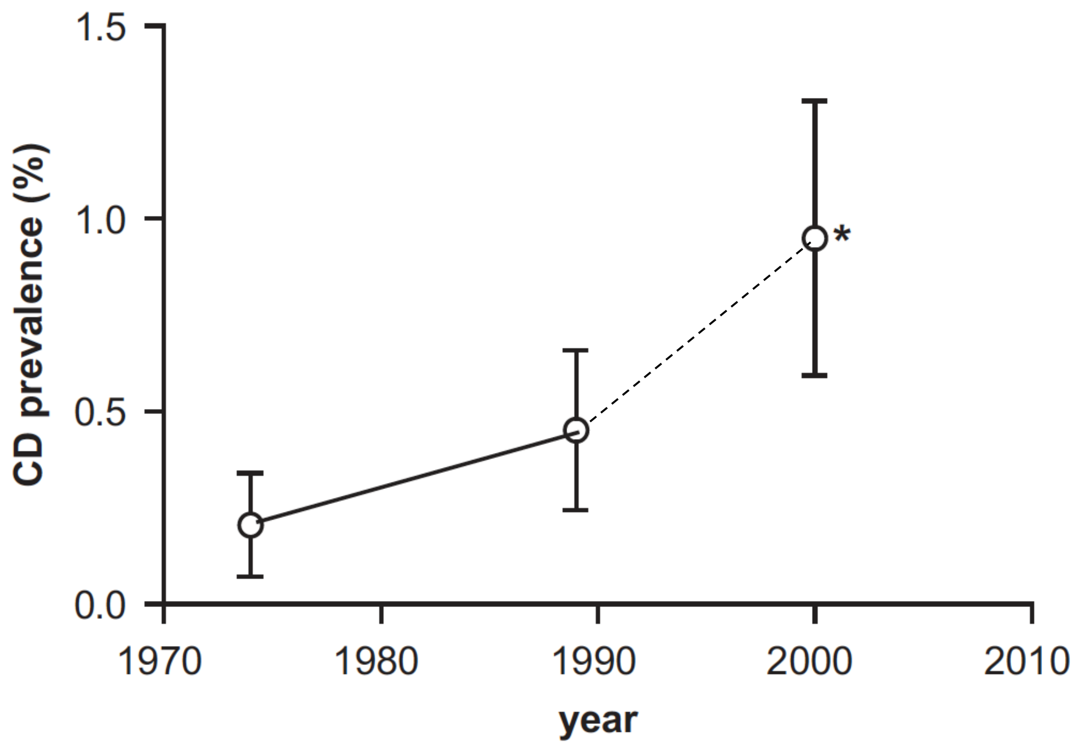
Adherence to GFD to improve symptoms, reduce the risk of complications, and confer health benefits *(i.e. improvement in bone mineral density)*.

However, studies have shown that **dietary transgressions** in patients with CD are common and can occur anywhere from **32% to 55%**

Silvester JA. Can J Gastroenterol.2007;21:557
Högberg L. Scand. J. Gastroenterol.2003;38:75

Increase of Prevalence

Natural history of celiac disease autoimmunity in a USA cohort followed since 1974



A recent US study showed that CD prevalence was only 0.2% in the year 1975, and increased 5-fold during the following 25 years

Catassi C. Ann Med. 2010;42:530

Increase of Prevalence: role of Environment

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changes in the quantity and quality of ingested gluten

infant feeding patterns

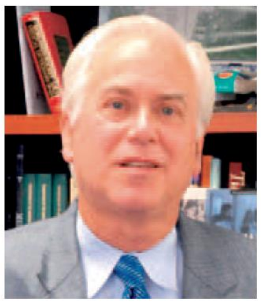
the spectrum of intestinal infections

gut microbiota colonization

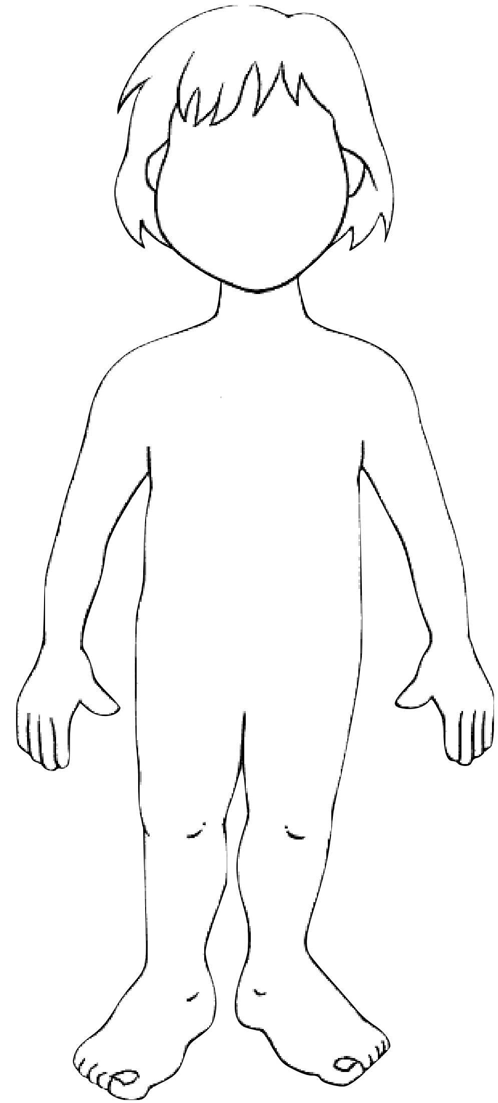
Who are we?

Indigenous microbes and the ecology of human diseases

Martin J. Blaser



Martin J. Blaser is the Frederick H. King Professor of Internal Medicine, the Chair of the Department of Medicine and a Professor of Microbiology at New York University School of Medicine, New York, USA.
E-mail: martin.blaser@med.nyu.edu



Blaser MJ. EMBO Rep. 2006;7:956

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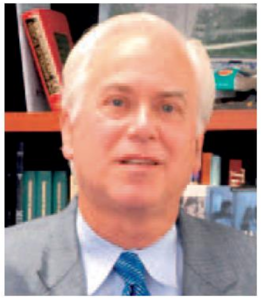
Who are we?

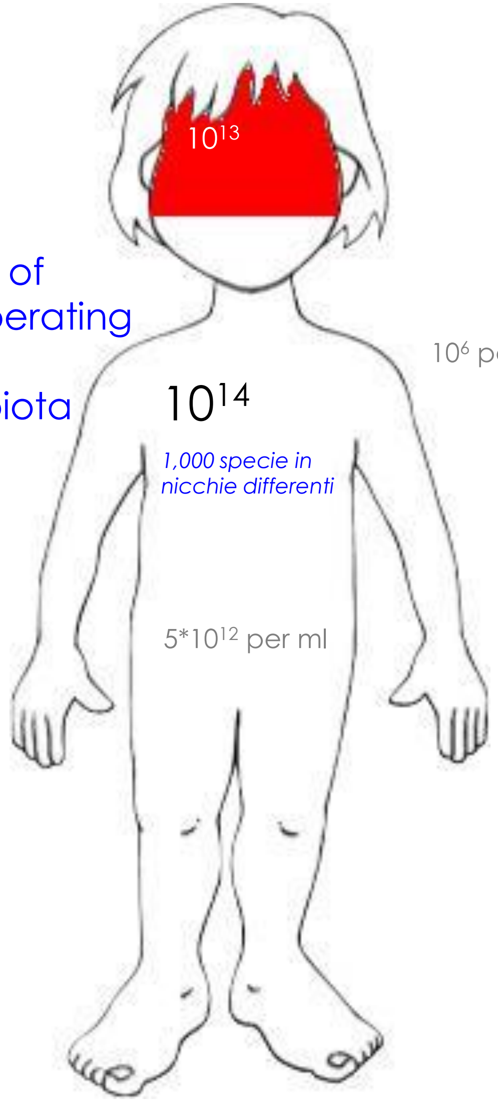
Indigenous microbes and the ecology of human diseases

Martin J. Blaser



Martin J. Blaser is the Frederick H. King Professor of Internal Medicine, the Chair of the Department of Medicine and a Professor of Microbiology at New York University School of Medicine, New York, USA.
E-mail: martin.blaser@med.nyu.edu

Massive assemblages of competing and cooperating microbes are known collectively as microbiota

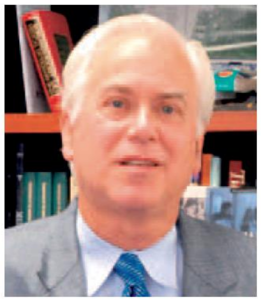


Blaser MJ. EMBO Rep. 2006;7:956

Who are we?

Indigenous microbes and the ecology of human diseases

Martin J. Blaser



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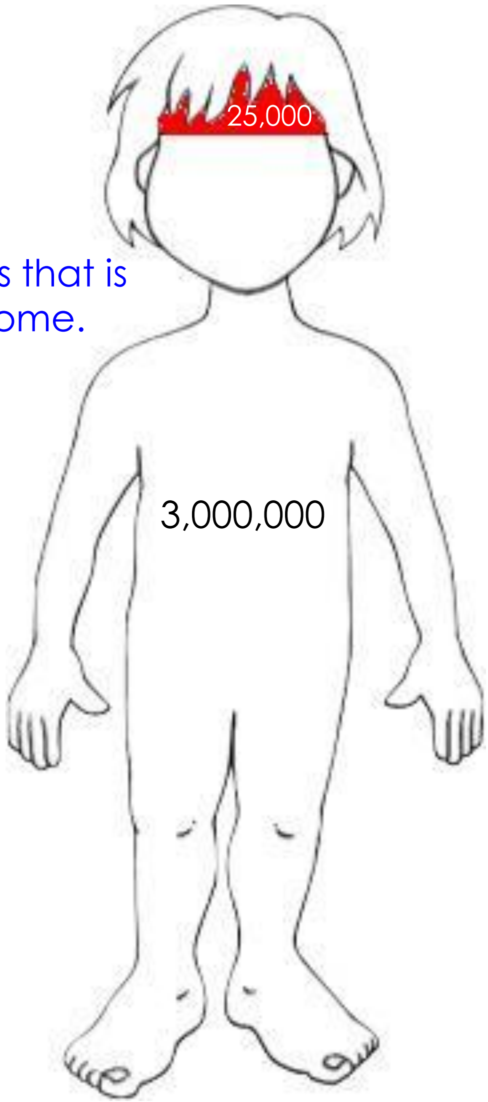
E-mail: martin.blaser@med.nyu.edu

The entire set of genes that is known as the microbiome.



Blaser MJ. EMBO Rep. 2006;7:956

Coevolution

Stable envrovment

Habitat

Food

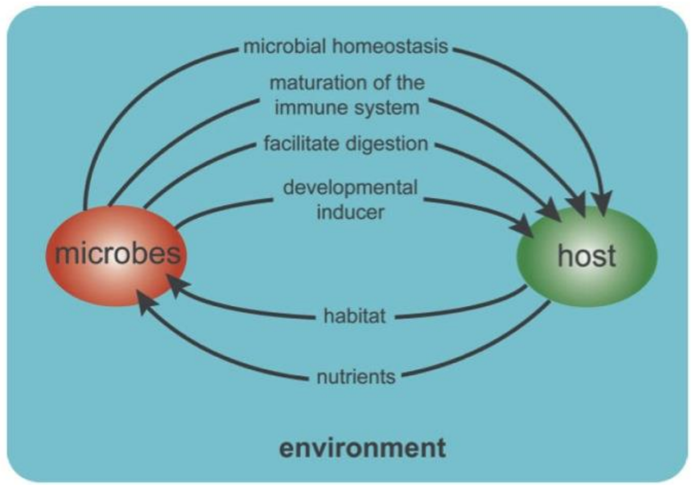
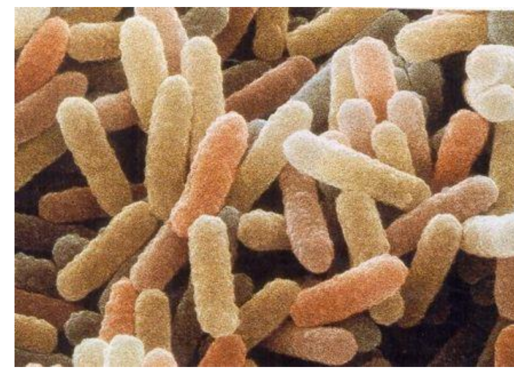


Figure 6. Schematic view of the interactions between host organisms and their microbiota.



Immune-modulation

Metabolic activities

Ann Rev Gen 2008;42:165

…biome

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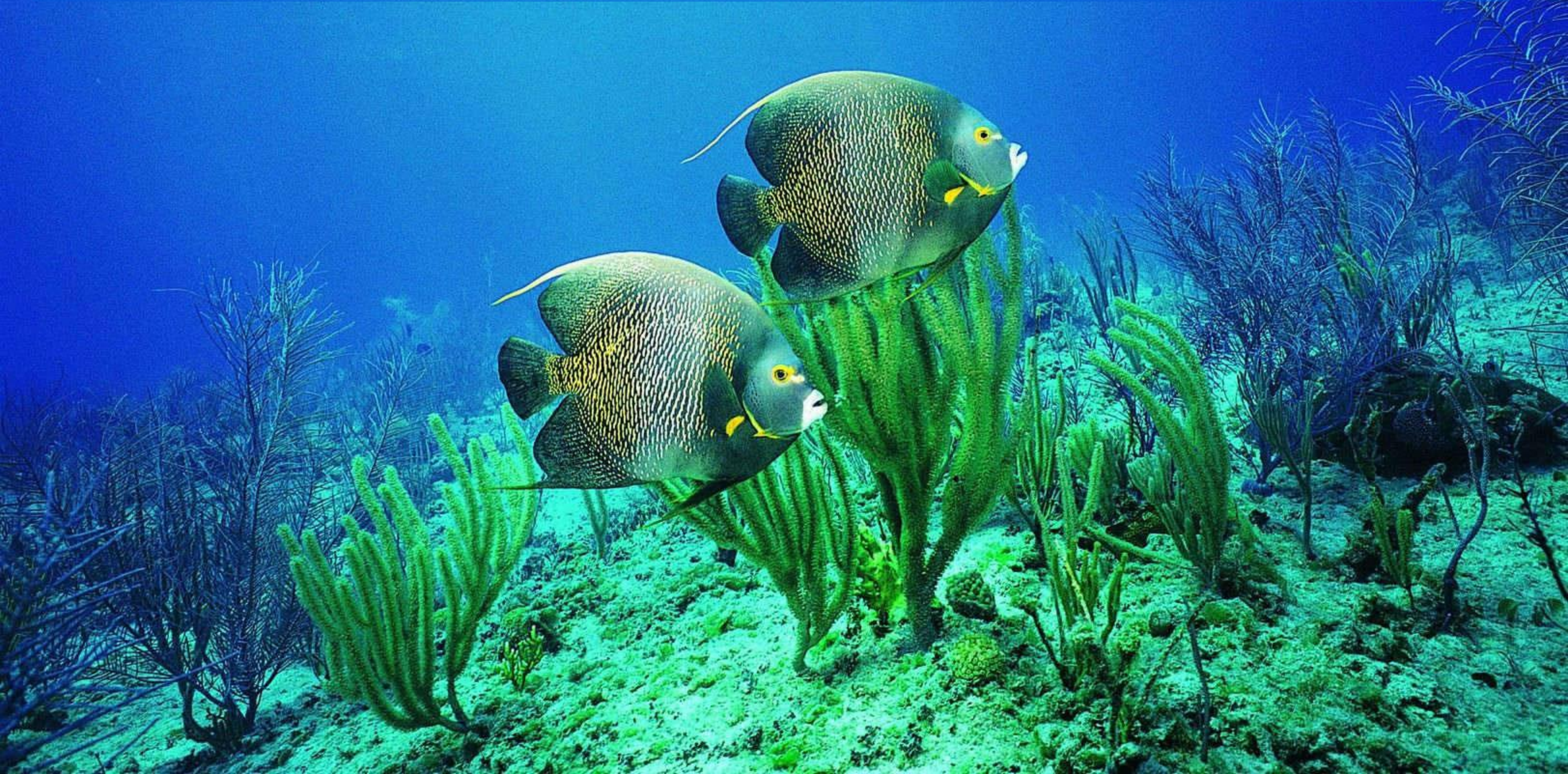
In ecology, **biome** refers to the sets of plants and animals in a community (such as a jungle, forest, or coral reef) in which an enormous diversity of species, large and small, interact to form complex webs of mutual support.

When a keystone species disappears or goes extinct the ecology suffers. It can even collapse



Disappearing Microbiota Hypothesis

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ESSAY

What are the consequences of the disappearing human microbiota?

Martin J. Blaser and Stanley Falkow

"For a number of reasons, we are losing our ancient microbe"

The loss of microbial diversity on and within our bodies is exacting a terrible price.

obesity, childhood diabetes, asthma, hay fever, food allergies, esophageal reflux and cancer, celiac disease, Crohn's disease, ulcerative colitis, autism, eczema

Disappearing Microbiota Hypothesis

ESSAY

What are the consequences of the disappearing human microbiota?

Martin J. Blaser and Stanley Falkow

Table 1 | **Changes in human ecology that might affect microbiota composition**

Change	Consequence
Clean water	Reduced faecal transmission
Increase in Caesarean sections	Reduced vaginal transmission
Increased use of pre-term antibiotics	Reduced vaginal transmission
Reduced breastfeeding	Reduced cutaneous transmission and a changed immunological environment
Smaller family size	Reduced early life transmission
Widespread antibiotic use	Selection for a changing composition
Increased bathing, showering and use of antibacterial soaps	Selection for a changing composition
Increased use of mercury-amalgam dental fillings	Selection for a changing composition

obesity, childhood diabetes, asthma, hay fever, food allergies, esophageal reflux and cancer, celiac disease, Crohn’s disease, ulcerative colitis, autism, eczema

Dysbiosis

Health

Disease

Diet

Hygene

Antibiotics

Life style

Pathogenic community

no single microbe is pathogenic alone. Instead, the community assemblage is an environmental risk factor that contributes to a disease state

Eubiosis

Dysbiosis

Ley R. Cell. 2006;124:837

Is this true for Celiac Disease?

Harmless bacteria

Lactobacillus, bifidobacteria

Potentially harmful bacteria

Bacteroides, Prevotella, E. Coli

regardless of whether CD was active or inactive (GFD)

Sanz Y. New York: Nova Science;2009.
Nadal I. J Med Microbiol.2007;56:1669.
Collado MC. BMC Microbiol.2008;22:232.
Collado MC. J Clin Pathol.2009;62:264.
De Palma G. BMC Microbiol.2010;10:63.
Di Cagno R. Appl Environ Microbiol. 2009;75:3963

CD dysbiosis and GFD

Some of these alterations *(e.g., increased numbers of enterobacteria or staphylococci)* are restored after adherence to a gluten-free diet, suggesting they are secondary consequences of the disease

Others *(increased Bacteroides, virulent-E. coli and decreased Bifidobacteria and Lactobacilli)* are associated with CD and, therefore, could play a more prominent role in this disorder

Sanz Y. New York: Nova Science;2009.
Nadal I. J Med Microbiol.2007;56:1669.
Collado MC. BMC Microbiol.2008;22:232.
Collado MC. J Clin Pathol.2009;62:264.
De Palma G. BMC Microbiol.2010;10:63.
Di Cagno R. Appl Environ Microbiol. 2009;75:3963

Dysbiosis and GFD

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Effects of a gluten-free diet on gut microbiota and immune function in healthy adult human subjects

Giada De Palma, Inmaculada Nadal, Maria Carmen Collado and Yolanda Sanz*

Table 1. Daily energy and nutrient intake before and after the gluten-free diet (GFD) intervention

(Mean values and standard deviations)

	Subjects before GFD (*n* 10)		Subjects under GFD (*n* 10)	
Diet composition	Mean	SD	Mean	SD
Energy				
kJ	7759·68	1446·91	7464·51	1263·28
kcal	1854·61	345·82	1784·06	301·93
Water (g)	2454·56	533·35	2764·96	464·18
Protein (g)	72·99	15·69	68·48	13·19
Energy from protein (%)	15·74	3·38	15·35	2·96
Fat (g)	78·69	21·12	71·95	19·00
Energy from fat (%)	38·19	10·25	36·30	9·58
Saturated fat (g)	23·21	11·17	22·42	6·55
Energy from saturated fat (%)	11·26	5·42	11·31	3·30
MUFA (g)	29·97	8·30	28·79	8·41
Energy from MUFA (%)	14·54	4·03	14·52	4·24
PUFA (g)	11·58	5·59	9·43	3·93
Energy from PUFA (%)	5·62	2·71	4·76	1·98
Cholesterol (mg)	262·36	181·37	266·76	115·07
CH (g)	212·41	55·42	218·87	69·05
Energy from CH (%)	45·81	11·95	49·07	15·48
Simple CH (g)	74·30	37·72	72·03	28·05
Energy from simple CH (%)	16·02	8·14	16·15	6·29
Polysaccharides (g)	116·63	51·62	62·95*	33·12
Energy from complex CH (%)	25·15	11·13	14·11	7·43
Dietary fibre (g)	19·52	10·78	17·56	9·13

CH, carbohydrates.
*Mean value was significantly different from that before the GFD ($P<0·05$; Student's *t* test).

This may contribute to the disruption of the delicate balance between the host and its intestinal microbiota which might favor the overgrowth of opportunistic pathogens and weaken the host defenses and not favor completely the normalization of gut ecosystem in treated CD patients.

De Palma G. Br. J. Nutr. . 2009;102:1159

CD dysbiosis and GFD

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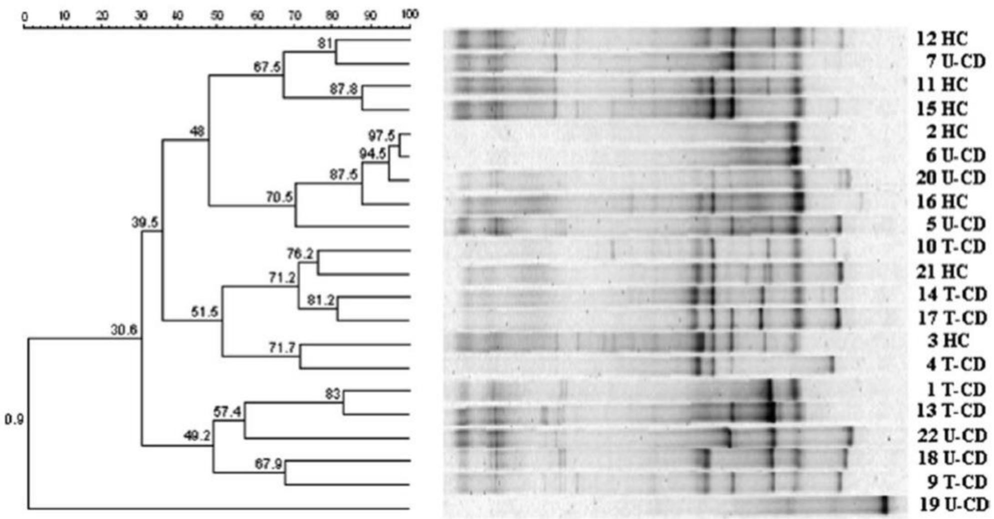
Different Fecal Microbiotas and Volatile Organic Compounds in Treated and Untreated Children with Celiac Disease∇†

Raffaella Di Cagno,[1] Carlo G. Rizzello,[1] Francesca Gagliardi,[2] Patrizia Ricciuti,[3] Maurice Ndagijimana,[4] Ruggiero Francavilla,[2] M. Elisabetta Guerzoni,[4] Carmine Crecchio,[3] Marco Gobbetti,[1] and Maria De Angelis[1]*



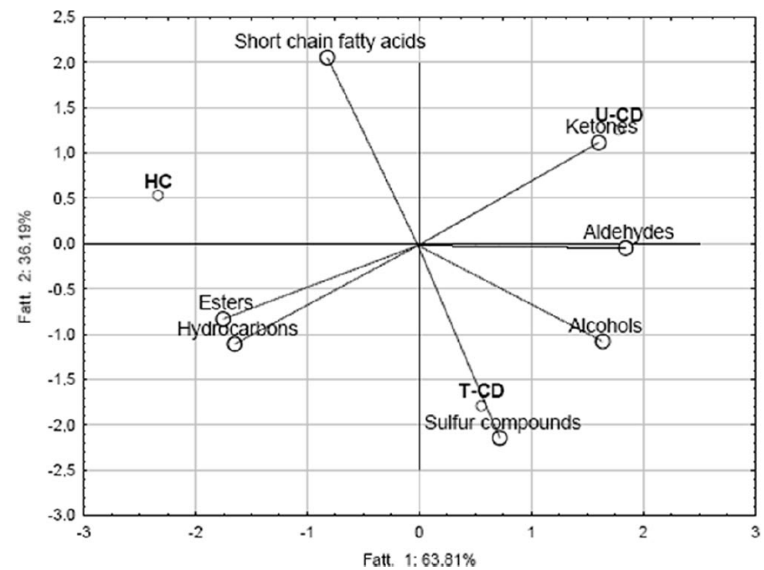


FIG. 4. Plot of the first and second principal components after PCA based on the median data for VOCs of T-CD, U-CD, and HC.

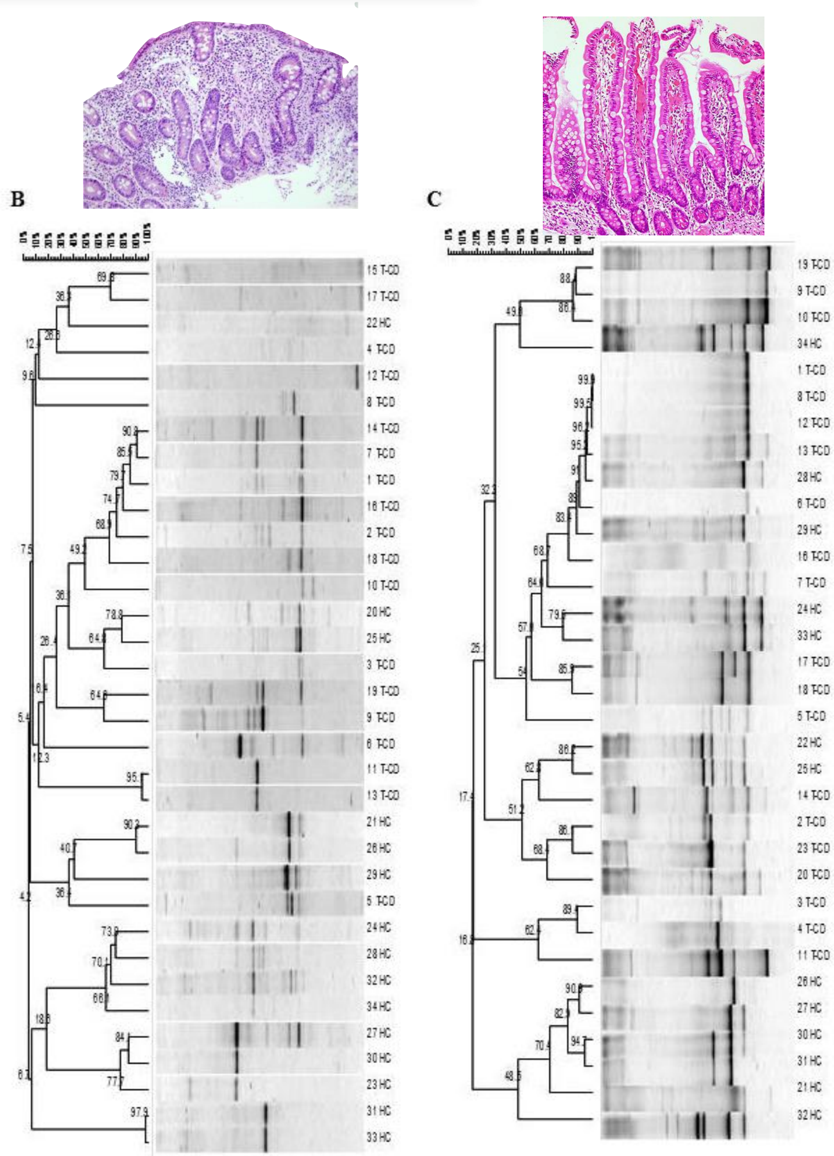
The percentages of Lactobacillus and Bifidobacterium species were lower in CD as compared to HC and remained lower after years of GFD. The median concentrations of volatile organic compounds varied markedly for HC, T-CD, and U-CD

Di Cagno R. Appl Environ Microbiol. 2009;75:3963

CD dysbiosis and GFD

Duodenal and faecal microbiota of celiac children: molecular, phenotype and metabolome characterization

Raffaella Di Cagno[1], Maria De Angelis[1*], Ilaria De Pasquale[1], Maurice Ndagijimana[2], Pamela Vernocchi[2], Patrizia Ricciuti[1], Francesca Gagliardi[3], Luca Laghi[2], Carmine Crecchio[1], Maria Elisabetta Guerzoni[2], Marco Gobbetti[1] and Ruggiero Francavilla[3]



We did not find bifidobacteria in biopsy specimens of CD subjects although present in fecal samples.

In addition, we showed a low level of microbiota diversity in biopsy specimens

Di Cagno R. BMC Microbiol. 2011;11:219

Increase of Prevalence: role of Environment

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Gut Microflora Associated Characteristics in Children with Celiac Disease

B. Tjellström, M.D.,[1,2] L. Stenhammar, M.D., Ph.D.,[2,3] L. Högberg, M.D., Ph.D.,[2] K. Fälth-Magnusson, M.D., Ph.D.,[3] K-E. Magnusson, Ph.D.,[4] T. Midtvedt, M.D., Ph.D.,[1] T. Sundqvist, Ph.D.,[4] and E. Norin, Ph.D.[1]

study of the SCFA pattern in fecal samples from children with CD: the results indicate that there is a difference in the metabolic activity of intestinal microbial flora in children with CD compared to that in HC.

Table 1. Short Chain Fatty Acid Levels of Children with Celiac Disease and Healthy Controls

	Short Chain Fatty Acids		
Type of Acid	Untreated CD	Treated CD	HC
Acetic acid†	50.6***	49.3***	25.4
	36	74	114
	(22.6)	(26.8)	(8.2)
Propionic acid	13.9	14.1*	11.6
	36	74	113
	(6.1)	(7.1)	(5.7)
i-Butyric acid	2.3**	2.2**	1.6
	36	74	113
	(1.0)	(1.3)	(1.1)
n-Butyric acid	15.4	15.7	14.9
	36	74	114
	(8.1)	(9.6)	(11.2)
i-Valeric acid	3.0**	2.8**	2.1
	36	74	114
	(1.4)	(1.9)	(1.6)
n-Valeric acid	1.8	1.8**	1.4
	36	74	114
	(1.0)	(1.1)	(1.2)
i-Caproic acid	0.3	0.2	0.2
	36	74	114
	(0.5)	(0.4)	(0.4)
n-Caproic acid	0.2	0.2	0.2
	36	74	114
	(0.3)	(0.3)	(0.3)
Total SCFA	87.4***	86.1***	57.1
	36	74	114
	(31.0)	(38.0)	(19.4)

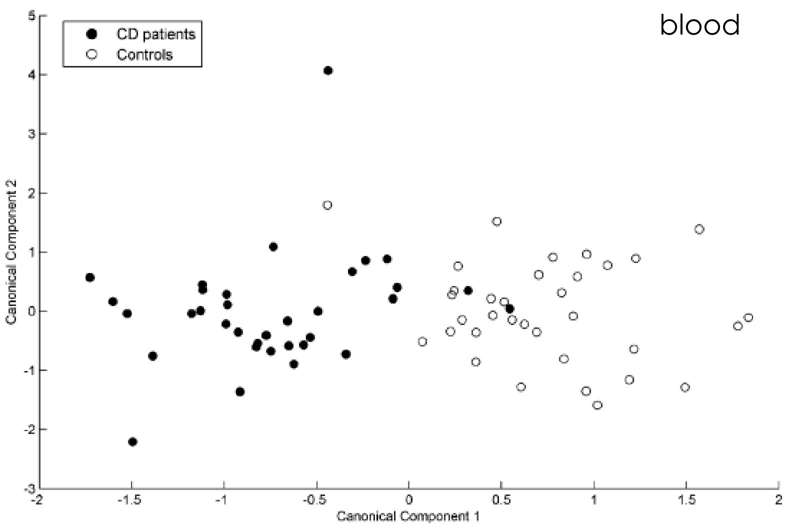
Tjellström B. Am J Gastroenterol. 2005;100:2784.

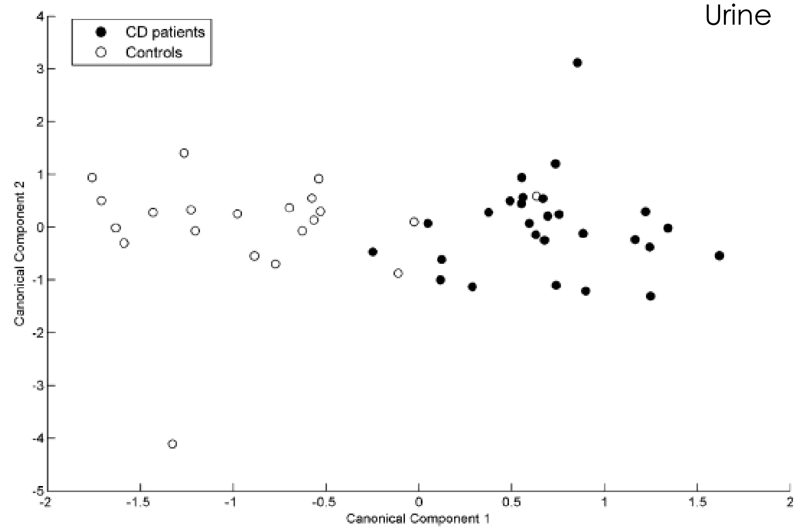
Metabonomic Signature of CD

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

The Metabonomic Signature of Celiac Disease

Ivano Bertini,*,†,‡ Antonio Calabrò,¶,# Valeria De Carli,¶,# Claudio Luchinat,†,§ Stefano Nepi,†,||,⊥
Berardino Porfirio,∇ Daniela Renzi,¶,# Edoardo Saccenti,†,||,⊥ and Leonardo Tenori†,||,⊥





NMR thus reveals a characteristic metabolic signature of celiac disease. Altered serum levels of glucose and ketonic bodies suggest alterations of energy metabolism, while the urine data point to alterations of gut microbiota

Bertini I. J Proteome Res. 2009;8:170

Probiotic in dysbiosis

Health

Disease

Eubiosis

Dysbiosis

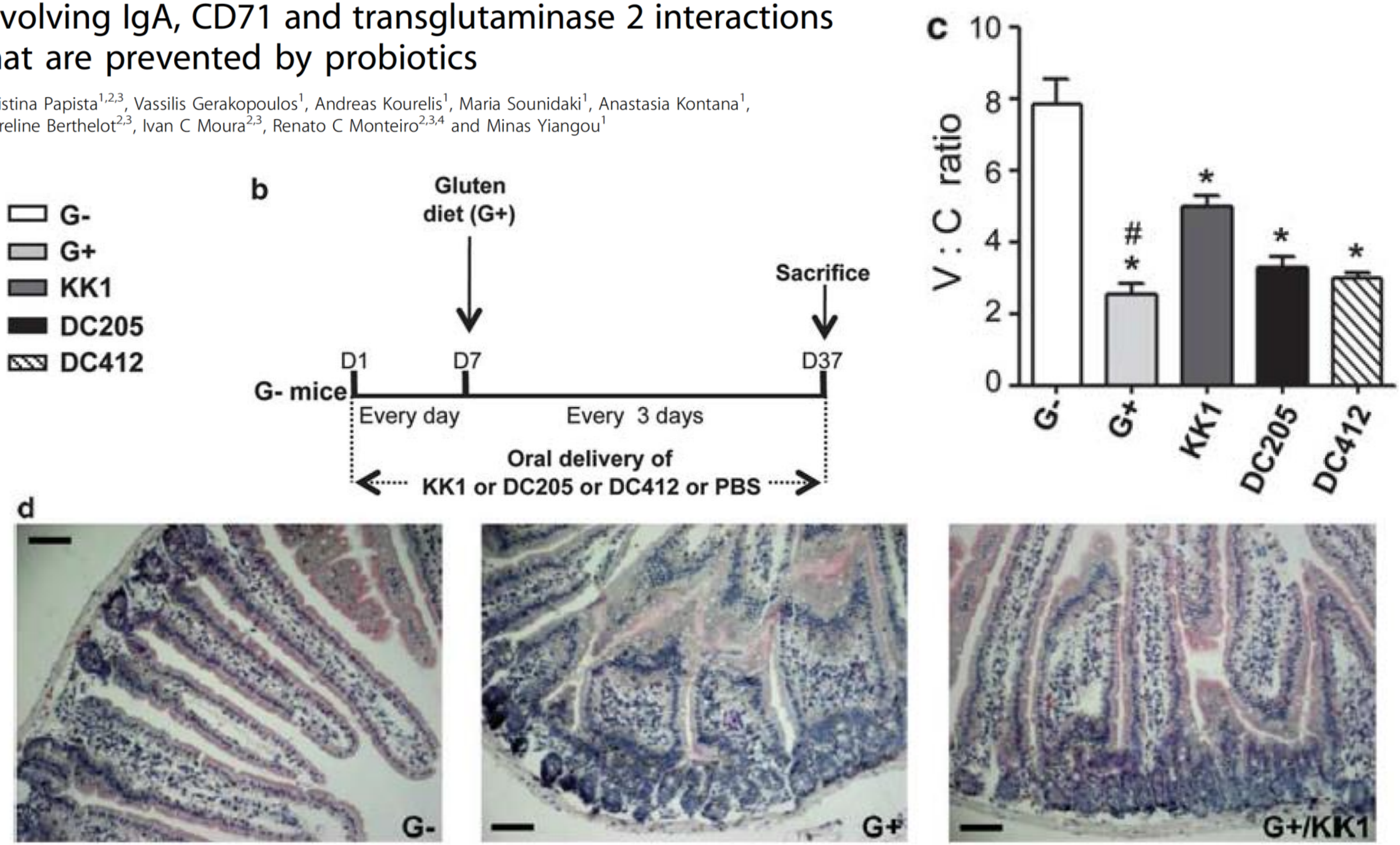
Ley R. Cell. 2006;124:837

Probiotic in CD – prof of concept

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

Gluten induces coeliac-like disease in sensitised mice involving IgA, CD71 and transglutaminase 2 interactions that are prevented by probiotics

Christina Papista[1,2,3], Vassilis Gerakopoulos[1], Andreas Kourelis[1], Maria Sounidaki[1], Anastasia Kontana[1], Laureline Berthelot[2,3], Ivan C Moura[2,3], Renato C Monteiro[2,3,4] and Minas Yiangou[1]



Papista C. Lab. Invest. 2012;92:625

Probiotic in CD – prof of concept

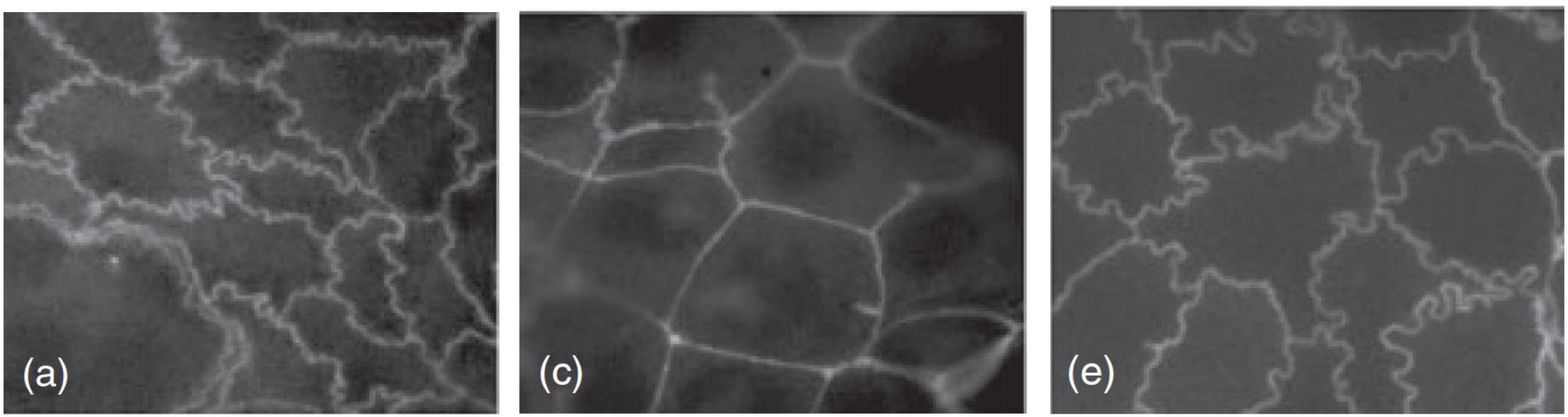
UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2008.03635.x

Live probiotic *Bifidobacterium lactis* bacteria inhibit the toxic effects induced by wheat gliadin in epithelial cell culture

K. Lindfors,* T. Blomqvist,* K. Juuti-Uusitalo,* S. Stenman,* J. Venäläinen,† M. Mäki* and K. Kaukinen‡
**Paediatric Research Centre, Medical School, University of Tampere, Finland, Department of Peadiatrics, Tampere University Hospital,*

The ability of live probiotics to inhibit gliadin-induced damage to human colon cells Caco-2



Normal tight junctions

Tight junctions after gliadin administration

Tight junctions after gliadin and probiotic administration

Lindfors K. Clinical and Experimental Immunology. 2008;152:552

Probiotic in CD at diagnosis

Exploratory, Randomized, Double-blind, Placebo-controlled Study on the Effects of *Bifidobacterium infantis* Natren Life Start Strain Super Strain in Active Celiac Disease

Edgardo Smecuol, MD,† Hui J. Hwang, MD,* Emilia Sugai, MD,* Laura Corso, MD,‡ Alejandra C. Cherñavsky, MD,§ Franco P. Bellavite, MD,* Andrea González, MD,‡ Florencia Vodánovich, MD,§ María L. Moreno, MD,* Horacio Vázquez, MD,*† Graciela Lozano, MD,* Sonia Niveloni, MD,*† Roberto Mazure, MD,* Jon Meddings, MD,‖ Eduardo Mauriño, MD,* and Julio C. Bai, MD*†#*

TABLE 3. Final/Baseline Ratios for Intestinal Permeability (Lactulose/Mannitol Ratio), Serology (IgA tTG and IgA DGP), and Immunologic Parameters (in Serum and in PBMC 24 h Culture Supernatant) in the Probiotic and Placebo Arms

Parameter	Probiotic Arm	Placebo Arm	*P*
Lactulose/mannitol ratio			
Final/baseline ratio, median (range)	1.11 (0.65-2.13)	0.99 (0.48-6.79)	
Immunologic markers			
Celiac disease serology (final/baseline antibody concentration ratio), median (range)			
IgA tTG	0.90 (0.26-1.19)	1.07 (0.78-2.40)	0.0558
IgA DGP	0.90 (0.57-1.71)	1.10 (0.68-2.07)	0.1809
Inflammatory mediators			
In serum (serum concentrations)			
MIP-1β (pg/mL), median (range)			
Baseline	99.3 (75.5-219.5)	104.8 (81.9-139.5)	
Final	129.9 (78.3-379.2)*	98.8 (52.4-136.5)	
In PBMC 24 h culture supernatant (final/baseline ratio), median (range)			
IL-12p70	0.9 (0.1-4.2)	3.5 (1.2-4.4)	< 0.02
IL-6	0.8 (0.1-1.4)	1.0 (0.2-7.2)	
IL-10/IL-12p70 ratio	1.0 (0.1-14.9)	0.5 (0.3-5.3)	

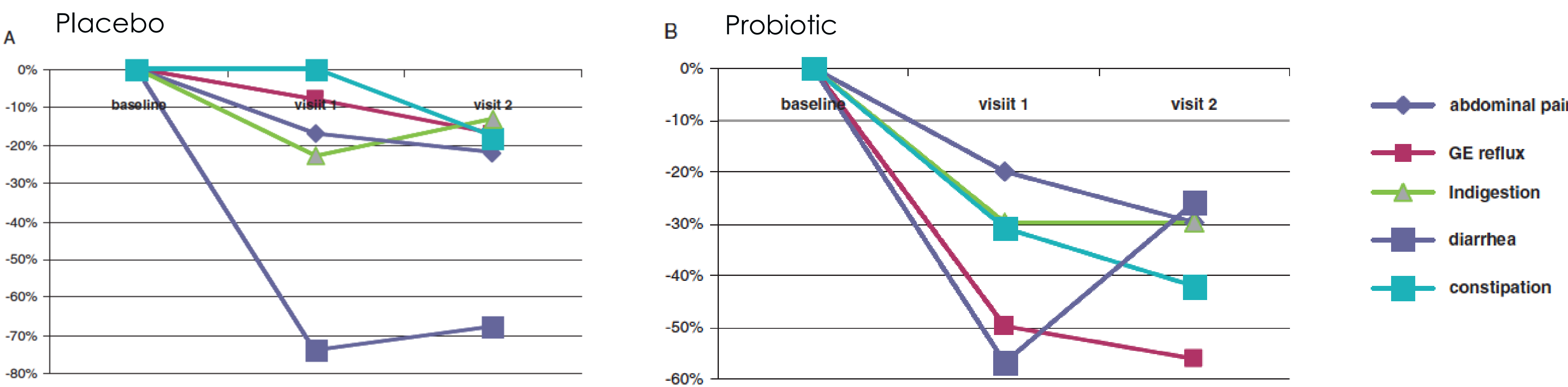
*$P < 0.04$.
PBMC indicates peripheral blood mononuclear cell.

Smecoul E. J Clin Gastroenterol 2013;47:139

Probiotic in CD at diagnosis

Exploratory, Randomized, Double-blind, Placebo-controlled Study on the Effects of *Bifidobacterium infantis* Natren Life Start Strain Super Strain in Active Celiac Disease

Edgardo Smecuol, MD,† Hui J. Hwang, MD,* Emilia Sugai, MD,* Laura Corso, MD,‡ Alejandra C. Cherñavsky, MD,§ Franco P. Bellavite, MD,* Andrea González, MD,‡ Florencia Vodánovich, MD,§ María L. Moreno, MD,* Horacio Vázquez, MD,*† Graciela Lozano, MD,* Sonia Niveloni, MD,*† Roberto Mazure, MD,* Jon Meddings, MD,‖ Eduardo Mauriño, MD,* and Julio C. Bai, MD*†#*



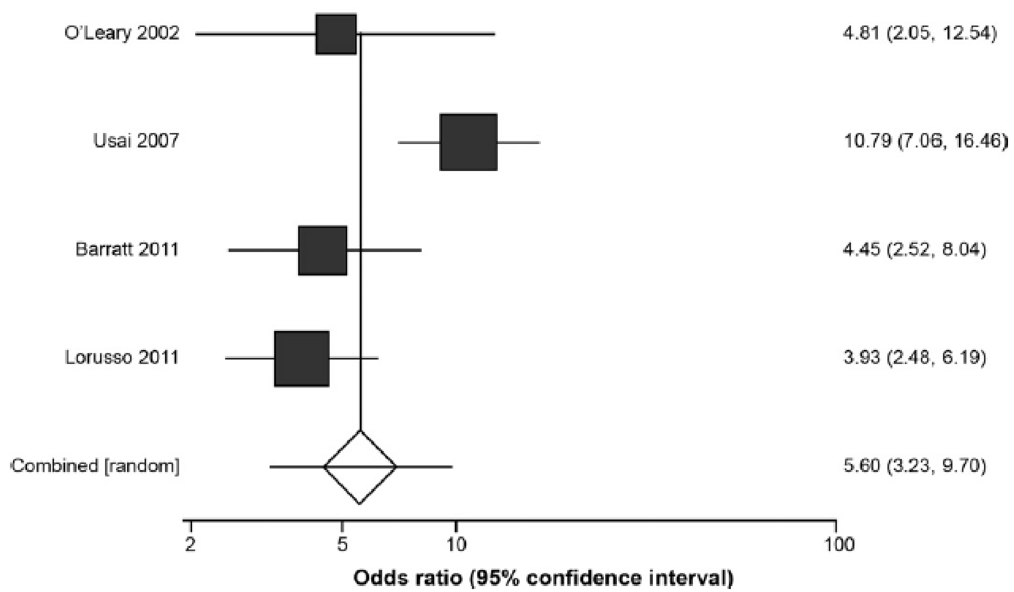
More than 70% of patients reported that these symptoms had improved with probiotics, whereas improvement occurred in 30% of patients with placebo. Once again, diarrhea was perceived as improved at the end of the trial by 80% of patients in both treatment arms.

Smecoul E. J Clin Gastroenterol 2013;47:139

Increase of Prevalence of IBS in CD

Prevalence of Irritable Bowel Syndrome–type Symptoms in Patients With Celiac Disease: A Meta-analysis

ANITA SAINSBURY,* DAVID S. SANDERS,‡ and ALEXANDER C. FORD*,§



The pooled OR for IBS-type symptoms was significantly higher in those with CD compared with controls. The odds of IBS-type symptoms were more than 5-fold higher (5.60; 95% CI, 3.23–9.70) among all patients with CD, regardless of adherence with a GFD, compared with controls without CD.

Sainsbury A. Clin Gastroenterol Hepatol. 2013;11:359

Increase of Prevalence of IBS in CD

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854 CD patients: 353 adults e 401 children

1237 control: 484 adults e 389 children

Cristofori F. SIGENP. 2014

Increase of Prevalence of IBS in CD

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

Dietary Supplement Use in Patients With Celiac Disease in the United States

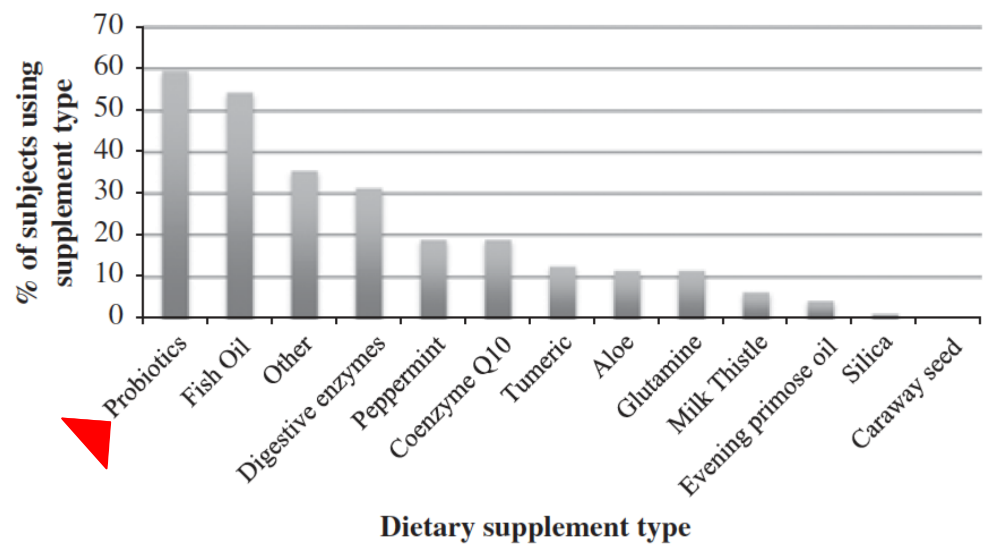
Samantha Nazareth, MD, Benjamin Lebwohl, MD, MSc,*† Christina A. Tennyson, MD,‡ Suzanne Simpson, RD,* Heather Greenlee, ND, PhD,†§ and Peter H. Green, MD**

CD patients completed a questionnaire on demographics, types of dietary supplement use, attitudes toward CAM

TABLE 2. Dietary Supplement Use and Attitudes Toward CAM

Variables	All Patients (423) [n (%)
Dietary supplement use	
No	323 (76.4)
Yes	100 (23.6)
Doctors should be supportive of CAM use	
Not at all	77 (18.2)
Slightly	105 (24.8)
Moderately	90 (21.3)
Quite a bit	71 (16.8)
A great deal	70 (16.5)

CAM indicates complementary and alternative medicine.



Nazareth S. J Clin Gastroenterol 2014; in press

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

Global and Deep Molecular Analysis of Microbiota Signatures in Fecal Samples From Patients With Irritable Bowel Syndrome

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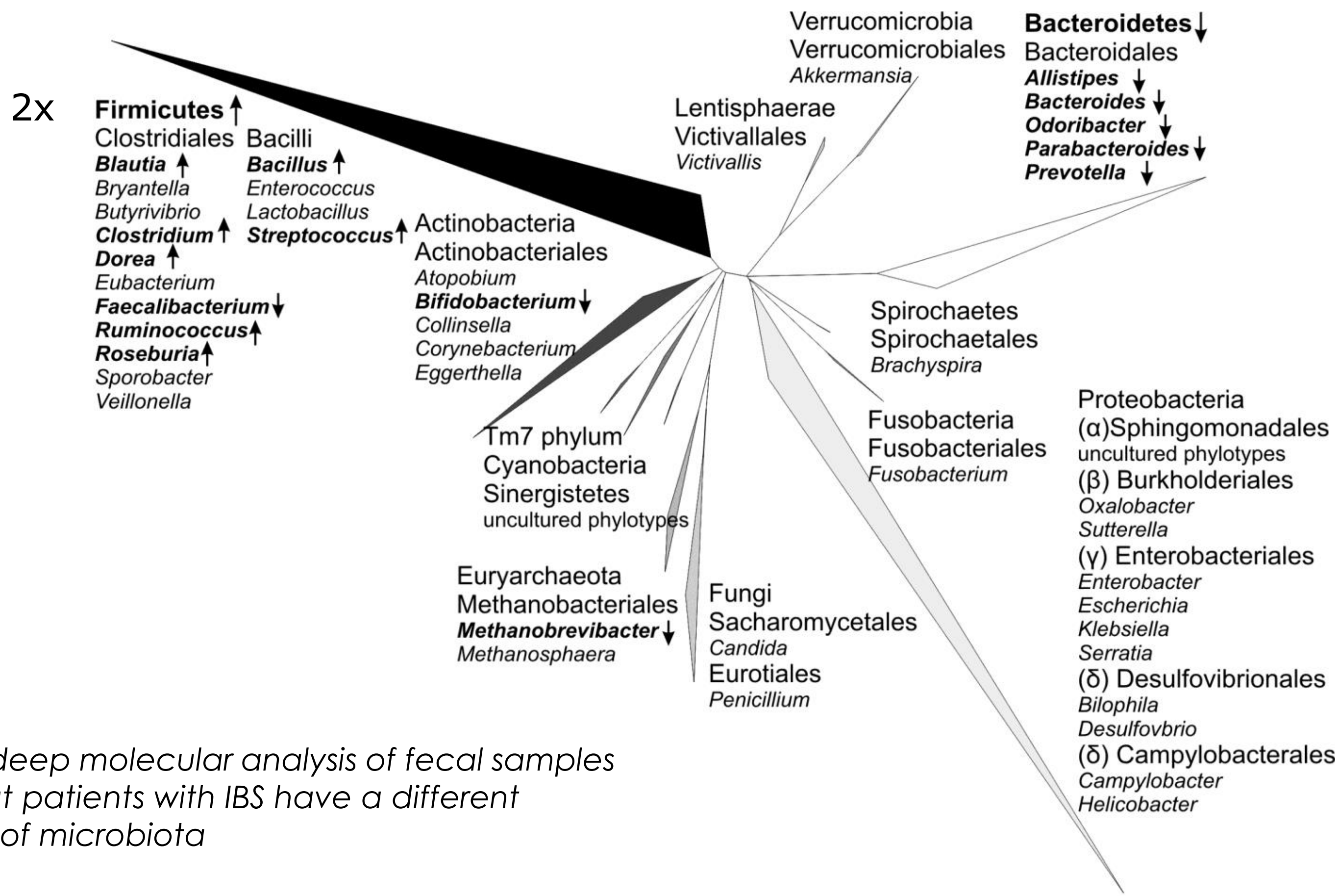
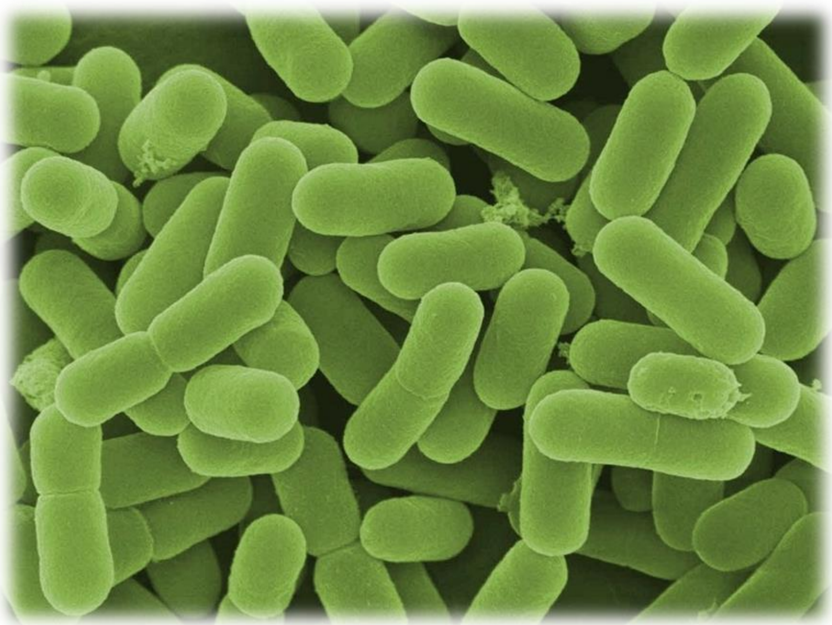


Global and deep molecular analysis of fecal samples indicates that patients with IBS have a different composition of microbiota

Rajilic ́-Stojanovic. Gastroenterology 2011;141:1792

Probiotic combination for CD&IBS patients



Lactobacillus plantarum CECT 4528

Lactobacillus casei 101/37 LMG P-17504

Bifidobacterium breve Bbr8 LMG P-17501

Bifidobacterium breve Bl10 LMG P-17500

Bifidobacterium animalis (Subsp. lactis) LMG P-17502

PROCEDO study

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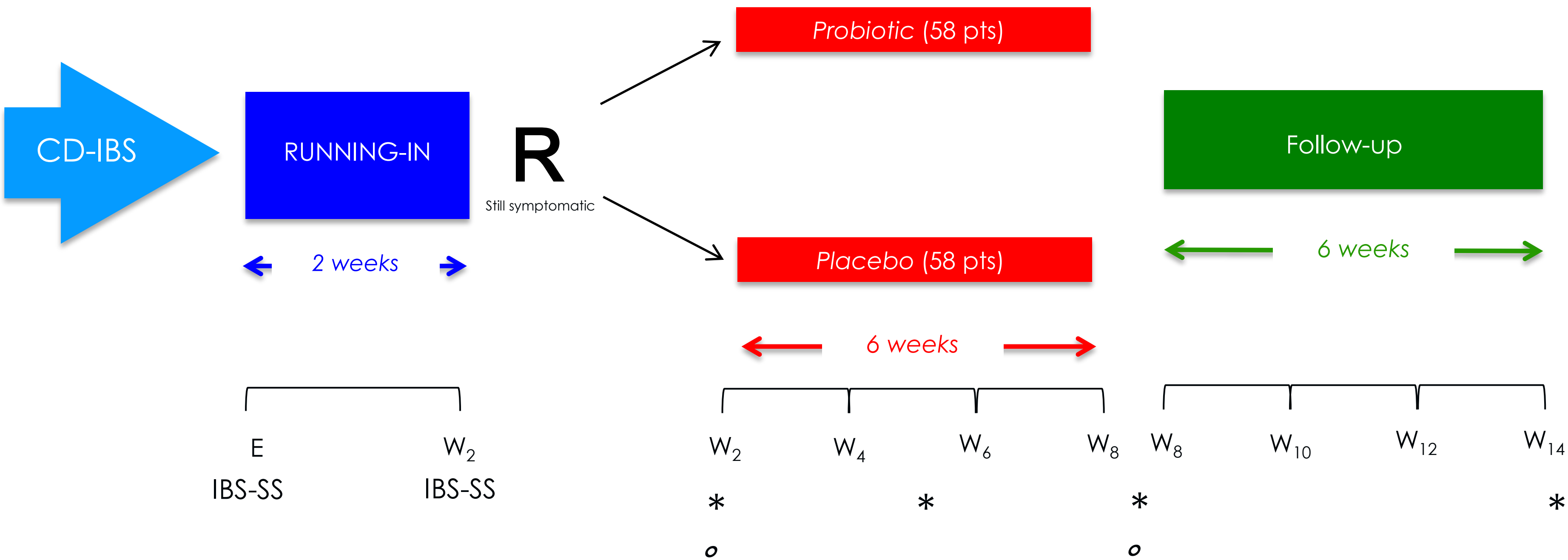
116 patients enrolled



* IBS severity score (IBS-SS) assessed by VAS

* Gastrointestinal Symptom Rating Scale (GSRS)

* Bristol Stool Chart (BSC)

* IBS Quality of Life (I-QOL)

* Symptom Check List (SL-90)

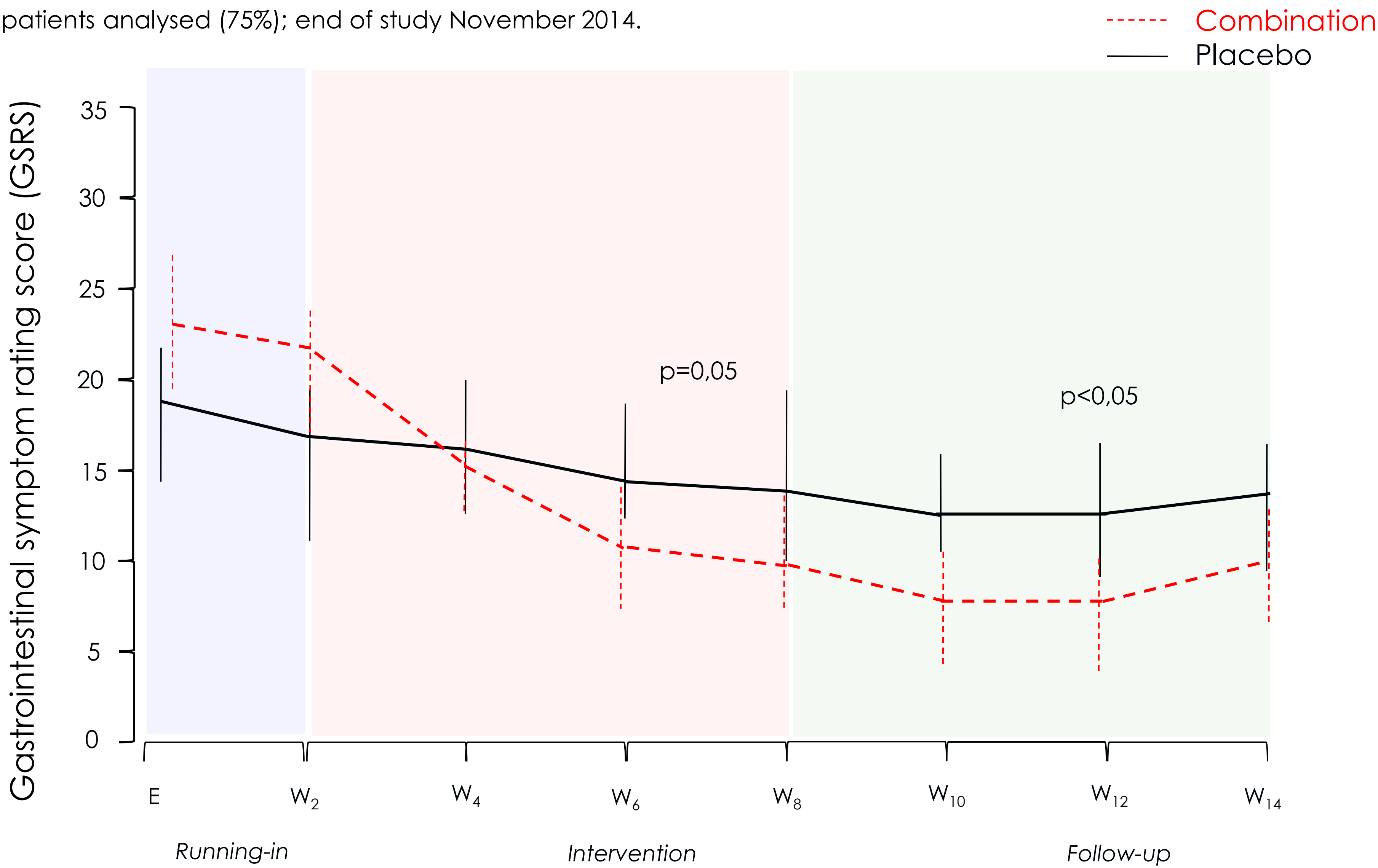
* Hospital Anxiety & Depression Scale (HADS)

° Urine - stools

ClinicalTrials.gov Identifier: NCT01699191

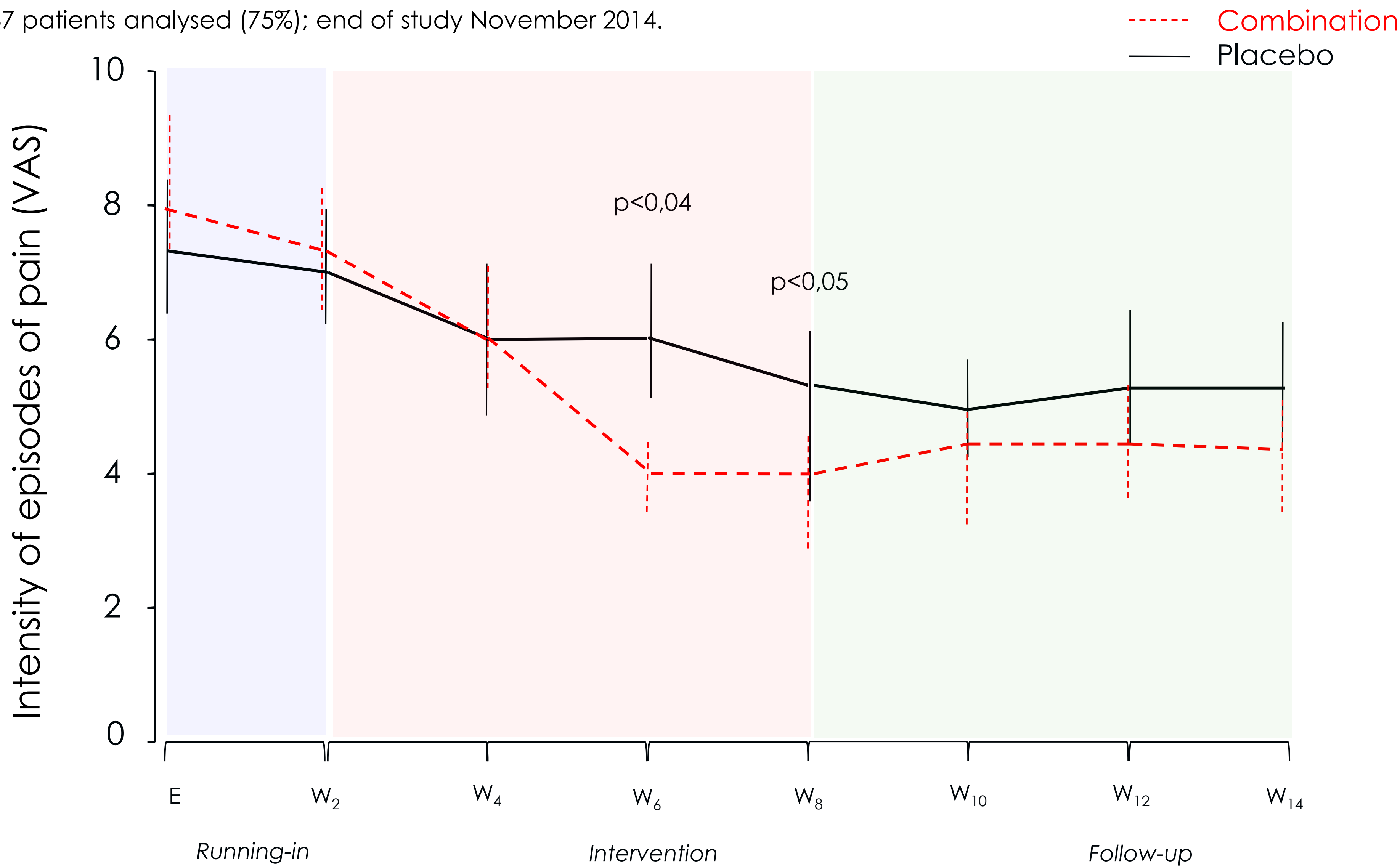
PROCEDO study: GSRS

87 patients analysed (75%); end of study November 2014.



PROCEDO study: IBS-SS by VAS

87 patients analysed (75%); end of study November 2014.



PROCEDO study: BSC

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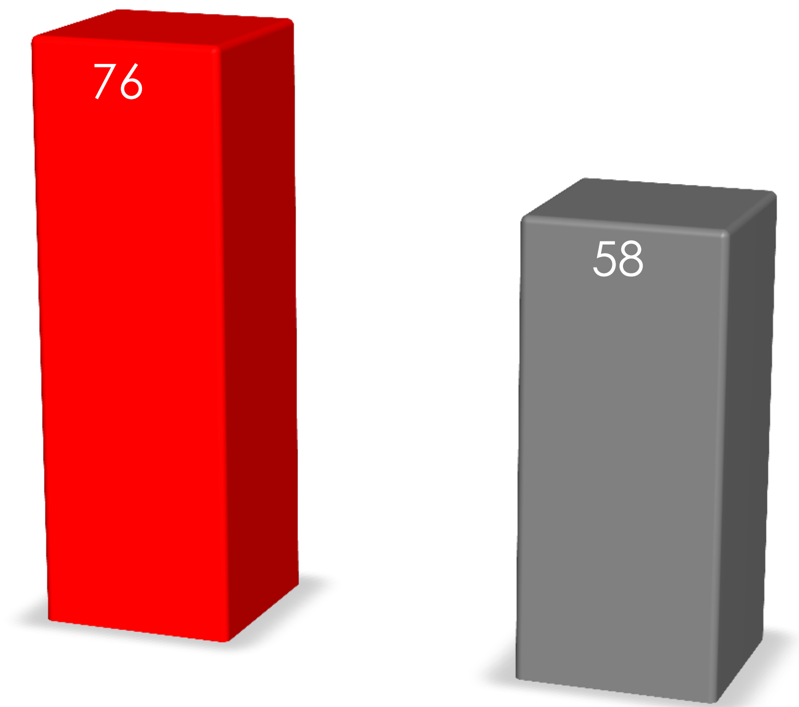
87 patients analysed (75%); end of study November 2014.

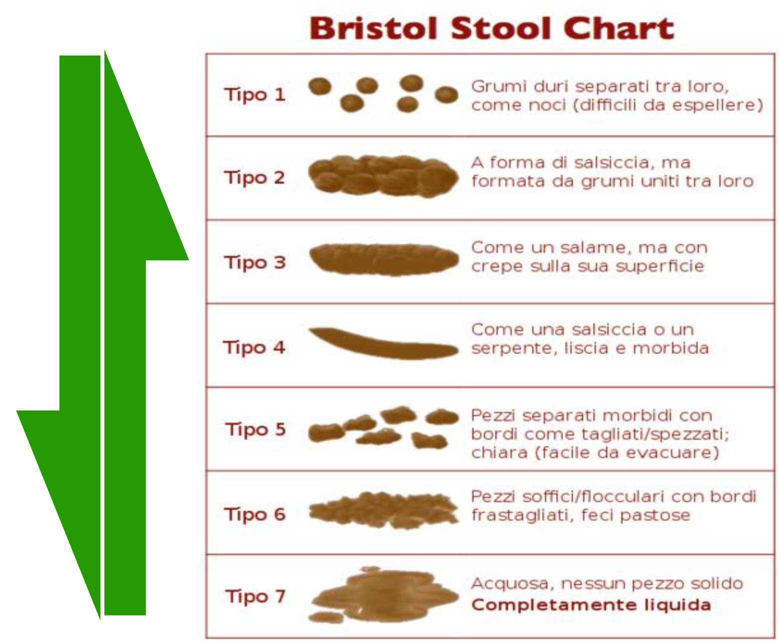
Improvement of defecation

p<0,04



Combination

Placebo



Probiotic combination for CD&IBS patients

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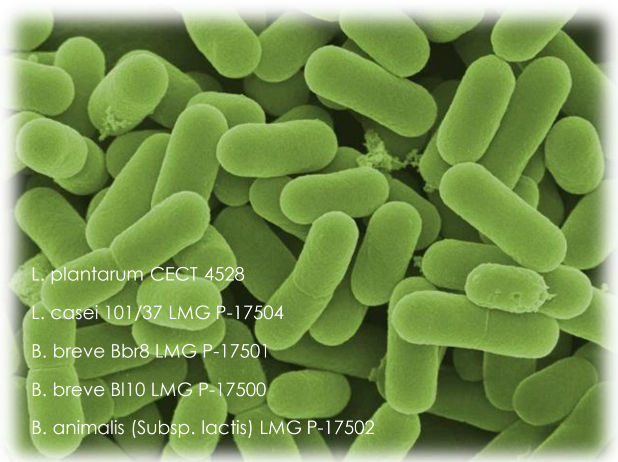
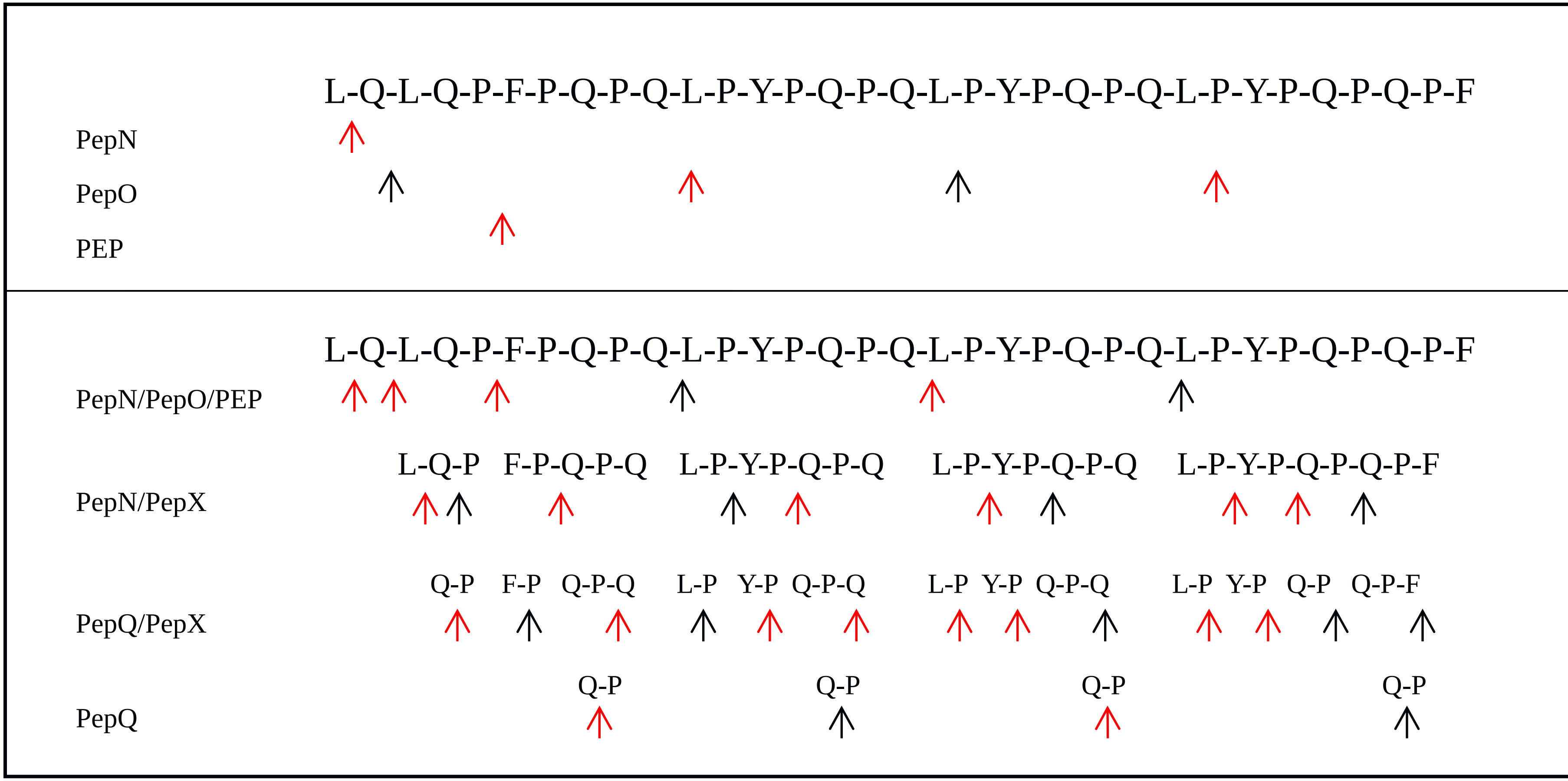


A 6-wk treatment with a probiotic mixture of 2 Lactobacilli and 3 Bifiodobacteria provided effective symptom relief in celiac patients suffering of symptoms suggestive for IBS

PROCEDO: effect on gliadin peptides

The probiotic combination has the ability to hydrolyze toxic gliadin polypeptides



PROCEDO: effect on gliadin peptides

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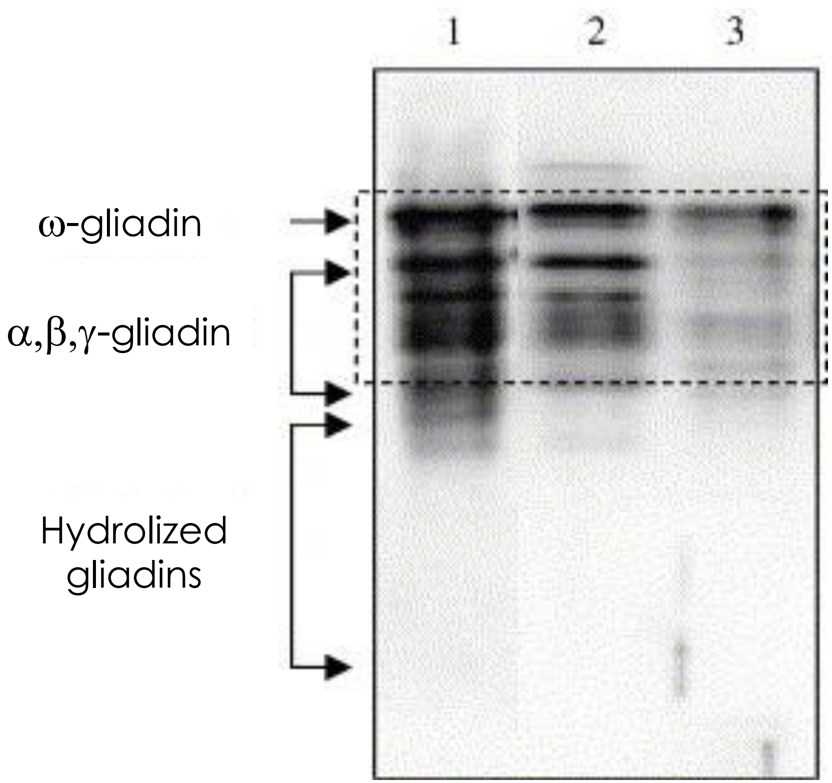
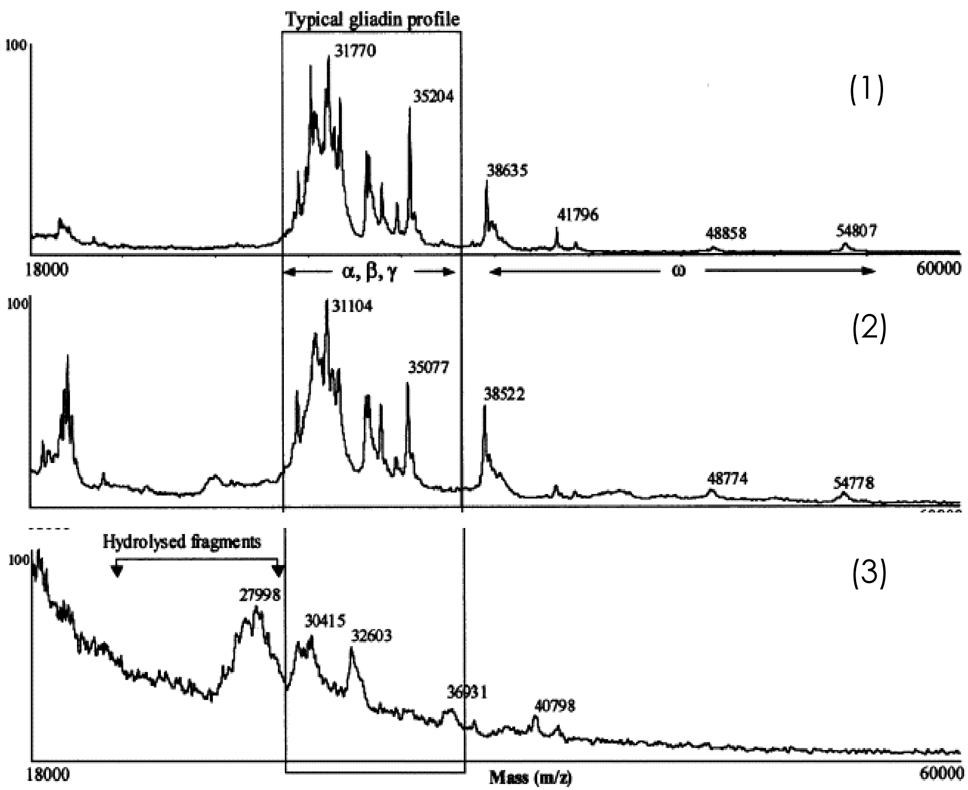


Western blot/R5 analysis of:

1) *European gliadin reference*
2) *Dough (control)*
3) *Dough incubated for 24 h at 37 ° C with the probiotic combination.*

PROCEDO: effect on gliadin peptides

The probiotic combination has the ability to hydrolyze toxic gliadin polypeptides



MALDI-TOF mass spectra of aqueous ethanol extract of wheat gliadin:

1) *European gliadin reference*
2) *Dough (control)*
3) *Dough incubated for 24 h at 37 ° C with the probiotic combination.*

Summary

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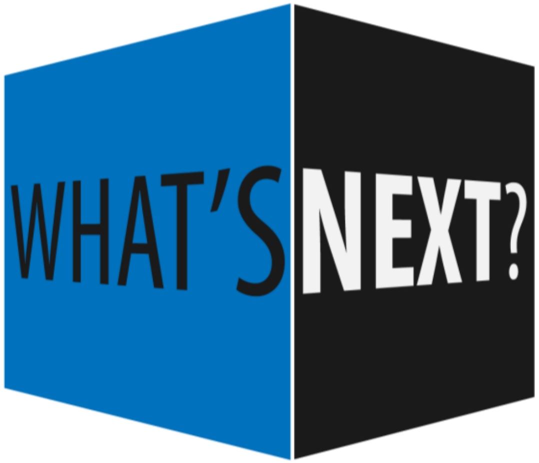

1. CD is characterized by a state of dysbiosis that do not completely reverse by GFD levels;

2. Bifidobacteria and lactobacilli are reduced in CD patients, and these bacteria can be considered a promising target for probiotic therapy at least to reduce GI symptoms that are common in this condition.

3. The identification of strains capable of producing enzymes that degrade gliadin peptides and induce anti-inflammatory effects needs to be studied.

4. Finally, studies including a larger sample size and involving international health and research centers would contribute to the design of common directions and guidelines for the use of probiotic and advance the knowledge regarding the importance of microbiota in CD.