THE EFFECT OF A CONSORTIUM PROBIOTIC ADMINISTRATION ON A MICROBIOTA MICROBIOLOGICAL STABILITY

Emanuel VAMANU[1]

e-mail: email@emanuelvamanu.ro

Abstract

Colonic microbiota role is appreciated more and more lately. Although food and physiological problems affect the microbial community in the colon, the use of probiotics and functional foods remain the best way to improve the friendly bacteria in the colon. The purpose of this study is to determine the effect of probiotics on three consortia microbiological stability of the microbiota in the training of a child. The studies were conducted using *in vitro* GIS2 simulator. It was used a negative control strain of *Bacillus subtilis*, and two positive control, a *Saccharomyces cerevisiae* yeast strain of *Bifidobacterium bifidum*-one. Each segment has shown strict microbiological characteristics, and characterized by a fermentative behavior characteristic. The administration has kept the number of microorganisms consortia favorable, which was characterized by a structure of microbial balance of the microbiota. The positive effect was most pronounced in the case of a consortium showed C9 noted, is made up of *Lactobacillus rhamnosus* E4.2, *Weisela paramesenteroides* FT1a and *Lactobacillus plantarum* 26.1 in the ratio of 1:1:1.

Key words: probiotic, *Lactobacillus*, *in vitro*, viability, microbiota

The colonic microbiota plays an important part in the digestion of food. Such products resulting from the bacterial fermentation process are further used by other microbial genres. Therefore, varied dietary fiber consumption may lead to corresponding microbiota diversity, providing a high health status at the individual level (Dore J., Blottiere H., 2015). Such a diet could eliminate the need to administer probiotics supplements, which have a limited period of administration.

Thus, the ratio between microbial strains of the microbiota is directly linked with the health of the individual. The microbiological action and structure of the microbiota is linked to the efficient use of food resources which may lead to obesity for example. From another point of view, certain inflammatory processes of the colon may be inhibited, processes that left untreated may determine cancer (Kemperman R.A. *et al*, 2013). An efficient assessment of the action of the microbiota is the *in vitro* simulators, which are capable to determine the effect of some products or food on the structure and activity of various groups of microorganisms. The microbiota may be assessed individually or, in general, in target groups.

The purpose of this research was represented by the effect that a probiotic consortium has on a forming microbiota, namely that of a child of maximum 12 months old. It was tracked whether administration of the consortium determines a stability of the microbiota in colon segments, by using the GIS2 *in vitro* system (www.gissystems.ro).

MATERIAL AND METHOD

Biological material

Three probiotic consortia have been used, which had the following formula: **C4** - *Weisela* (*W.*) *paramesenteroides* FT1a, *Lactobacillus* (*L.*) *paracasei ssp paracasei* 409, *Lactobacillus fermentum* 428ST; **C5** – *Lactobacillus paracasei ssp paracasei* 47.1a, *Lactobacillus sp.* 34.1, *Lactobacillus rhamnosus* E 4.2 and **C9** – *Lactobacillus rhamnosus* E 4.2, *Weisela paramesenteroides* FT1a, *Lactobacillus plantarum* 26.1, all strains having a 1:1:1 ratio. We used a negative control, the *Bacillus* (*B.*) *subtilis* strain and two positive controls, a *Saccharomyces* (*S.*) *cerevisiae* yeast strain and one of *Bifidobacterium* (*B.*) *bifidum*, in the collection of the faculty. These strains were stored in 20% glycerol, at – 80^0C, and the revitalization was performed in LB, YPG medium and, MRS (Vamanu E., 2014).

In vitro simulation of the human colon

We used the GIS2 *in vitro* simulator (*figure 1*), which used faeces from a seven years old child, and the detailed description of the operating and

[1] University of Agronomic Science and Veterinary Medicine Bucharest - Faculty of Biotechnology

processing conditions of the samples was performed in a previous study. Experiments developed in parallel have been preceded by a 14 days period, during which stabilization of the microbiota was installed. Samples were collected at half of the stationing time at the level of each segment of the colon, considering the point in which the balance status of the microbiota is installed. Samples were stored in a refrigerator, at – 10˚C, until microbiologically and biochemically analyzing it (Kemperman R.A. *et al*, 2013).

.

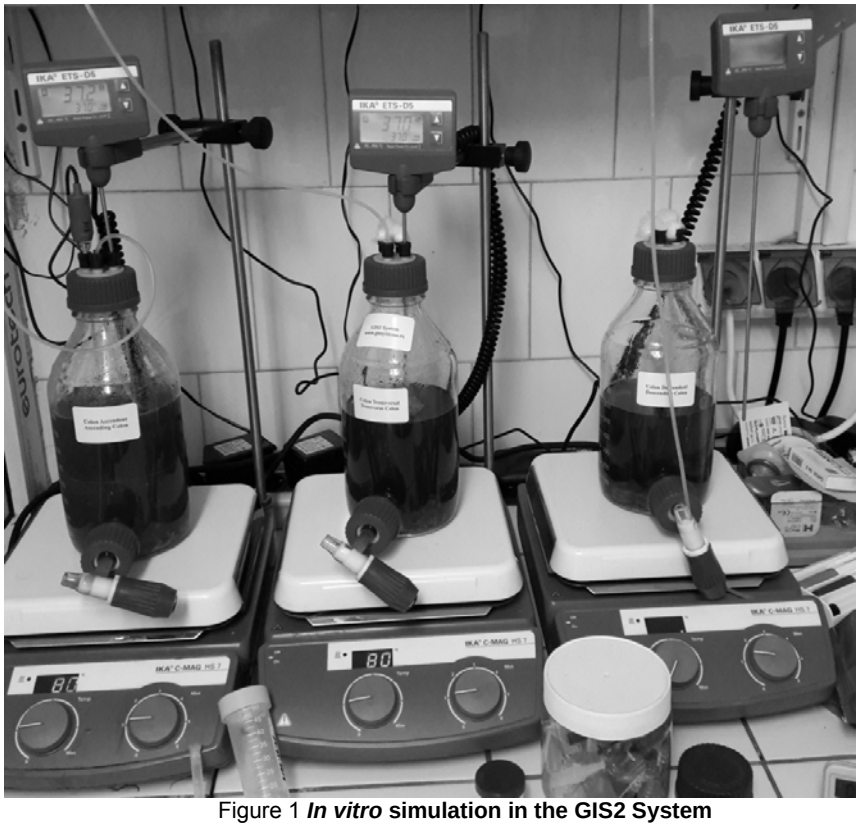

Figure 1 ***In vitro*** **simulation in the GIS2 System**

RESULTS AND DISCUSSIONS

The administration of certain probiotic consortia (combination of probiotic strains in variable proportions) is recognized to determine a series of benefits for the health, such as: simulation of the immunity, improvement of the intestinal transit, decrease of cholesterol etc. At the level of the human colon an action takes place on a microbiota and is based on an improvement of the microbiological report between favorable and pathogenic strains (Kemperman R.A. *et al*, 2013; Kemperman R.A. *et al*, 2013 Vamanu E. *et al*, 2015).

Table 1 presents the effects of administration of the three probiotic consortia in the GIS2 in vitro simulator. The continuous fermentation system simulates with accuracy the dynamics of the intestinal transit of the human colon. Consortia used have showed a different behavior, with weak results for C4 consortium. Although it contained, the FT1a strain joint with the C9 consortium, it did not determine a behavior corresponding the use as probiotic. If compared only microbiologically, the probiotic effect was expressed by the strains of the C9, E 4.2 and 26.1 consortiums. Administration of consortia has mainly kept the favorable microorganisms number. The difference came from the microbial load of the rest of the strains determined. The most important favorable effect was determined by C9, followed by C5 and C4. Compared to the untreated microbiota, the decrease of the coliform bacteria number with the use of C9 was approximately 30±0.2%.

It was noted that upon administration of C5, of positive control *S. cerevisiae*, as well as partly, for negative control *B. subtilis* no enterococci were identified. In return, C9 has demonstrated a balance of the microbial structure of the microbiota, also characterized by the presence of a microbial group. The microbial association presented in consortium C9 has determined a gradual decrease of the pathogenic strains, in average with 0.5 UFC/mL. This behavior was not

identified for the C5 consortium, for example. A positive control (*B. bifidum*) had a similar effect, while the rest of the controls allowed a stabilization of the number of microorganisms, around 5 UFC/mL.
.

Table 1

Microbiological analysis after *in vitro* simulation

Sample	The average number of microorganisms (log CFU/mL)						
	Total anaerobes	Facultative anaerobes	Coliforms	Enterococci	Clostridia	Bifidobacteria	Lactobacilli
Ascending colon C4	5.15±0.5	4.60±0.1	3.84±0.5	4.77±0.0	4.95±0.9	4.60±0.2	5.15±0.5
Transverse colon	5.48±0.2	4.73±0.3	5.39±0.7	4.67±0.2	4.77±0.0	4.64±0.3	5.42±0.8
Descending colon	5.02±0.1	5.25±0.3	4.86±0.9	4.65±0.3	5.08±0.6	4.79±0.1	5.75±0.9
Ascending colon C5	4.75±0.5	5.01±0.1	5.52±0.3	0.00±0.0	5.07±0.5	4.53±0.1	5.71±0.4
Transverse colon	4.75±0.5	5.66±0.2	4.75±0.5	0.00±0.0	5.02±0.5	4.47±0.3	4.95±0.1
Descending colon	4.69±0.0	4.92±0.1	5.55±0.0	0.00±0.0	5.02±0.3	4.36±0.1	4.81±0.1
Ascending colon C9	4.79±0.1	4.96±0.5	5.58±0.9	4.91±0.9	5.13±0.9	4.44±0.3	5.13±0.3
Transverse colon	5.12±0.3	4.92±0.6	4.07±0.9	4.54±0.4	4.63±0.3	4.71±0.4	5.27±0.4
Descending colon	4.94±0.9	5.77±0.5	4.78±0.5	4.55±0.6	4.88±0.6	4.71±0.6	5.77±0.4
Ascending colon S.c.	4.20±0.4	4.89±0.3	4.38±0.0	0.00±0.0	4.79±0.1	5.30±0.1	4.83±0.2
Transverse colon	5.63±0.1	5.31±0.3	4.36±0.1	0.00±0.0	4.63±0.3	4.55±0.4	5.33±0.2
Descending colon	5.52±0.3	5.32±0.6	4.27±0.2	0.00±0.0	4.47±0.3	4.63±0.3	5.56±0.1
Ascending colon B.b.	4.56±0.2	5.68±0.4	4.90±0.3	4.47±0.3	5.18±0.3	4.73±0.2	5.36±0.5
Transverse colon	5.73±0.5	5.62±0.5	4.47±0.3	4.66±0.2	4.77±0.0	5.46±0.3	5.57±0.2
Colon descendent	4.99±0.1	5.39±0.4	4.47±0.7	4.69±0.0	4.17±0.4	5.19±0.2	5.57±0.8
Ascending colon B.s.	Peste 7.00	5.56±0.5	4.74±0.2	0.00±0.0	4.75±0.5	4.90±0.3	4.92±0.4
Transverse colon	Peste 7.00	5.27±0.1	4.94±0.1	0.00±0.0	4.63±0.3	5.37±0.2	5.69±0.0
Descending colon	Peste 7.00	5.03±0.3	4.76±0.3	0.00±0.0	4.86±0.3	5.05±0.4	5.34±0.3

Administration of the three consortia also has a direct impact on fermentation processes in the simulated colon. Intensification of the favorable fermentation processes was characterized by a decrease of the pH. This phenomenon was noticed mainly for C9 together with passing from a segment to another of the *in vitro* simulation. C4 and C5 have showed a slow decrease of the pH, mainly when passing from the transversal to the descending segment. Positive controls have determined a constant behavior of the pH profile, while the negative one has determined a slow increase of the pH. This was opposite to the number of anaerobe strains, mainly in the two segments, where they were present in a number that exceeds 7 UFC/mL.

This behavior was explained by the presence of some facultative anaerobic strains (with pathogen potential) of the *Staphylococcus* type, whose number was determined by a separate analysis. Such a microbial load was determined for this microbiota in a previous study, but it was not considered this time. The simulated microbiota, in stabilized phase, indicated a presence of approximately 5 UFC/mL (Vamanu E. *et al*, 2013). The presence of the stimulated negative control and other negative strains, which influenced the microbial load negatively (*table 1*). These values have also influenced the fermentation activity, by inhibition of favorable fermentation processes, which produce, for example, lactic acid.

CONCLUSIONS

This study has offered a detailed image of the microbiological structure of the microbiota after administration of three probiotic consortia, compared to two positive controls and one negative control. Each independent simulated segment has manifested strict microbiological features, characterized by a characteristic fermentation behavior. Studies have to be continued by a molecular analysis and confirmation of the impact on the microbiota of the adult.

ACKNOWLEGMENTS

This work was supported by UEFISCSU,project number PN-II-PT-PCCA-2011-3.10969/2012 (http://www.gissystems.ro/ & http://prolab77.freewb.ro/). The probiotic samples (three probiotic consortia) were provided by the research team of Microgen Center, Faculty of Biology, University of Bucharest.

REFERENCES

Dore J., Blottiere H., 2015 - *The influence of diet on the gut microbiota and its consequences for health*. Current Opinion in Biotechnology, 32:195–199.

Kemperman R.A., Gross G., Mondot S., Possemiers S, Marzorati M., van de Wiele T., Doré J., Vaughan E.E., 2013 - *Impact of polyphenols from black tea and red wine/grape juice on a gut model microbiome*. Food Research International, 53:659–669.

Vamanu E., Pelinescu D., Avram I., Nita S., Vamanu A., 2013 - *Study of PROBAC product influence on infant microbiota in a single chamber colonic fermentation model GIS1*. Annals of Microbiology, 63:1029–1038.

Vamanu E., 2014 - *Testing in vitro viability of a thermophilic probiotic bacterial strain in simulated gastrointestinal conditions*. Annals of Microbiology, 64:1439-1442.

Vamanu E., Sarbu I., Nedelcu I., Pelinescu D., 2015 - *Study of PROEXO product influence on infant microbiota in an in vitro colonic fermentation system*. Annals of Microbiology, 65:1189-1193.