Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2023 20(4):986-992. **OPEN ACCESS**

The impacts of feeding an artificial sweetener "Steviana" on rats' Gut microbial diversity

Alaa T. Qumsani

Biology Department, Al-Jammoum University College, Umm al-Qura University, Makah, **Saudi Arabia**

***Correspondence: atqumsani@uqu.edu.sa** **Received 21 Sep., 2023, Revised: 17 Oct., 2023, Accepted: 18 Oct., 2023 e-Published: 20 Oct., 2023**

Steviana (ST) is a natural sweetener obtained from the leaves of the Stevia rebaudiana plant and has the potential to be widely used in our diets. We monitored the bactreial population and species in the rat gut using viable bacterial counts and denaturing gradient gel electrophoresis (DGGE) methods to examine the impact of ST on changes in bacterial diversity in the intestinal tract. The viable cell count and DGGE data showed that the ST had no influence on the total bacteria, enter bacteria, and lactobacilli composition. Although it was found that steviana might improve lactobacilli diversity, a low dose only little changed the diversity whereas a high dose significantly increased the species of lactobacilli, thereby enhancing host health. In conclusion, ST appears to have little impact on the makeup of bacteria, indicating that it is safe for gut microbes.

Keywords: Artificial Sweetener, Steviana, Intestinal Bacteria , Bacterial Diversity , DGGE.

INTRODUCTION

The impact of dietary components on the gut flora has been extensively studied. The most attention has been paid to dietary fiber, primarily because the human body is unable to use it and because the gut microbiota may ferment it utilizing a variety of enzymes that are not encoded in the human genome (Rowland *et al.,*2018).The gut microbiota is influenced by a Mediterranean diet, which includes more legumes, grains, fruit, and vegetables and has health-promoting effects (Libik-Konieczny *et al*., 2021).Food additives and artificial sweeteners are increasingly prevalent in our diets. The health of the host is considered when evaluating the impacts of food additives, but the long-term host health and the impact on the human gut flora are not considered (Konstantinos *et a*l., 2020). According to current animal study,food additives can negatively impact intestinal and cardiovascular health by altering the intestinal flora and intestinal mucus layer.(Sara Jarmakiewicz-Czaja *et a*l.,2022). The development of microbial dysbiosis has been shown to have a negative impact on rodent metabolism, according to research on artificial sweeteners (Arna Shil and Havovi Chichger,2021).Stevia, the major component of steviana sweetener, displayed antiviral properties by reducing the activation of nuclear factor kappa B (NF-B), mitogen-activated protein kinase (MAPK) signaling, and the creation of pro inflammatory cytokines. (Arezina *et a*l., 2022), immunomodulatory activity, and anti-inflammatory properties in vitro and in vivo [Sael Casas-Grajales *et al*., 2019). Although the underlying mechanism has not been fully elucidated, stevioside (asteviol glycoside) demonstrated antiglycemic actions in rats by boosting insulin secretion, lowering plasma glucose concentrations, and inhibiting glucagon levels (Rizwan *et al.*, 2019). Stevia stabilizes atherosclerotic plaque and prevents future growth while also improving insulin signaling, having an antioxidant impact in adipose tissue, and reducing blood pressure (systolic and diastolic) levels (Islam *et al*., 2019).

Additional animal studies have demonstrated that chemicals derived from stevia can reduce hepatic steatosis and repair liver and kidney damage. In vitro tests on a variety of cancer cell lines, including breast, prostate, stomach, and colon cancer cell lines, revealed that steviol glycoside derivatives have antiproliferative and anticancer potential (Hasan *et a*l., 2020). The mitochondrial apoptotic pathway was responsible for this outcome. The gut microbiota is now thought of as an organ that promotes the host's health, regulates metabolism, and governs cellular immunity ([Raghad Khalid *et a*l., 2023).

This study looked at the effects of steviana "natural sweetener" on rat's gut major bacteria in vivo, as well as its impacts on specific bacteria. Denaturing gradient gel electrophoresis (DGGE) was used to analyze DNA isolated from the microbial environment directly and to monitor the diversity of microorganisms in animal digestive tracts. Furthermore, performing viable bacterial count with DGGE will allow to evaluate how well steviana influences gut flora.

MATERIALS AND METHODS

Animal design and steviana preparation

In the current study, fifteen rats (6 - 8 weeks old and 120.0 ± 20.0 g body weight) were used. The animals were housed individually and kept at 24°C with a light-dark cycle of 12/12 hours. After one week of acclimatization in the laboratory, rats were randomly separated into three groups (5 rats per group) and given steviana orally, by using rat stomach tube , every day for 30 days at 0 mg/kg bw (Control), 5 mg/kg bw (Low dose, LD), and 10 mg/kg bw (High dose, HD).Food and drink were provided by Adlibetium. At the end of 30 days, fecal samples were collected from 12 rats (4 rats per group) and utilized for bacterial cell count and DNA extraction.

The current study was approved by the umm-alqura university's biomedical research ethics committee (Approval No. HAPO-02-K-012-2023-06-1646). The trials were conducted in conformity with national laws and regulations and the recommendations of the International Animal Ethics Committee.

Steviana Sweetener was purchased from a mega-hyper mall in the form of a powder Jar (200 grams) made by Said Salim Bawazir Sons for toothpaste and sweeteners Ltd.Co,Jeddah-Saudi Arabia. The dosage was calculated to meet the FDA's (5 mg/kg) allowed daily intake in the United States (Uswa *et al*., 2020).It was filtered via a 0.22 m Millipore filter (Carrigthohill, Co. Cork, Ireland) after being diluted with distilled water to a final concentration of 10% (w/v). The two doses used in the current study were 5 (LD) and 10 mg/kg body weight (HD) .Doses were adjusted weekly to account for changes in weight gain.

Count of viable bacterial cells

Fresh feces were processed within 2 hours.Samples were serially diluted 10 times in saline before plating 300uL drops for every dilution on brain-heart infusion agar , (BHI)Land Bridge Technology Co. Ltd, Beijing, China) supplemented with sterile skimmed milk10% for total anaerobic bacteria (incubated an aerobically at 37°C for 36 h), MAC MacConkey agar (MAC) (OXOID Ltd, England) for enter bacteria (aerobically, 37°C, 24 h), and MRS agar for lactobacilli (an aerobically, 37°C, 24 h), respectively (Sikder *et a*l.,2018). The plate count method was used to count the visible bacterial colonies. The CFU/ml value is calculated by multiplying the number of visible colonies (CFU) on an agar plate by the dilution factor.

Pcr amplification and dna extraction

The total genomic DNA was extracted using the bead-beating method from four fecal samples from each group (Chalasani *et a*l.,2018). The DNA was extracted and denatured according to manufacturer's instructions and kept at -20°C for subsequent tests.PCR amplification was performed by Biosci PCR equipment. Table (1) lists the primers used for the PCR amplification of the microbial population following the manufacturer's recommendations. To determine the size and quantity of amplicons, aliquots of 5 uL were electrophoreses on an agarose gel (1.0%) and the results were evaluated.

Denaturing gradient gel electrophoresis (dgge)

The DGGE sequence separation was carried out using the previously described and analyzed amplicons, and it was done so in accordance with Fiona and Andrew's technique [Donskey et al.,2003) The DGGE patterns were evaluated using Bio-Rad software after normalization. During analysis, the background was removed, lane intensity discrepancies were adjusted with computing the correlation matrix. The similarity % was calculated using Pearson correlation and the un weighted pair group method with arithmetic mean (UPGMA) (Strathdee et al., 3013). According to the equation of Yan et al.,2019), similarity indices including the Shannon-Wiener index (H), richness (S), and evenness (E_H) were computed to examine genetic diversity in the gut bacterial communities of control and experimental rats.

Dgge band sequencing

To release DNA from the polyacrylamide matrix, the sections of DNA we wanted were cut out from the gels using a clean blade. Then, they were put into 20 micro liters of deionized water and stored at a cold temperature (4°C) overnight. Given the earlier mentioned conditions, these DNA samples were used using the same primers but without a GC clamp. The DNA samples were cleaned using a special kit, cloned with a specific system, transferred to a type of bacteria, and then analyzed at a different laboratory. Ltd, short for Limited, is a term used in business to indicate that a company is a separate legal entity with limited liability. Shanghai is a city in China. The NCBI website disagreed with or found fault with these conclusions.

In order to allow DNA to diffuse out of the polyacrylamide matrix, the interested bands of DNA were sliced using a sterile blade from the gels, placed in 20 uL of deionized water, and left 12h at 4°C. Under the previously mentioned circumstances, these DNA samples were employed in subsequent PCR amplifications using homologous primers but without a GC clamp. The PCR products were purified with a commercial PCR purification kit, transformed into an Escherichia coli DH5 competent cell, and sequenced at Sangon Biotech Co. Ltd. (Shanghai, China). The NCBI website disputed these findings.

Statistical analysis

The data are all provided as mean ± SE. The SPSS 13.0 program was used for data analysis, with independent one-way ANOVA testing. The LSD test was used to examine the differences between rat groups at ($p \leq 0.05$

Table 1: The PCR primers

Target Bacteria	Primers	Sequence	References
Total bacteria	Forward(TPU1) Reverse(RTU8)	(AGAGTTTGATCMTGGCTCAG) (AAGGAGGTGATCCANCCRCA)	(Chalasani et al.,2018)
Entrobacteriaceae	Forward(Ec1055) Reverse(Ec1392)	(ATGGCTGTCGTCAGCT -GC clamp- -ACGGGCGGTGTGTAC-	(Sikder et al.,2018) (Jeyaram et al.,2008) (AbriouelEt al.,2019)
Lactobacilli	Forward(Lac) Reverse(Lac)	-AGCAGTAGGGAATCTTCCA- -GC clamp- - ATTYCACCGCTA-	

- GC clamp (CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGG)

RESULTS AND DSCUSSON

Microbial count

The intestine is a home to a large number of microbes that form a complex ecological community. This community influences normal physiology and is crucial for maintaining human health because it provides defense against enteropathogens (da Silva_*et al*.,2022), extracts nutrients and energy (Guan *et al*.,2020), and supports healthy immune function (Chen *et al*.,2019) If the microbial equilibrium was disturbed, certain diseases would develop, including cancer, neurological disorders, inflammatory bowel disease, obesity, malnutrition, and malnutrition. Steviana should not encourage the formation of infections or disturb the usual bacterial balance when used as a food ingredient (Gomez-Brandon *et a*l.,2020 ; Alam *et a*l.,2020 ; Deng *et a*l.,2020).

This study examined the impact of ST on the alive counts of total bacteria, enterococci, enterobacteria, and lactobacilli.The results revealed no appreciable variations in all bacterial populations (Fig. 1). This was in line with earlier findings (Uddin *et al*.,2022) and suggested that steviana did not significantly change the composition of the intestinal micro biota after administration.

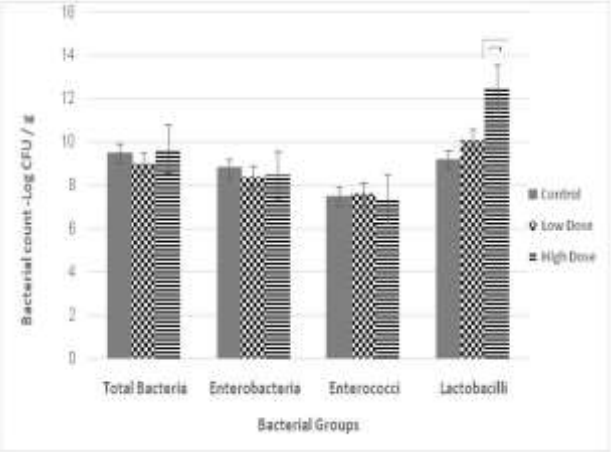


Figure 1: Numbers of intestinal bacterial groups in rats supplemented with steviana sweetener for 30 days. ( ) Significant at (*p≤0,05*)

Denaturing gradient gel electrophoresis (dgge)

Total bacteria

As shown in table (2), Bands 1,2,4,6,7,8,and 9 were occupied dominant positions throughout the administration, indicating tolerance to steviana concentrations . As shown in table (5), The control group was stable because all of the rats in it clustered into one group and had a minimum similarity of 79%. The minimal similarity of the LD group (85%) , HD group (82%), and the minimal similarity of the control, LD, and HD groups (72%), offered additional proof that ST had just a little impact.Also,there were no detectable changes in the bacterial diversity indices (Table 6).

Enterobacteria

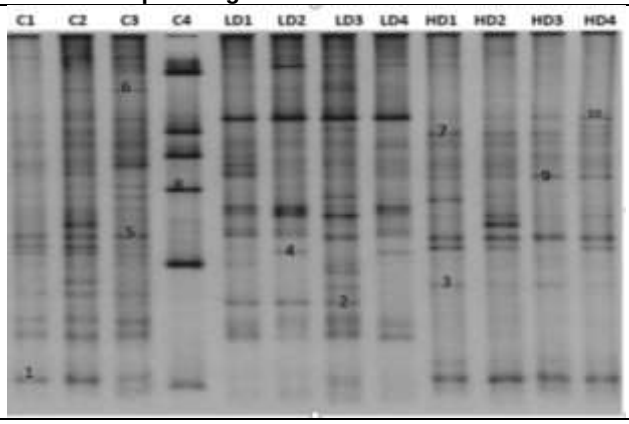
The large gram-negative bacilli group known as entero-bacteria frequently resides in the gastrointestinal tracts of both humans and animals. The common microbiota in the digestive system includes enterobacteria, which can develop into pathogens and cause disease in conjunction with altered internal conditions (Walburga Dieterich *et al*.,2018).

Table (3) revealed that among the three groups, bands 1 and 6 had held the dominating positions and had undergone no change; however, bands 2, 3, and 4 had the dominant position in the HD group, indicating that steviana had boosted their counts in the intestinal tracts of rats. These groups had greater than 63.0% similarity according to the UPGMA results (Table 5), and the diversity indices showed no appreciable changes (Table 6).

Lactobacilli

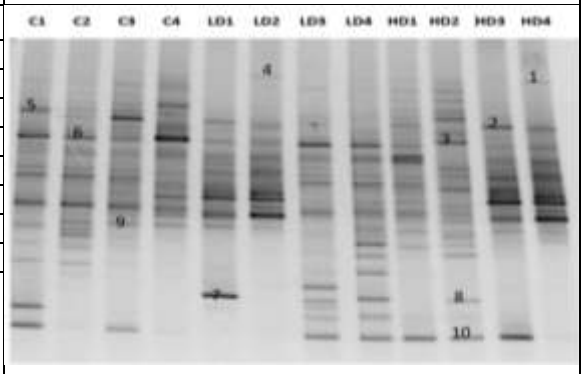
The lactobacilli in the human gastrointestinal tract are essential for preserving intestinal health and regulating host immunity (Elaine and Sinéad · 2022). Table (4) showed that while bands 6 and 8 held the prominent positions in the intestinal tracts of rats and had no change when given varying concentrations of steviana, bands 1, 2, 3, 5, and 7 remained unchanged.

Table 2: DGGE bands Sequencingproduced by PCR from fecal DNA using universal bacterial primers for bacterial identification.

Strain No	Nearest relative	Gene bank- ID	Sequencing of bacterial DNA in feces
1	Uncultured bacterium	AB702754.1	
2	Uncultured bacterium	JQ695608.1	
3	Uncultured bacterium	JQ894202.1	
4	Uncultured Bacteroides sp.	JF710634.1	
5	Bacteroides chinchillae	AB547637.1	
6	Uncultured bacterium	JQ894297.1	
7	Uncultured bacterium	JX013201.1	
8	Uncultured bacterium	JF259521.1	
9	Escherichia coli	JX290090.1	
10	Not detectable	-	

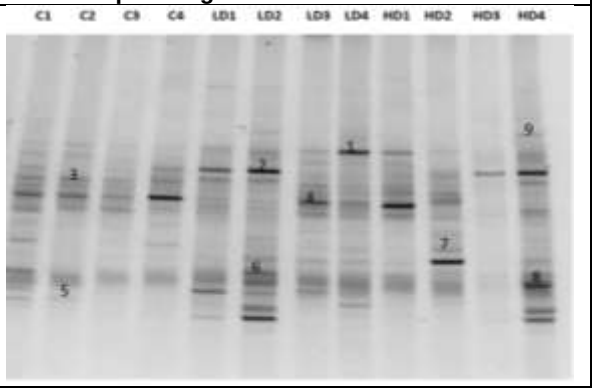
- Sequences were contrasted with those found in the GenBank database.Control (C), low dose (LD), and high dose (HD).

Table 3: DGGE bands Sequencing produced by PCR from fecal DNA using Enterobactereacae primers for bacterial identification.

Strain No	Nearest relative	Gene bank- ID	Sequencing of bacterial DNA in feces
1	Shigella flexneri	JX307691.1	
2	Stenotrophomonas maltophilia	JX293297.1	
3	Pseudomonas geniculata	AB734811.1	
4	Mucispirillum schaedleri	NR_042896.1	
5	Xanthomonas retroflexus	JQ890537.1	
6	Uncultured bacterium	HM124111.1	
7	Unidentified bacterium	AY345532.1	
8	Akkermansia muciniphila	CP001071.1	
9	Escherichia coli	JQ863234.1	
10	Not detectable		

- Sequences were contrasted with those found in the GenBank database.Control (C), low dose (LD), and high dose (HD).

Table 4: DGGE bands Sequencing produced by PCR from fecal DNA using Lactobacilli primers for bacterial identification.

Strain No	Nearest relative	Gene bank- ID	Sequencing of bacterial DNA in feces
1	Lactobacillus johnsonii	JQ989153.1	
2	Lactobacillus taiwanensis	HE573918.1	
3	Lactobacillus crispatus	AY335500.1	
4	Lactobacillus intestinalis	FR683097.1	
5	Lactobacillus animalis	JN713320.1	
6	Lactobacillus agilis	JQ837458.1	
7	Lactobacillus reuteri	EF439674.1	
8	Lactobacillus taiwanensis	HE573918.1	
9	Uncultured bacterium	JQ085223.1	
10	Not detectable		

- Sequences were contrasted with those found in the GenBank database.Control (C), low dose (LD), and high dose (HD).

Table 5: Diversity indices

Primers	Groups	Diversity Indices			
		Richness *(S)*	H'^{A}	$H'max^{B}$	Enveness(E)
Universal bacteria	C	30 . 4 ± 0 . 50	3 . 46 ± 0 . 10	3 . 88 ± 0 . 02	0 . 980 ± 0 . 001
	LD	29 . 0 ± 0 . 70	2 . 91 ± 0 . 20	2 . 83 ± 0 . 02	0 . 890 ± 0 . 004
	HD	28 . 4 ± 0 . 80	3 . 40 ± 0 . 10	3 . 90 ± 0 . 03	0 . 895 ± 0 . 001
Enterobacteracae	C	19 . 2 ± 2 . 10	3 . 01 ± 0 . 10	3 . 05 ± 0 . 15	0 . 880 ± 0 . 002
	LD	19 . 4 ± 0 . 50	2 . 92 ± 0 . 15	2 . 96 ± 0 . 07	0 . 885 ± 0 . 004
	HD	18 . 8 ± 1 . 70	2 . 80 ± 0 . 17	3 . 88 ± 0 . 08	0 . 990 ± 0 . 001
Lactobacilli	C	9 . 9 ± 0 . 80	2 . 90 ± 0 . 08	2 . 08 ± 0 . 09	0 . 89 ± 0 . 01
	LD	12 . 8 ± 1 . 10	1 . 97 ± 0 . 12	2 . 19 ± 0 . 10	0 . 92 ± 0 . 14
	HD	13 . 2 ± 0 . 84*	2 . 86 ± 0 . 09	2 . 25 ± 0 . 09*	0 . 95 ± 0 . 04

- (*n = 4*) , H'A, Shannon-Wiener index; H'max B, the maximum Shannon-Wiener index ; C, control ; LD, low dose , HD,hight dose . Data are mean ± SE,(*) values are significant at ($p \leq 0.05$).

Table 6: Minimal similarity % of fecal microbiota within / among groups calculated through UPGMA analysis

Primers	Groups	Similarity %	
		Within group	Among groups
Universal bacteria	C	0.79	0.72
	LD	0.82	
	HD	0.85	
Enterobacteracae	C	0.78	0.63
	LD	0.75	
	HD	0.68	
Lactobacilli	C	0.73	0.42
	LD	0.68	
	HD	0.69	

- (*n = 4*), Data are % value ; C, control ; LD, low dose , HD,hight dose ;UPGMA, un-weighted pair group method with arithmetic mean

In the HD group, the maximum Shannon-Wiener (H'max B) and species richness indices were considerably higher than those in the control group ($p \leq 0.05$) (Table 6). As a result, even though there is a minimum similarity of 60% between the control and the LD groups, while, it is only 42% between the HD, control and LD groups (Table 5). It appeared that ST could increase the diversity of lacto-bacilli;the LD of ST only minimally altered the diversity of lactobacilli, whereas a large dose dramatically increased the species of lactobacilli, perhaps improving host health.

CONCLUSION

The obtained results revealed that steviana had no influence on the diversity of gut bacteria. These results could be due to gut bacteria in the gastrointestinal tract degrading steviana into other safe metabolites. Nonetheless, it appeared that steviana could increase the diversity of lactobacilli; the LD minimally altered the diversity of lactobacilli, whereas a large dose dramatically increased the species of lactobacilli, perhaps improving host health. Finally, the results indicated that steviana sweetener had no effect on the composition of intestinal bacteria proved its safety for the gut microbiota.

Supplementary materials

The supplementary material / supporting for this article can be found online and downloaded at: https://www.isisn.org/article/10.3390/antiox12081524/s1,
.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT

The author thanks Dr. Mohmed E. Elbeeh for supporting this work. This study got no specific funding from government, commercial, or non-profit organizations.

AUTHOR CONTRIBUTIONS

The author designed the article, searched the literature, wrote the first draft, reviewed the revised draft of the manuscript, completed the manuscript, and approved

Alaa T.Qumsani
the submitted version.

Funding statement
Not funded.

Institutional Review Board Statement
The study was approved by the Bioethical Committee of the Umm Al-Qura University Ethical committee.
Informed Consent Statement
Not applicable.

Data Availability Statement
All of the data is included in the article/Supplementary Material.

Acknowledgments
We thank ABCD for editing the English text of a draft of this manuscript. Add acknowledgements here

REFERENCES

Abriouel, H., Pérez Montoro, B., de la Fuente Ordoñez, J.J. *et al.* (2019).New insights into the role of plasmids from probiotic *Lactobacillus pentosus* MP-10 in Aloreña table olive brine fermentation. *Sci Rep* **9**, 10938 . https://doi.org/10.1038/s41598-019-47384-1

Alam, M.T., Amos, G.C.A., Murphy, A.R.J. *et al.* (2020).Microbial imbalance in inflammatory bowel disease patients at different taxonomic levels. *Gut Pathog* **12**, 1.

Arezina N. Kasti, Maroulla D. Nikolaki, Kalliopi D. Steviana and gut microbial diversity Synodinou,et al. (2022).The Effects of Stevia Consumption on Gut Bacteria: Friend or Foe?. Microorganisms. Apr; 10(4): 744. doi: 10.3390/microorganisms10040744

Arna Shil and Havovi Chichger. (2021). Artificial Sweeteners Negatively Regulate Pathogenic Characteristics of Two Model Gut Bacteria, E. coli and E. faecalis. Int J Mol Sci. May; 22(10): 5228. doi: 10.3390/ijms22105228

Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. (2018).The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatol. (Professional society guidelines). 67 (1):328-357.

Chen, H., Li, D., Mao, Q., Xiao, K., Wang, K., (2019). Resource limitation of soil microbes in karst ecosystems. Sci Total Environ, 650(Part 1): 241-248. http:// doi.org/10.1016/j.scitotenv..09.036

Deng T., X., Chen, L., Xiang, W., (2020). The soil properties and their effects on plant diversity in different degrees of rocky desertification. Science of Total Environment, 736: 139667. http://doi.org/10.1016/j. scitotenv.2020.139667

Donskey, C.J., Hujer, A.M., Das, S.M., Pultz, N.J., Bonomo, R.A., and Rice, L.B. (2003). Use of denaturing gradient gel electrophoresis for analysis of the stool microbiota of hospitalized patients. J. Microbiol Meth., 54, 249-256

Elaine Dempsey and Sinéad C. Corr. (2022).Lactobacillus spp. for Gastrointestinal Health: Current and Future Perspectives. Front Immunol. ; 13: 840245.doi: 10.3389/fimmu.2022.840245

Gomez-Brandon, M., Probst, M., Siles, J.A., Peintner, U., Bardelli, T., Egli, M., Insam, H., Ascher-Jenull, J., (2020). Fungal communities and their association with nitrogen-fixing bacteria affect early decomposition of Norway spruce deadwood. Scientific Reports, 10: 8025. http://doi.org/10.1038/s41598-020- 64808-5

Guan, H.; Ke, W.; Yan, Y.; Shuai, Y.; Li, X.; Ran, Q.; Yang, Z.; Wang, X.; Cai, Y.; Zhang, X. (**2020).**Screening of natural lactic acid bacteria with potential effect on silage fermentation, aerobic stability and aflatoxin B1 in hot and humid area. *J. Appl. Microbiol.* , *128*, 1301–1311

Hasan M, Mahmud AA, Alam MJ, Siddiqui SA, Arman MSI, Mahmud MH, Amin MN, Imtiaz O, Shahriar M, Jakaria M. (2020). Subacute oral toxicity of ayurvedic anti-diabetic preparation Jambadyarista in Sprague-Dawley rats. Toxicol Rep.; 7:1616-1621.

Islam MAF, Barua A, Hassan MB, Alam MJ, Ershad M, Choudhuri MSK, Masuma R. (2019).Pre-clinical investigation of anti-diarrheal and CNS depressant effect of Wedelia trilobata in Swiss albino mice. Orient Pharm Exp Med. 19(4):435–444.

Jeyaram, K., Mohendro Singh, W., Premarani, T., Devi,

A.R., Chanu, K.S., Talukdar, N., and Singh, M.R. (2008). Molecular identification of dominant microflora associated with 'Hawaijar'—A traditional fermented soybean (Glycine max (L.)) food of Manipur, India. Int. J. Food microbiol., 122, 259-268.

Konstantinos Gerasimidis, Katie Bryden, Xiufen Chen, Eleftheria Papachristou, Anais Verney, Marine Roig et al. (2020).The impact of food additives, artificial sweeteners and domestic hygiene products on the human gut microbiome and its fibre fermentation capacity. Eur J Nutr. ; 59(7): 3213–3230.doi: 10.1007/s00394-019-02161-8

Libik-Konieczny M, Capecka E, Tuleja M, Konieczny R. (2021).Synthesis and production of steviol glycosides: recent research trends and perspectives. Appl Microbiol Biotechnol.; 105(10):3883-3900.

Raghad Khalid AL-Ishaq,Peter Kubatka and Dietrich Büsselberg. (2023).Sweeteners and the Gut Microbiome: Effects on Gastrointestinal Cancers. *Nutrients* , *15*(17), 3675; https://doi.org/10.3390/nu15173675

Rizwan F, Yesmine S, Banu SG, Chowdhury IA, Hasan R, Chatterjee TK. (2019).Renoprotective effects of stevia (Stevia rebaudiana Bertoni), amlodipine, valsartan, and losartan in gentamycin-induced nephrotoxicity in the rat model: Biochemical, haematological and histological approaches. Toxicol Reports.; 6:683-691.

Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, (2018).Tuohy K. Gut microbiota functions: metabolism of nutrients and other food components.Eur J Nutr. Feb;57(1):1-24. doi: 10.1007/s00394-017-1445-8.

Sara Jarmakiewicz-Czaja, Dominika Piątek, and Rafał Filip. (2022).The impact of selected food additives on the gastrointestinal tract in the example of nonspecific inflammatory bowel diseases. Arch Med Sci. ; 18(5): 1286–1296. doi: 10.5114/aoms/125001

Sael Casas-Grajales, Erika Ramos-Tovar, Esmeralda Chávez-Estrada, Diana Alvarez-Suarez Erika Hernández-Aquino,et al. (2019). Antioxidant and immunomodulatory activity induced by stevioside in liver damage: in vivo, in vitro and in silico assays. Life Sci. May 1; 224: 187–196. doi: 10.1016/j.lfs.2019.03.035

Sikder MM, Zulfiquar TN, Chowdhury IH, Akter K, Asha UH, Haque E, Chakraborty P, Choudhuri MSK. (2018).Preclinical Lipid Profile Studies of a Classical Ayurvedic Preparation "Siddha Makardhwaja (SMD)" after Chronic Administration to Male Sprague-Dawley Rats. Biol Med.(Aligarh).; 10(3):439.

Silva da , É.B.; Liu, X.; Mellinger, C.; Gressley, T.F.; Stypinski, J.D.; Moyer, N.A.; Kung, L. (**2022).**Effect of dry matter content on the microbial community and on the effectiveness of a microbial inoculant to improve the aerobic stability of corn silage. *J. Dairy Sci.* , *105*, Steviana and gut microbial diversity 5024–5043.

Strathdee F, Free A. (2013).Denaturing gradient gel electrophoresis (DGGE). Methods Mol Biol.;1054:145-57. doi: 10.1007/978-1-62703-565-1_9. PMID: 23913290.

Uddin A. , M., Akter, F., H. Chowdhury, I., H. Asha, U., Z. Tanny, S., A. Sony, T., Neon, N., Saha, N., M. Sikder, M., & Yesmine, S. (2022). Toxicological Studies of Leaf extract of Stevia rebaudiana Bertoni in Sprague-Dawley Rats. *Tropical Journal of Natural Product Research (TJNPR)*, *6*(5), 714–720.

Uswa A, Rabia SA, Muhammad S, Ali I, Muhammad H, Farzana S, Zarina Y, Ijaz A, Ashir M, Abdul B. (2020).Haematological and toxicological effects of aqueous leaf extract of Stevia rebaudiana Bertoni in normal rat modals. Pak J Pharm Sci.; 33(Suppl 5):2249-2255.

Walburga Dieterich, Monic Schink, and Yurdagül Zopf. (2018).Microbiota in the Gastrointestinal Tract. Med Sci (Basel). ; 6(4): 116.doi: 10.3390/medsci6040116

Yan, Y.; Li, X.; Guan, H.; Huang, L.; Ma, X.; Peng, Y.; Li, Z.; Nie, G.; Zhou, J.; Yang, W.; et al. **(2019**).Microbial community and fermentation characteristic of Italian ryegrass silage prepared with corn stover and lactic acid bacteria. *Bioresour. Technol.* , *279*, 166–173.