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# Composition of cultivable intestinal microbiota in patients suffering from obesity and type II diabetes

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## ABSTRACT

Obesity and the metabolic syndrome represent a real threat to human health. The rate of obesity has increased significantly worldwide. Excess body weight is one of the most important factors that contribute to the etiology of many diseases. Worldwide, nearly 1 billion adults and 10% of children are classified as overweight or obese. Their average life span is reduced and the main consequences are cardiovascular diseases and type II diabetes. Recently other causes of obesity were recognized; in this regard the intestinal microbiota is a new accepted factor. The colonic microbiota is diversified and very complex, composed from a wide range of microorganisms, usually in symbiosis with the host. The microbiota could be regarded as a metabolic organ that can break down indigestible food components, degrade potentially toxic compounds, and synthesize certain vitamins and amino acids. Other physiological functions include preventing the proliferation of pathogens and the boosting of the local immunity, production of essential nutrients to maintain the energy balance and adjusting the energy storage. Sometimes this balance is lost and a state of dysbiosis occurs. A dysbiosis of the microbiota can lead to host metabolic imbalances, as metabolic syndrome for example. The purpose of this study was to evaluate plasma levels of hepatic transaminase, lipase, cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, ESR, the C-reactive protein and fibrinogen in relation to the composition of the gut microbiota in obese people with diabetes and dyslipidemia.

**Keywords:** *intestinal microbiota, biochemical tests, obesity, type II diabetes.*

## 1. INTRODUCTION

The human intestinal tract contains about 2000 species of microorganisms, collectively known as microbiota, of which about 500 species are commensal and are very important in the process of food digestion, as well as in the control of intestinal epithelial homeostasis (Ianiro et al., 2014; Hartstra et al., 2015). A dynamic relationship between the host and gastrointestinal microorganisms begins shortly after birth (Rajilic-Stojanovic et al., 2007; Shen et al., 2013). Generally, the colon microbiota consist in a wide variety of bacterial species (Eckburg et al., 2005), and can be influenced by many factors such as age, geographical origin, dietary habits, antibiotics or probiotics (Cox et al., 2013; Turnbaugh et al., 2009). The commensal microbiota plays an important role in maintaining the health of the host by absorption of metabolites, vitamins, short chain fatty acids (Bassaganya-Riera et al., 2012; Festi et al., 2014), but the effects of the interactions between the host and its microbiota are still being investigated.

The balance between different bacterial strains and the intestinal immunity effectors influences commensal microbiota and the presence of pathologic inflammation, which depends on the number and virulence of the pathogenic microorganisms (Greabu et al., 2014; Ruemmele et al., 2003). Disbiosis of the gastrointestinal tract microbiota is an important factor in triggering inflammatory bowel disease, irritable bowel syndrome, colon cancer and certain systemic diseases, such as obesity, type II diabetes and non-alcoholic fatty liver disease (Morales et al., 2010; Cani et al., 2007).

## 2. EXPERIMENTAL SECTION

The analyzed samples were isolated from patients suffering from at least three conditions associated with the metabolic syndrome, namely obesity, type II diabetes and dyslipidemia, hospitalized in the cardiology ward of the Prof.Dr. Constantin Angelescu Hospital.

### 2.1. Isolation, identification and characterization of intestinal microbiota.

Intestinal microbiota variations were studied on a number of 22 stool samples. The bacterial species were identified using biochemical conventional tests and the automatic Vitek 2 system. An analysis of the bacterial virulence patterns was done using specific culture media according to the method described by Lazar et al., 2004. The presence of hemolysins was determined by spotting the strains on blood agar plates with 5% sheep blood. Media supplemented with 2.5% yolk, gelatin and Tween 80 respectively were used to determine the expression of lecithinase, gelatinase and lipase. For the caseinase assay a medium containing 15% soluble casein was used. The DNA agar medium was used for the evaluation of DNA-se presence. The expression of amylase was determined on a medium supplemented with starch. For aesculin hydrolysis a medium with $Fe^{3+}$ citrate was used and inoculated by spotting.

### 2.2. Biochemical analyses.

Laboratory investigations of blood samples were undertaken to determine the following biochemical parameters: a'jeun glucose, the levels of total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol, lipids, liver transaminases, the speed of blood sedimentation, C-reactive protein and fibrinogen.

For analysis an automatic analyzer Dirui 300 was used. The C reactive protein tests were done using the Immulite automated immunology analyzer. To determine the erythrocyte sedimentation rate classic methods were used and the fibrinogen was assessed with the Sismex CA-650. These data were correlated with the body mass index and height-weight ratio.

## 3. RESULTS SECTION

From the 22 stool samples, a number of 29 bacterial strains were isolated. The predominant microorganisms were the Gram-negative bacteria from the *Enterobacteriaceae* family, belonging to the following genera and species: 13 *Escherichia coli* (45%), 4 *Klebsiella sp.* (14%) and *2 Citrobacter freundii* (7%) strains. 9 isolates were represented by Gram-positive cocci, namely 7 *Enterococcus sp.* (24%) and 2 *Staphylococcus aureus* (7%) strains. 1 *Pseudomonas sp.* strain was also isolated (Fig. 1).

Regarding the virulence factors expressed by the respective strains, the most prevalent was represented by the hydrolysis of esculin in contrast to gelatinase, DN-ase, caseinase and amylase which were not expressed at all. Some members of *Enterobacteriaceae* family expressed also some pore forming toxins (hemolysins, lecithinase and lipase) (Fig. 2).

The low expression of the virulence factors can be linked to the fact that these microorganisms are part of the normal intestinal microbiota, but also can suggest a latent pathogenity which could manifest in particular situations when the body is suffering from a disease that incapacitates or weakens the immune response. The presence of pore forming toxins confers the capacity of the producing microorganisms to diseminate from intestine to other extra-intestinal sites, leading to serious infectious complications, sometimnes systemic with a high mortality rate especially in imunosupressed patients.

The results obtained in this study show that in the intestinal microbiota of patients with metabolic syndrome the most prevalent were species of *Enterobacteriaceae* family. These results are confirmed also by the study conducted by Hartstra et al. on a group of 345 patients with type II diabetes, which showed that their intestinal microbiota presented an increased prevalence of opportunistic Gram-negative pathogens (*Escherichia coli*) (Hartstra et al., 2015). Regarding the investigated biochemical parameters, the values obtained were presented in tabel 1, using the following reference values: glucose 70-115 mg/dL, total cholesterol 125-200mg/dL, aspartate aminotransferase (AST) 0-35 IU/L, alanine aminotranferase (ALT) 0-41 IU/L, HDL-cholesterol 35-80 mg/dL, LDL-cholesterol 0-100 mg/dL, triglycerides 50-200 mg/dL, lipids 500-800 ml%, Reactive C Protein 0-1,0 mg/dL, fibrinogen 200-400 mg/dL and erytrocyte sedimentation rate between 0-15 mm/h. In table 2, a correlation between isolated strains and obesity degree is presented. The results of biochemical tests, toghether with body mass index fit the patients into the category of those suffering from metabolic syndrome.

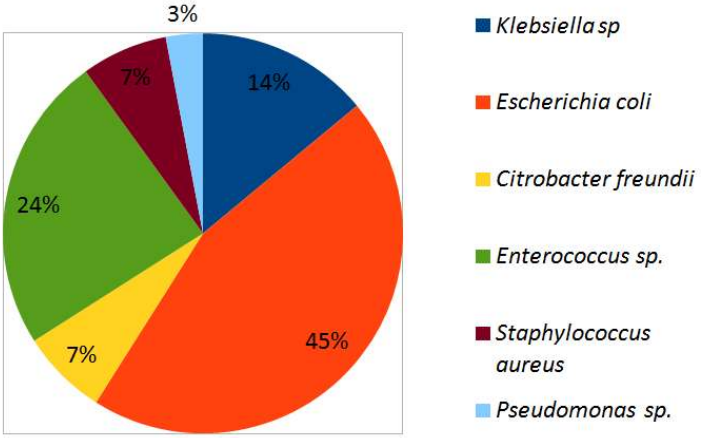


**Figure 1.** Graphic representation of the percentage distribution of microbial species isolated from the analyzed stool samples.

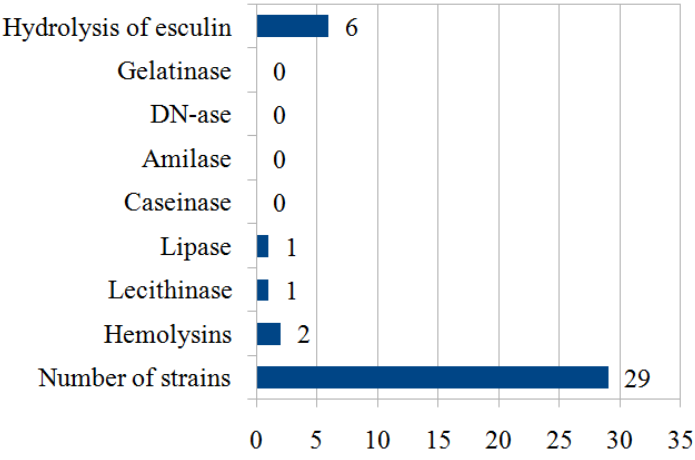


**Figure 2**.The enzymatic virulence factors expressed by isolated strains

**Table 1.** The values of biochemical parameters investigated in the analyzed patients

| Pacient no. | Isolated strains | | Glucose mg/dL | Cholesterol mg/dL, | AST/ ALT IU/L | HDL-cholesterol mg/dL | LDL-cholesterol mg/dL | Triglycerides mg/dL | Lipids ml%, | CRP mg/dL | VSH mm/h |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gram-negative bacteria | Gram-positive bacteria | | | | | | | | | |
| 1 | *E. coli* | *Enterococcus sp.* | 113 | 270 | 26/22 | 37.18 | 171.65 | 222.23 | 893.5 | 0.95 | 7.00% |
| 2 | *E. coli* | *Enterococcus sp.* | 96 | 302.41 | 45/64 | 180.57 | 190.49 | 406.57 | 920 | 0.23 | 9 |
| 3 | *E. coli* | *Enterococcus sp.* | 147 | 262 | 18/25 | 63.26 | 154.62 | 223.41 | 897 | 1.01 | 15 |
| 4 | *E. coli*, *Klebsiella sp.* | *Enterococcus sp.* | 140 | 139 | 19/17 | 32 | 40.70 | 124 | 720 | 1.23 | 21 |
| 5 | *E. coli*, *Klebsiella sp.* | | 331 | 234 | 26/50 | 37.5 | 80.7 | 175 | 752 | 1.23 | 25 |
| 6 | *Citrobacter freundii* | | 144 | 246 | 10/25 | 39.80 | 166 | 184 | 827.5 | 1.34 | 28 |

| Pacient no. | Isolated strains | | Glucose mg/dL | Cholesterol mg/dL, | AST/ ALT IU/L | HDL-cholesterol mg/dL | LDL-cholesterol mg/dL | Triglycerides mg/dL | Lipids ml%, | CRP mg/dL | VSH mm/h |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gram-negative bacteria | Gram-positive bacteria | | | | | | | | | |
| 7 | *E. coli* | *Enterococcus sp.* | 203 | 216 | 18/24 | 81.50 | 117.10 | 109 | 685 | 2.05 | 32 |
| 8 | *Pseudomonas* | *Enterococcus sp.* | 418 | 332 | 41/82 | 48.2 | 214.5 | 366 | 1203 | 11.5 | 76 |
| 9 | | *Staphylococcus aureus* | 118 | 380 | 36/71 | 57.5 | 169 | 348 | 1293 | 6.90 | 46 |
| 10 | *E. coli* | | 128 | 303 | 21/40 | 69.5 | 155.5 | 472 | 1243.8 | 4.7 | 54 |
| 11 | *E. coli* | *Staphylococcus aureus* | 112 | 260 | 21/42 | 41.4 | 164.6 | 421 | 1096 | 0.67 | 32 |
| 12 | *Klebsiella sp.* | | 192 | 326 | 30/51 | 39.5 | 226.5 | 381 | 1204.5 | 3.90 | 34 |
| 13 | *E. coli* | | 121 | 284 | 15/26 | 36 | 230 | 241 | 970 | 8.79 | 53 |
| 14 | *E. coli, Klebsiella sp.* | | 131 | 268 | 50/61 | 46.5 | 214.5 | 221 | 914 | 18.22 | 120 |
| 15 | *Klebsiella sp.* | | 112 | 274 | 10/19 | 46.1 | 176.9 | 280 | 986.5 | 0.90 | 25 |
| 16 | *Citrobacter freundii*, *Klebsiella sp.* | *Enterococcus sp.* | 171 | 248 | 24/33 | 59 | 146 | 217 | 865 | 9.60 | 85 |
| 17 | *Enterobacter asburiae* | | 147 | 219 | 12/3 | 32.1 | 123.9 | 313 | 895.8 | 1.25 | 45 |
| 18 | *E. coli* | | 124 | 336 | 14/34 | 53.8 | 240.2 | 210 | 1056 | 1.25 | 35 |
| 19 | *E. coli* | *Enterococcus sp.* | 198 | 232 | 17/24 | 41.5 | 187 | 456 | 1068 | 11.5 | 75 |
| 20 | *E. coli* | | 110 | 409 | 25/26 | 50.9 | 225 | 314 | 1324 | 10.8 | 58 |
| 21 | *E. coli* | | 125 | 306 | 89/99 | 22.9 | 241.1 | 209 | 987.5 | 6.9 | 45 |
| 22 | *Klebsiella sp.* | | 130 | 299 | 25/42 | 56.5 | 217.5 | 123 | 885.8 | 6.9 | 37 |

**Table 2.** Correlation between obesity degree of the patients and the isolated strains

| Patient no. | Isolated strains | | Body mass index | | | | |
|---|---|---|---|---|---|---|---|
| | Gram-negative bacteria | Gram-positive bacteria | G(kg)/ H(cm) | Excess body weight | Obesity type I | Obesity type II | Morbid obesity degree |
| 1 | *E. coli* | *Enterococcus sp.* | | | | | |
| 2 | *E. coli* | *Enterococcus sp.* | 86/175 | X | | | |
| 3 | *E. coli* | *Enterococcus sp.* | 103/170 | | | X | |
| 4 | *E. coli*, *Klebsiella sp.* | *Enterococcus sp.* | 89/165 | | X | | |
| 5 | *E. coli*, *Klebsiella sp.* | | 103/165 | | | X | |
| 6 | *Citrobacter freundii* | | 120/175 | | | X | |
| 7 | *E. coli* | *Enterococcus sp.* | 115/185 | | X | | |
| 8 | *Pseudomonas* | *Enterococcus sp.* | 109/175 | | | X | |
| 9 | | *Staphylococcus aureus* | 109/165 | | | | X |
| 10 | *E. coli* | | 98/176 | | X | | |
| 11 | *E. coli* | *Staphylococcus aureus* | 77/162 | X | | | |
| 12 | *Klebsiella sp.* | | 99/160 | | | X | |
| 13 | *E. coli* | | 98/168 | | X | | |
| 14 | *E. coli*, *Klebsiella sp.* | | 102/170 | | | X | |
| 15 | *Klebsiella sp.* | | 102/162 | | | X | |
| 16 | *Citrobacter freundii*, *Klebsiella sp.* | *Enterococcus sp.* | 121/180 | | | X | |
| 17 | *Enterobacter asburiae* | | 95/156 | | | X | |
| 18 | *E. coli* | | 95/168 | | X | | |
| 19 | *E. coli* | *Enterococcus sp.* | 115/163 | | | | X |
| 20 | *E. coli* | | 110/168 | | | X | |
| 21 | *E. coli* | | 97/172 | | X | | |
| 22 | *Klebsiella sp.* | | 90/160 | | | X | |

The dyslipidaemic syndrome is one of the major risk factors concerning the development of cardiovascular disease in the case of individuals suffering from type II diabetes. The characteristics of diabetic dyslipidemia are the high concentration of plasma triglycerides, and imbalance of HDL cholesterol and LDL cholesterol lipoproteins. These changes are caused among other things by the increased flow of fatty acids, insulin resistance and an increase of the inflammatory response (Chehade et al., 2013; Clements et al., 2012).

Recently, several mechanisms linking obesity and the development of intestinal microbiota associated disorders (type II diabetes) have been discovered. The mechanisms involved in obesity and metabolic diseases include: (i) energy extraction from the diet by improving the conversion of dietary fibers to short

chain fatty acids; (ii) increased intestinal permeability by bacterial LPS in response to the consumption of fatty diets resulting in a systemic LPS level and in a state of inflammation (Tanti et al, 2013). Because the gastrointestinal tract and liver are connected through the portal venous system, the liver is more vulnerable to bacterial translocation, bacterial products, endotoxins or cytokines presented and secreted in the gastrointestinal tract (Blaser et al., 2009). The microbiota can alter liver function by stimulating hepatic triglycerides and lipid metabolism modulation indirectly impacting the fatty deposits in the liver (Macfarlane et al, 2012). Also, the intestinal microorganisms modulate the host immune system and affect lipid metabolism (Nieuwdorp et al., 2014).

The presence of bacteria and bacterial metabolites are therefore responsible for some of the pathological effects occurred in obesity and metabolic diseases.The increase of metabolic endotoxemia, namely increased plasma levels of LPS is one of the major triggering factors, leading to the development of metabolic inflammation. Growing evidence suggests that microbes contribute to the onset of inflammation that characterizes these disorders associated with metabolic mechanisms that contribute to the occurrence of intestinal barrier dysfunctions (Geurts et al., 2014).

LPS endotoxin is one of the virulence factors associated with Gram-negative bacteria, which have been shown to predominate in the stool of the patients analyzed in this study, and has a major role in acute and chronic inflammation (Zhang et al., 2008). These glycolipids are located on the outer membrane of the bacteria and enter the bloodstream at a rate of 80-97%. They can be neutralized in the bloodstream by HDL (Pussinen et al., 2011). Lipopolysaccharides are primarily bound to the HDL, and VLDL, increasing their affinity for low-density lipoproteins, during an infection (Ha et al., 2014). Dietary fats in the intestine are incorporated into lipoproteins, triglycerides, chylomicrons who promote the absorption of LPS (O'Keefe, 2008). This is consistent with observations that endotoxin activity is positively correlated with serum triglycerides.

This is a clear example about how intestinal microbiota influences aspects of the physiology and metabolism of the host organism (Ostaff et al, 2013). Several microbial structural components interact directly with the host intestinal cells and tissues to affect nutrient absorption (Blaut, 2014). The imbalance of these host-microbiota interactions is recognized as a risk factor in the emergence of metabolic disorders (Jumpertz et al., 2011).
Bacterial endotoxins produced by the intestinal microbiota can trigger inflammation and oxidative stress response to diets and can therefore act as triggers linking inflammation to the metabolic syndrome and diabetes. Pussinen et al. concluded that endotoxemia is a key player in the pathogenesis of diabetes, involving the intestinal microbiota. The chronic inflammation occurred in obesity and type II diabetes is partially attributed to endotoxins produced by Gram-negative bacteria. It activates both adaptive and innate immune systems, releasing cytokines, antibodies and other inflammatory mediators that can promote hepatic insulin resistance (Pussinen et al., 2011).

## 4. CONCLUSIONS

The issue approached in this paper is important as such literature data regarding patients from Romania are scarce. The metabolic syndrome obesity, diabetes and dyslipidemia are three distinct diseases that can be triggered in cascade if symptoms appear for only one of them. The results obtained proved that the carbohydrate and lipid metabolism are closely interrelated and influenced by compounds resulted from the metabolism of intestinal bacteria. The members of the *Enterobacteriaceae* family were the most prevalent strains in the collected samples. The analysis of the virulence patterns of the isolated strains has shown that although they were not very virulent, the present identified virulence factors could however be responsible for producing afflictions if an imbalance occurs. Extended studies on larger cohorts, molecular analysis of the microbiota and follow up of the patients' evolution in time is needed for a better evaluation of the relationships between the abundance and composition of intestinal microbiota on one side, and the obesity and metabolic diseases, on the other one.

## 5. REFERENCES

**[1]** Bassaganya-Riera J., Viladomiu M., Pedragosa M., De Simone C., Hontecillas R., Immunoregulatory mechanisms underlying prevention of colitis associated colorectal cancer by probiotic bacteria, *PLoS ONE*, 7, 4, **2012.**

[2] Blaser M.J., Falkow S., What are the consequences of the disappearing human microbiota?, Nature reviews, *Microbiology*, 7, 12, 887-894, **2009.**

[3] Blaut M., Gut microbiota and energy balance: role in obesity, *The proceedings of the Nutrition Society*, **2014.**

[4] Cani P.D., Amar J., Iglesias M.A., Poggi M., Knauf C., Bastelica D., Neyrinck A.M., Fava F., Tuohy K.M., Chabo C., Waget A., Delmee E., Cousin B., Sulpice T., Chamontin B., Ferrières J., Tanti J.F., Gibson G.R., Casteilla L., Delzenne N.M., Alessi M.C., Burcelin R., Metabolic endotoxemia initiates obesity and insulin resistance, *Diabetes*, 56, 7, 1761-1772, **2007.**

[5] Chehade J.M., Gladysz M., Mooradian A.D., Dyslipidemia in type 2diabetes: prevalence, pathophysiology and management, *Drugs*, 73, 4, 327-339, **2013.**

[6] Chifiriuc C., Mihăescu G., Lazăr V.; Microbiologie şi virologie medical, *Ed. Universităţii din Bucureşti*, 72, **2011.**

[7] Clements A., Young J.C., Constantinou N., Frankel G., Infection strategies of enteric pathogenic Escherichia coli, *Gut Microbes*, 3, 2, 71-87, **2012.**

[8] Cox L.M., Blaser M.J., Pathways in microbe induced obesity, *Cell Metab*, 17, 6, 883-894, **2013.**

[9] Festi D., Schiumerini R., Eusebi L.,H., Marasco G., Taddia M., Colecchia A., Gut microbiota and metabolic syndrome, *World journal of gastroenterology*, 20, 43, 16079-16094, **2014.**

[10] Haslam D.W., James W.P., Obesity, *Lancet*, 366, 9492, 1197-1209, **2005.**

[11] Dominic S.N., Diabetic dyslipidemia: from evolving pathophysiological insight to emerging therapeutic targets, *Canadian Journal Diabetes*, 37, 5, 319-326, **2013.**

[12] Eckburg P.B., Bik E.M., Bernstein C.N., Purdom E., Dethlefsen L., Diversity of the human intestinal microbial flora, *Science*, 308, 5728, 1635-1638, **2005**.

[13] Geurts L., Neyrinch A.M., Delzenne N.M., Knauf C., Cani P.D., Gut microbiota controls adipose tissue expansion, gut barrier and glucose metabolism: novel insights into molecular targets and interventions using probiotics, *Beneficial microbes*, 5, 1, 3-17, **2014.**

[14] Greabu M., Totan A, Mohora M., Dricu A., Pârvu A.E., Foia L., Motoc M., Ghid de biochimie medicală, *Editura Curtea Veche*, 60, **2014.**
[15] Ha C.W., Lam Y.Y., Holmes A.J., Mechanistic links between gut microbial community dynamics, microbial functions and metabolic health, *World journal of gastroenterology*, 20, 44, 16498-16517, **2014.**
[16] Harris K., Kassis A., Major G., Chou C.J., 2012, Is the gut microbiota a new factor contributing to obesity and its metabolic disordes?, *Journal of obesity*, 1-14, **2012.**
[17] Hartstra A.V., Bouter K.E., Bäckhed F., Nieuwdorp M., Insights into the role of the microbiome in obesity and type 2 diabetes, *Diabetes Care*, 38, 1, 159-165, **2015.**
[18] Ianiro G., Bruno G., Lopetuso L., Beghella F.B., Laterza L., D'Aversa F., Gigante G., Cammarota G., Gasbarrini A., Role of yeasts in healthy and impaired gut microbiota: the gut mycome, *Current pharmaceutical design*, 20, 28, 4565-4569, **2014.**
[19] Jumpertz R., Le DS Turnbaugh P.J., Trinidad C., Bogardus C., Gordon J.I., Krakoff J., Energy balance studies reveals associations between gut microbes, caloric load, and nutrient absorption in humans, *The American journal of clinical nutrition*, 94, 1, 58-65, **2011.**
[20] Lazar V., Herlea V., Cernat R., Balotescu M.C., Bulai D., Moraru A., Microbiologie generala*, Ed. Univ. Bucureşti,* **2004.**
[21] Ley E.R., Bäckhed F., Turnbaugh P., Lozupone C.A., Knight R.D., Gordon J.I., Obesity alters gut microbial ecology*, Proceedings of the National Academy of Sciences of the United States Of America*, 102, 31, 11070-11075, **2005.**
[22] Macfarlane G.T., Macfarlane S., Bacteria, colonic fermentation and gastrointestinal health, *Journal of AOAC International*, 95, 1, 50-60, **2012.**
[23] Morales P., Brignardello J., Gotteland M., The association of intestinal microbiota with obesity, *Revista medica de Chile*, 138, 8, 1020-1027, **2010.**
[24] Nieuwdorp M., Gilijamse P.W., Pai N., Kaplan L.M., Role of the microbiome in energy regulation and metabolism, *Gastroenterology*, 146 6, 1525-1533, **2014.**
[25] O'Keefe S.J., Nutrition and colonic health: the critical role of the microbiota, *Current opinion in gastroenterology*, 24, 1, 51-58, **2008.**
[26] Ostaff M.J., Stange E.F., Wehkamp J, Antimicrobial peptides and gut microbiota in homeostasis and pathology, *EMBO molecular medicine*, 5, 10, 1465-1483, **2013.**
[27] Pussinen P.J., Havulinna A.S., Lehto M., Sundvall J., Salomaa V., Endotoxemia is associated with an increased risk of incident diabetes, *Diabetes care*, 34, 2, 392-397, **2011.**
[28] Rajilic-Stojanovic M.**,** Smidt H., de Vos W.M., Diversity of the human gastrointestinal tract microbiota revisited, *Environmental Microbiology*, 9, 9, 21-25, **2007.**
[29] Ruemmele F.M., Schwartz S., Seidman E.G., Dionne S., Lew E., Lentze M.J., Butyrate induced Caco-2 cell apoptosis is mediated via the mitochondrial pathway, *GUT*, 52, 1, 94-100, **2003.**
[30] Shen J., Obin M.S., Zhao L., The gut microbiota, obesity and insulin resistance, *Molecular aspects of medicine*, 34, 1, 39-58, **2013.**
[31] Tanti J.F., Ceppo F., Jager J., Berthou F., Implication of inflammatory signaling pathways in obesity-induced insulin resistance, *Frontiers in endocrinology*, 3, 181, **2013.**
[32] Turnbaugh Peter J. and Gordon Jefrey I., The core gut microbiome, energy balance and obesity, *The Journal of Physiology*, 587, 17, 4153-4158, **2009.**
[33] Zhang Y.M., Rock C.O., Membrane lipid homeostasis in bacteria, *Nature reviews, Microbiology*, 6, 3, 222-233, **2008.**

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