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Immunological Impacts of Fermented *Pentaclethra macrophylla* Candy

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Abstract

This study investigated the immunological impact of fermented Pentaclethra macrophylla (Oil bean) candy, aiming to address the issue of chemical-fortified candies affecting gut immunity in Nigeria. The researchers prepared and fermented Pentaclethra macrophylla seeds, then mixed them with Lactobacillus rhamnosus and packaged them as candy. The immunological effects were evaluated using in vivo techniques, gravimetric and instrumentation methods. The results showed significant improvements in immune response, including increased Phagocytic Index: Enhanced ability of immune cells to engulf pathogens; Elevated Antibody Titre: Higher levels of antibodies, indicating a stronger immune response; T Cell Population Increase: Boosted cell-mediated immunity, Pronounced Delayed Type Hypersensitivity (DTH): Enhanced immune response to pathogens. The study used Lactobacillus acidophilus strain DSM20079 and Bacillus subtilis strain PK5-17 as fermenters and demonstrated the candy's safety and immunostimulatory potential. The findings suggest that this fermented candy could be a potential natural remedy for boosting gut immunity. Further research could explore its applications in human health and potential integration into existing confectionery products, like those offered by Sweetco Foods Limited, a Nigerian candy manufacturer known for its high-quality products

.Keywords: *Pentaclethra,* Lactobacillus, *In vivo*, Fermenters

INTRODUCTION

Candy is basically made of sugar, in addition to some other ingredients, according to the kind of candy; such as fats, colors and flavors. The nutritional value of candy is confined to the fact that it is a source of considerably big energy, but it does not provide the body with the necessary nutrients, like proteins, vitamins and minerals (Zulkani *et al*., 2017). Chocolate, however, could be exempted, as it contains a number of proteins, owing to milk being one of its ingredients. Sugars or sweets are not always harmful. On the contrary, the body is always in need of sugars to produce the energy necessary for motion and activity. However, people, especially children, should rationalize the use of sweets to avert the harmful effects that might come up as a result of the excessive use of them. Tooth decay and obesity may result. **Some additives**, like caffeine which affects the nervous system and causes nervousness, tension and insomnia. It could also accelerate pulse and cause many other problems, especially for children (Feng *et al.,* 2019). Research had shown that candy can be produced from natural sources such as oil bean ((*Pentaclethra macrophylla*). Oil bean is a nutritious food found majorly in the Eastern part of Nigeria. It is a nutrient dense condiment containing the essential vitamins and minerals required for healthy living. Pytochemical composition of oil bean includes terpenoids, cardiac glycosides and saponin. Medically, oil bean had been optimized in lowering cholesterol level, reducing risk of cancer, insomnia and cataract, promoting weight loss and healthy heart (Bell *et al*., 2018).

Recently, bioactive compounds associated with fermented plant products and activities of probiotics especially *Lactobacillus* species have been considered as an important strategy to retaining the nutritive values of candy in order to maintain the steady-state of the gut and boost the body immunity. In Nigeria, the choice of fermenters and plants with high nutritive values and beneficial phytochemical constituents remain the major aspect that needs urgent attention. Hence, the present study focuses on the immunological impact of fermented *Pentaclethra macrophylla* (Oil bean) seed candy

MATERIALS AND METHODS

Isolation and Characterization of the Fermenters

Isolation of the fermenters: The inocula used in this study were yoghurt and garden soil samples. The yoghurt

sample was prepared in 1:10 dilution whereas 1:10 diluted garden soil sample was further diluted to 1:1000 dilution. The media used for this isolation includes de Man Rogosa and Sharpe broth (MRS/BIOTECH) and Nutrient agar (NA/BIOTECH). The soil sample was boiled, allowed to cool in an aseptic condition, and 0.1 mL of the prepared soil sample was collected using a sterile pipette and aseptically plated onto solidified Nutrient Agar plate (90 mm x 15 mm) which was prepared according to the manufacturer's instruction, and this was spread using a spreading rod, and incubated using electronic laboratory incubator (STXB128) at 35 ± 2^0C for 24 h. Also 10 mL of the prepared yoghurt sample was collected using sterile pipette and aseptically inoculated into sterile 200 mL conical flask (Glassco) containing MRS broth (BIOTECH) which was prepared according to the manufacturer's instruction, and this was incubated in a microaerophic environment (containing candle used to evacuate all traces of oxygen thereby creating an environment having only carbon iv oxide). The incubation was done for 24 – 72 h at (30 ± 2^0C).

Purification of the isolates: The plate that showed discrete colonies were selected after 24 h and each colony was aseptically streaked using a sterile wire loop on a sterile poured plate (90 mm x 15 mm) containing nutrient agar (BIOTECH) prepared according to the manufacturers description. Similar procedure was repeated on deMan Rogosa Sharpe (MRS) agar plate (90 mm x 15 mm) that was prepared according to the manufacturer's instruction after which it was incubated at their required growth conditions.

Characterization of the bacteria pure isolates: The pure isolates were characterized using the morphological, biochemical and molecular characteristics as described by Iheukwumere *et al.* (2018).

Morphological characteristics of the bacteria isolates: The cultural descriptions (size, appearance, edge, elevation, colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described in the study published by Iheukwumere *et al.* (2018).

Immunostimulatory Potential of the Prepared Candy

Antigen preparation: This was carried out using the method described in the

study published by Nfeimbi *et al.* (2015). Fresh blood sample was collected from healthy sheep reared in Ipupe, Abia State, and this was mixed with sterile Alsever's solution (1:1). The sample was centrifuged at 2000 xg for 5 min to enable the red blood cells (RBCs) settled at the bottom of the test tube. Then the supernatant was discarded and the sediment was collected as the sheep red blood cells (SRBCS). The SRBC was then washed three times with pyrogen-free phosphate buffered saline (PH 7.2). This was then kept under refrigeration for the study.

Experimental protocols for the *in vivo* models: A total of 64 albino Wistar rats were used for this study. The albino Wistar rats were grouped 4 groups (Normal control, test, dexamethasone and levamisole group) and each group contained 16 rats. A total of 8 rats in each group were sensitized intraperitoneally with 0.1 mL SRBC containing $1x10^8$ cells. Then all the rats in control group (C) were giving normal saline (0.85% Nacl), the test group (T) were fed with the candy (0.5 g/100g), dexamethasone group (D) were giving dexamethasone (200 mg / kg bwt) and levamisole group (L) were giving levamisole (50 mg/kg bwt) for 7 days. The Immunostimulatory activity of the set- up was determined using the parameters below.

Delayed type hypersensitivity (DTH): This was carried out using the modified method described and published by Anarthe *et al.,* (2014). Here, four (4) rats that were not injected SRBCs were selected from each group for this study. On the 7th day prior to injection, right hand footpad thickness of the albino rats (4 rats of 16 in each group) were measured with micrometer screw gauge. Then the rats were challenged by injecting 20 mL of 1 % SRBC into the right hind foot pad. On the 8th and 9^{th} days, the footpad thickness of the experimented rats were again measured and the mean differences of the pretreated and post treated rats were calculated and recorded in millimeters.

Antibody titer: This was carried out using the modified methods described and published by Anarthe et al. (2014) and Nfeimbi *et al*. (2015). Here, four (4) rats that were not injected SRBCs were selected from each group for this study. On 7th day before challenge, blood samples were drawn from retro-orbital plexus of the rats. The blood samples were centrifuged for 2000 xg for 5 min, and the sera were collected. Two -fold serial dilution was carried out on each of the serum (i.e. 50 µl of serum was added

in each microtiter well dilution in order to get up to the 24th well. Then 50 µl of 1 % SRBC prepared using normal saline was added into each well, and the plates were incubated at 37ºC for 1 h. The reciprocal of the highest dilution that showed visible agglutination was considered as the hemagglutination antibody titer (HA units /NL).

Population of T cell: This was carried out using the modified method described and published by Anarthe *et al*. (2014). Here, four (4) rats that were injected SRBCs were selected from each group for this study. On the 11th day, blood samples were collected from retro-orbital plexus of the rats (another 4 rats in each group) and these were mixed with Alsever's solution in test tubes. These were kept, in sloping position (45º) and incubated at 37ºC for 1 h. The RBCs were allowed to settle at the bottom of the test tubes, and supernatant was collected from each test tube using micropipette and this contains the Lymphocytes. Differential white blood cell count was carried out to determine percentage population of lymphocytes using Automated Hematology Analyzer (MIN DRAY BC – 360) as described in the work published by Agiang *et al.* (2017)

Phagocytic activity (Carbon clearance assay): This was carried out using the modified method described and published by Anarthe *et al.* (2014). Here, four (4) rats that were injected SRBCs were selected from each group for this study. After 7days, the experimented rats (another 4 rats from each group) were stabilized for 2 days. On the 11th day, the selected rats were intravenously (through the tail vein) injected with carbon suspension (1:50 dilution of Indian ink) in a dose of 0.5 mL/100 g bwt. Blood samples were withdrawn from the retro-orbital venous plexus before injection, at 5 min and 15 min after injection of the carbon suspension. Then 0.05 mL of each blood sample was lyzed with 4 mL of 0.1 % Na_2CO_3 and the optical density was measured spectrophotometrically at 650nm wavelength. The phagocytic index (K) was calculated using the equation below

$$K = \frac{\text{Log}(ODa) - \text{Log}(ODt)}{t}$$

ODo = Optical density at 0 min

ODt = Optical density at 5 min and 15 min

t = time (5 min and 15 min)

Statistical Analysis

The data obtained in this study were presented in Tables. One way Analysis of Variance was used to determine the significance of the sample sources 95% confidence level. Pairwise comparison was carried out using student "t" test (Iheukwumere *et al*. 2018).

RESULTS

Characteristics of the Fermenters

The cultural and morphological characteristics of the two used fermenters is shown in Table 1. The two isolates were Gram positive rods but differed in their elevation, edge, surface nature, optical character, motility and ability to produce endospores as shown in Table 1. Isolate P had low convex elevation, smooth edge, and surface, transparent and non-motile whereas isolate Q had flat elevation, irregular edge, rough surface, opaque, centrally positioned endospore and motile. Isolate P was catalase, oxidase, citrate, urease, methyl red and Voges Prokauer negative, and was not able to hydrolyze gelatin whereas as shown in Table 2. The two isolates showed varying utilization of sugars and sugar alcohols, and isolate Q utilized D- mannitol and this pointed to *Bacillus subtilis.* The ratio of the absorbance of nucleic acids extracted from the two isolates at 260 nm and 280 nm were within the range 1.80 – 1.90, and this pointed that the nucleic acids were Deoxyribonucleic acid (DNA) as shown in Table 3. The molecular identities of isolate P and Q revealed *Lactobacillus acidophilus* strain DSM 20079 (LADSM) and *Bacillus subtilis* strain PK5-17 (BSPK5) as shown in Table 4 with 100% identities.

Immunostimulatory Effect of the Candy

Delayed type hypersensitivity (DTH), mean antibody titre, mean lymphocyte population, phagocytic index and percentage lymphocytes were used as yardstick for monitoring the Immunostimulatory activity of the melded candy as shown in Table 7. There were significant ($P < 0.05$) increased in DTH, mean antibody titre, mean lymphocyte population, phagocytic indices and percentage lymphocytes among the experimented rats fed with the melded candy compared to the normal control group who were giving the normal feed and water without the melded candy. There was no significant difference ($P > 0.05$) between the values of DTH, mean antibody titre, mean lymphocyte population, phagocytic indices and percentage lymphocytes from the rats fed with 100 mg/g dose and those fed with 200 mg/g dose of the melded candy, only that slight increase was detected among the rats fed with 200 mg/g dose of the melded candy. It was also observed that the values of DTH were detected more after 24 h and the values slightly decelerated after 48 h in both rats fed with 100 mg/g and 200 mg/g of the melded candy, and this decrease was statistically non-significant ($P > 0.05$).

Table 1: Cultural and morphological characteristics of the fermenters

Parameter	Isolate P	Isolate Q
Appearance	Cream-white on MRS agar	Cream/white on Nutrient agar
Elevation	Low-convex	Flat
Edge	Smooth	Irregular
Surface	Smooth	Rough
Optical Nature	Transparent	Opaque
Gram Reaction	+	+
Cell Morphology	Rods	Rods with round ends
Spore	_	+
Position of Spore	_	Central
Motility	_	+

+ + Positive; - = Negative

Table 2: Biochemical characteristics of the fermenters

Parameter	Isolate P	Isolate Q
Catalase	_	+
Citrate	_	+
Oxidase	_	+
Urease	_	_
Gelatin	_	+
Methyl Red	_	_
Voges Proskauer	_	+
Glucose	+	+

D-mannitol	+/_	+
Lactose	+	_
Maltose	+	+
Xylose	_	+
Inositol	+/_	+
Fructose	+	+
Sorbitol	_	+
Trehalose	+/_	+
Dulcitol	+/_	+
Possible Isolate	*Lactobacillus* species	*Bacillus* species

Table 3: Authentication of nucleic acids extracted from the fermenters

Sample ID	Nucleic Acid Conc(µg/mL)	260 nm	280 nm	260/280
P	142.40	3.1915	1.7440	1.83
Q	126.20		1.7220	1.81

Table 4: Molecular identities of the fermenters

Parameter	Isolate P	Isolate Q
Max Score	6593	4783
Total Score	10535	4783
Query Cover (%)	100	100
E-Value	0.0	0.0
Identity (%)	100	100
Accession Length	2009973	4086149
Accession Number	CP020620.1	CP026037.1
Description	*Lactobacillus acidophilus*	*Bacillus subtilis* strain

strain DSM20079 Chromosome Complete genome (LADSM)	PK5-17 Chromosome Complete genome (BSPK5)	

Table 7: Immunostimulatory effect of the candy

Parameter		Control	100 mg/g	200 mg/g
DTH	24 h	5.00±0.00	9.50±0.00	9.67±0.14
	48 h	4.50±0.00	8.67±0.14	9.00±0.00
Mean Antibody Titre(HA Units/µL)		32.00±0.00	512.00±0.00	512.00±0.00
Mean Lymphocytes Population		147.22±1.82	286.44±.62	289.31±1.22
Phagocytic Index		0.041±0.001	0.086±0.001	0.087±0.001
Lymphocytes (%)		49.80±1.08	86.50±1.22	87.20±1.41

DTH = Delayed Type Hypersensitivity 24/48 h ($P > 0.05$); 100 mg/ control ($P < 0.05$) 200 mg/control ($P < 0.05$); 200 mg/ 100 mg ($P > 0.05$).

DISCUSSION

The characteristics of *Lactobacillus acidophilus* and *Bacillus subtilis* strains used for this study agrees with the findings of many researchers (Oz *et al*., 2017; Salam *et al*., 2017; Zhu *et al*., 2017; Anggraeni *et al.,* 2019; Rizal *et al*., 2020). The characteristic features of the prepared candy and the level of water activity conform with World Health Organization (WHO) and National Industrial Standard (NIS) stipulation for preserved and safety food product. Fermentation of the product introduced the biological method of preserving the food product, reducing the microbial load due to the presence of organic acids, ethanol and bacteriocins, producing new and desirable food product. Similar deduction was made by Marco *et al*. (2017). The microbial degradation results in degradation of various anti-nutrient, and increases the amount of small peptides and essential amino acids content of the candy, making the product more palatable, safe and nutritive. Similar deduction was made by Mukherjee *et al*. (2016) and Bell *et al.* (2018). The joint activities of *Lacobacillus acidophilus* and *Bacillus subtilis* strains produced extracellular enzymes that could have fermented the food product in order to boost the nutritive values, majorly the protein content and fat content as reported by Mukherjee *et al*. (2016).

The increase in delayed type hypersensitivity (DTH), antibody titre (AT), phagocytic indices (PI), population of T cells (PTC) and percentage of lymphocytes (PL) are indications of immune stimulation induced by the candy and these agree with the findings of Anarthe *et al*. (2014), Ramesh *et al.* (2016) and Obi *et al*. (2019). Anarthe *et al*. (2014) reported that DTH involved initial sensitization and effector phases. The initial sensitization phase involves activation of TH1 cells as a result of presentation of processed antigen by antigen presenting cell (APC) using major histocompatibility class 11 (MHC 11) molecule whereas effector phase involves induction of DTH as a result of subsequent exposure to the antigen, and secretion of cytokines and other non-specific inflammatory mediators. The significant increase in antibody titre is an indication of B cell activation and secretion of plasma cells that produce significant antibodies. The activation of B cells was due to the interaction of macrophage, T cells and B cells leading to immune stimulation through humoral activity as described by Anarthe *et al.* (2014).

Study has shown that IL-1β induced by commensal bacteria is critical for the

differentiation of Th 17 cells, and fermented foods that contain active lactic acid bacteria play active role in maintaining the population of Th 17 cells which are the prominent population among the T cells present in the intestinal lamina propria that maintain the intestinal homeostasis (Hur *et al*., 2014; Bell *et al*., 2018; Pandiyan *et al*., 2019; Shahbazi *et al.,* 2021). Th 17 is a subset of $CD4^+$ T cells and primarily secretes IL – 17, which has important effects on the intestinal epithelium through improving the barrier function and stimulating mucin production, as well as on the function of tight junction and transport of Ig A. In the present study, the study increase in the population and percentage of T cells could be attributed to the proliferation of Th 17 cells which was induced by IL - 1 β secreted by the commensal bacteria and activities of autochthonous and allochthonous probiotics. Similar findings were reported by many researchers (Zulkani *et al*., 2017; Bell *et al*., 2018; Birhanu *et al*., 2018; Yahfoufi *et al*., 2018; Pandiyan *et al*., 2019; Shahbazi *et al*., 2021).

CONCLUSION

This study has shown that candy prepared from fermentation of *Pentaclethra macrophylla* using *Lacobacillus acidophilus* strain DSM20079 (LADSM) *and Bacillus subtilis* strain PK5-17 (BSPK5) had good quality. The blood samples drawn from rats fed with the candy showed elevation of delayed type hypersensitivity, antibody titre, phagocytic index, T cell population and percentage of lymphocytes. Therefore, the candy is recommended since it is safe and had pronounce immunostimulatory potential.

REFERENCES

Abdallah, A., Zhang, P., Zhong, Q. and Sun, Z. (2019). Application of traditional Chinese herbal medicine by-products as dietary feed supplements and antibiotic replacements in animal production. *Current Drug Metabolism* 20: 54 – 64.

Adams, S., Che, D., Qin, G., Rui, H., Sello, C.T. and Hailong, J. (2018). Interactions of dietaryfibre with nutritional components on gut microbial composition, function and health in monogastrics. *Current Protein Peptide Science* 19: 1011 – 1023

Adams, S., Kong, X., Che, D., Qin, G. and Jiang, H. (2019). Effects of dietary supplementation of alfalfa (*Medicago sativa*) fibre on the blood biochemistry, nitrogen metabolism, and intestinal morphometry in weaning piglets. *Applied Ecological and Environmental Research* 17: 2275 – 2295

Adedokun, I.I., Okolie, S.U., Onyeneke, E.N. and Anoruo, S.A. (2013). Evaluation of yield, sensory and chemical characteristics of soft unripened cheese produced with partial in corporation of bambara nut milk. *Acedemia Journal of Food Research* 1(1): 14 – 18.

Ademiluyi, A.O., Oboh,G. and Omojokun, O.S. (2019). Traditional fermented condiments modulate biochemical indices in high cholesterol diet-fed- rats. *Journal of Sustainable Technology* **10**: 1–12.

Agiang, M.A., Dongo, B.S., Williams, I.O. and Utu-Baku, A.B. (2017). Assessment of the haematological indices of albino rats supplemented with jack fruit bulb and seed. *International Journal of Biological and Chemical Sciences* **11**(1): 397−407.

Anarthe, S.J., Rani, S.D.S. and Raju, M.G. (2014). Immunomodulatory activity of methanolic extract of Trigunella foenum graecum whole plant in wistar albino rats. American Journal of phytomedicine and clinical Therapeutics **2(9)**: 1081–1092.

Anggraeni, S.D., Husmaini, H., Sabrina, S., Zulkarnain, Z. and Rossi, E. (2019). Viability of *Lactobacillus planetarium and Lactobacillus pentosus* isolated from solid waste of soy milk as candidate probiotic for poultry. *International Conference of Bio-Based Economy and Agricultural Utilization* **497**: 1 – 7.

Association of Official Analytical Chemists (2019). Official methods of Analysis of Association of Official Analytical Chemists, 21st Ed. AOAC, Washington DC, PP. 301 – 369.

Azab, A., Nassar, A. and Azab, A.N (2016). Anti-inflammatory activity of natural products. *Molecules* 21: 1321 – 1329

Bested, A.C., Logan, A.C. and Selhub, E.M. (2013). Intestinal microbiota, probiotics and mental health: From Metchnikoff to modern advances: Part II–contemporary contextual research. *Gut Pathogens* 5: 3

Blaabjerg, S., Artzi, D.M. and Aabenhus, R. (2017). " Probiotics for the prevention of antibiotic- associated diarrhea in outpatients-A systematic review and meta-analysis". *Antibiotics* **6** (4): 21

Bell, V., Ferrao, J., Pimentel, L., Pintado, M. and Fernandes, T. (2018). One health, fermented foods, and gut microbiota. *Foods* 7: 195 – 201

Birhanu, B.T., Kim, J.Y., Hossain, M.A., Choi, J.W., Lee, S.P. and Park, S.C. (2018). An *in vivo* immunostimulatory and anti-inflammatory study of fermented Dendropanax morbifera leaf extract. *BMC Complementary Alternative Medicine* 18: 222 – 231

Campbell, C. and Rudensky, A. (2020). Roles of rgulatory T Cells in tissue

pathophysiology and metabolism. *Cell Metabolism* 31(1): 18 – 25.

Chan, L.Y., Takahashi, M., Lim, P.J., Aoyama, S., Makino, S., Ferdinandus, F., Ng, S.Y.C., Arai, S. and Fujit Tan, H.C. (2019). *Eurotium cristatum* fermented okara as a potential food ingredient to combat diabetes. *Science Representation* 9: 1–9

Cameron, D., Hock, Q.S., Kadim, M., Mohan, N., Ryoo, E., Sandhu, B., Yamashiro, Y., Jie, C., Hoekstra, H. and Guarino, A. (2017). "Probiotics for gastrointestinal disorders: Proposed recommendations for children of the Asia-Pacific region". *World Journal of Gastroenterology* **23** (45): 7952–7964.
de Vrese, M., Laue, C., Papazova, E., Petricevic, L. and Schrezenmeir, J. (2019). "Impact of oral administration of four *Lactobacillus* strains on Nugent score - systematic review and meta-analysis". *Beneficial Microbes* **10** (5): 483 – 496.

Ding, Y., Yan, Y., Chen, D., Ran, L., Mi, J., Lu, L. and Cao, Y. (2019). Modulating effects of polysaccharides from the fruits of Lycium barbarum on the immune response and gut microbiota in cyclophosphamide-treated mice. *Food and Function* 10(6): 3671–3683.

Duar, R.M., Lin, X.B., Zheng, J., Martino, M.E., Grenier, T. and Pérez-Muñoz, M.E. (2017).

"Lifestyles in transition: evolution and natural history of the genus *Lactobacillus*". *FEMS Microbiology Reviews* ***41****: 27–48*

Enidiok, S.E., Bello, F.A. and Udo, I.I. (2017). Nutritional evaluation and sensory attributed of Ekoki produced from maize and enhance with soy and tigernut milk. *Journal of Advances in Food Science and Technology* **4(4):** 131 – 136.

Feng, Y., Huang, Y., Wang, Y., Wang, P., Song, H. and Wang, F. (2019). Antibiotics induced intestinal tight junction barrier dysfunction is associated with microbiota dysbiosis, activated NLRP3 inflammasome and autophagy. *Plos One* 14: 384 – 395

Fong, F. L. Y., Kirjavainen, P., Wong, V. H. Y. and El-Nezami H. (2015a). Immunomodulatory effects of *Lactobacillus rhamnosus* GG on dendritic cells, macrophages and monocytes from healthy donors. *Journal of Functional Foods* 13: 71–79

Fong, F. L. Y., Shah, N. P., Kirjavainen, P. and El-Nezami, H. (2015b). Mechanism of action of

probiotic bacteria on intestinal and systemic immunities and antigen-presenting cells. *International Reviews of Immunology* 25: 1–11.

Ganguli, K. (2015). *Lactobacillus rhamnosus* GG and its SpaC pilus adhesin modulate inflammatory responsiveness and TLR-related gene expression in the fetal human gut. *Pediatric Research* 77: 528 – 535

Greenbaum, S., Greenbaum, G., Moran-Gilad, J. and Weintraub, A.Y. (2019). Ecological dynamics of the vaginal microbiome in relation to health and disease. *American Journal of Obstetrician and Gynecology* 220: 324–335

Hojsak, I., Fabiano, V., Pop, T.L., Goulet, O., Zuccotti, G.V., Çokuğraş, F.C., Pettoello-Mantovani, M. and Kolaček, S. (2018). "European guidance on paediatric use of probiotics states that benefits are limited to several conditions and urges caution with specific vulnerable groups". *Acta Paediatrica.* ***107*** *(6): 927–937*

Hwang, D., Charchoghlyan, H., Lee, J.S. and Kim, M. (2015). Bioactive compounds and antioxidant activities of the Korean lotus leaf (*Nelumbo nucifera*) condiment: Volatile and nonvolatile metabolite profiling during fermentation. *International. Journal of Food Science and Technology* 50: 1988–1995

Hur, S.J., Lee, S.Y., Kim, Y.C., Choi, I. and Kim, G.B. (2014). Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry* 160: 346 – 356

Hur, Y.M., Kang, M.N. and Kim, Y.J. (2021). Vaginal health in women and the possibility of predicting preterm birth through microbiome analysis. *Journal of Korean Medical Association* 64: 833–840

Hu, Y., Piao, C., Chen, Y., Zhou, Y., Wang, D., Yu, H. and Xu, B. (2019). Soybean residue(okara) fermentation with the yeast *Kluyveromyces marxianus*. *Food Bioscience* 31: 100 – 139 .

Iheukwumere, I.H., Olusola, T.O. and Chude, C. (2018). Molecular characterization and diversity of enteric bacteria isolated from chicken feeds. *Journal of Natura Sciences Research***8**: 21–33.

Kang, M.J., Bae, I.Y. and Lee, H.G. (2018). Rice noodle enriched with okara: Cooking property, texture, and in vitro starch digestibility. *Food Bioscience* 22: 178 –183

Kho, Z.Y. and Lai, S.K. (2018). The human gut microbiome – A potential controller of wellness and disease. *Frontier in Microbiology* 9: 1835 – 1842

Kaushal, D. and Kansal, V. K. (2014). Dahi containing *Lactobacillus acidophilus* and

Bifidobacterium bifidum improves phagocytic potential of macrophages in aged mice. *Journal of Food Science and Technology* 51(6): 1147–1153.

Kieffer, D.A., Martin, R.J. and Adams, S.H. (2016). Impact of dietary fibers on nutrient

management and detoxification organs: Gut, liver, and kidneys. *Advanced Nutrition* 7: 1111 – 1121

Jang, H. J., Yu, H.-S., Lee, N.-K. and Paik, H.D. (2020). Immune-stimulating Effect of *Lactobacillus plantarum* Ln1 Isolated from the Traditional Korean Fermented Food Kimchi. *Journal of Microbiology and Biotechnology* 30(6): 926–929

Jang, S.-E., Joh, E.-H., Ahn, Y.-T., Huh, C.-S., Han, M. J., & Kim, D.-H. (2013). *Lactobacillus casei* HY7213 ameliorates cyclophosphamide-induced immunosuppression in mice by activating NK, cytotoxic t cells and macrophages. *Immunopharmacology and Immunotoxicology* 35(3): 396 – 402.

Lim, E.Y., Song, E.J., Kim, J.G., Jung, S.Y., Lee, S.Y., Shin, H.S., Nam, Y.D. and Kim, Y.T. (2021). *Lactobacillus intestinalis* YT2 restores the gut microbiota and improves menopausal symptoms in ovariectomized rats. *Beneficial Microbes* 12: 503–516.

Liu, D.D. and Gu, C.T. (2020). "Proposal to reclassify Lactobacillus zhaodongensis, Lactobacillus zeae, Lactobacillus argentoratensis and Lactobacillus buchneri subsp. silagei as Lacticaseibacillus zhaodongensis comb. nov., Lacticaseibacillus zeae comb. nov., Lactiplantibacillus argentoratensis comb. nov. and Lentilactobacillus buchneri subsp. silagei comb. nov., respectively and Apilactobacillus kosoi as a later heterotypic synonym of Apilactobacillus micheneri". International Journal of Systematic and *Evolutionary Microbiology* ***70*** *(12): 6414–6417*

Ludwig, I. S., Broere, F., Manurung, S., Lambers, T. T., van der Zee, R. and van Eden, W. (2018). *Lactobacillus rhamnosus* GG-Derived Soluble Mediators Modulate AdaptiveImmune Cells. *Frontiers in Immunology* 9(1): 1546–1552

Macro, M.L., Heeney, D., Binda, S., Cifelli, C.J., Cotter, P.D., Foligne, B., Ganzle, M., Kort, R., Pasin, C. and Pihlanto, A. (2017). Health benefits of fermented foods; microbiota and beyond. *Current opinion in Biotechnology* 44: 94 – 102

Maizels, R. M., Smits, H. H., & McSorley, H. J. (2018). Modulation of Host Immunity by Helminths: The Expanding Repertoire of Parasite Effector Molecules. *Immunity* 49 (5): 801 – 818

Meng, Y., Li, B., Jin, D., Zhan, M., Lu, J. and Huo, G. (2018). Immunomodulatory activity of*Lactobacillus plantarum* KLDS1.0318 in cyclophosphamide-treated mice. *Food and Nutrition Research* 62(5): 1296 –1305

Mirnejad, R., Vahdati, A. R., Rashidiani, J., Erfani, M. and Piranfar V. (2013).The antimicrobial effect of *lactobacillus casei* culture supernatant against multiple drug resistant clinical isolates of *Shigella sonnei* and *Shigella flexneri in vitro. Iranian Red Crescent Medical Journal* 15: 122–126.

Nishinari, K., Fang, Y., Guo, S. and Phillips, G. (2014). Soy proteins: A review on composition, aggregation and emulsification. *Food Hydrocoll* 39: 301–318

.Nfeimbi, J., Ebosa, G.S., Sembajire, L.F' Gakunga, J. and Kasolo, J. (2015). Immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in wister albino rats, *Journal of Basic Clinical and Physiological Pharmacology* **26 (6):** 603 – 611.

Nwobodo,E.I.,Nwosu,D.C.,Ogbodo, S.O., Ugwuene, F.O., Ihim, A.C., Ani,N.O., Nnodim, J.K. Ani, O. (2018). Effects of Azadirachita indica leaf aqueous extract on the antioxidant enzymes in paracetamol-induced hepatoxicity in Wistar rats. *International Journal of Biological and Chemical Sciences*.**12**:1–10.

Obi, A., Egwurugwu, J.N., Ojefa, S.O., Ohamaeme, M.C., Ekweogu, C.N. and Ogunnaya, F.U. (2019). Immunomodulatory effects of hydromethanolic extract of *Moringa oleifera* leafon male Wistar rats. *Nigeria Journal of Experimental and Clinical Biosciences* **6**:26 – 32.

Onderdonk, A.B., Delaney, M.L. and Fichorova, R.N. (2016). The human microbiome during bacterial vaginosis. *Clinical Microbiology Review* 29: 223–238.

Olletti, A., Attrovio, A., Boffa, L., Mantegna, S. and Cravotto, G. (2020). Valorisation of By-Products from Soybean (Glycine max (L.) Merr.) Processing. *Molecules* 25: 2129.

Oz, E., Kaban, G., Baris, O. and Kaya, M. (2017). Isolation and identification of lactic acid bacteria from pastirma. *Food Control* **77**: 158 –162.

Pandiyan, P., Bhaskaran, N., Zou, M., Schneider, E., Jayaraman, S. and Huehn, J. (2019). Microbiome dependent regulation of Tregs and Th 17 cells in mucosa. *Frontier in Microbiology* 10: 426 – 433

Peng, S., Yang, Y., Li, S., Wu, Q., Shah, N. P., Wei, H., & Xu, F. (2014). Immunomodulatory activities of *Lactobacillus rhamnosus* ZDY114 and donkey milk in BALB/c mice. *International Dairy Journal* 34(2): 263 – 266.

Pino, A., Rapisarda, A.M.C., Vaccalluzzo, A., Sanfilippo, R.R., Coman, M.M., Grimaldi, R.L.,Caggia, C., Randazzo, C.L., Russo, N. and Panella, M.M. (2023). Oral intake of the commercial probiotic blend synbio for the Management of Vaginal Dysbiosis. *Journal of Clinical Medicine* 12:27

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G.A.D., Gasbarrini, A. and Mele, M.C. (2019). *What is health gut microbiobiota composition*? A changing ecosystem ac ross age, environment, diet and diseases. *Microorganisms* 7: 14 – 19

Rastall, R.A. and Gibson, G.R. (2015). Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. *Current Opinion in Biotechnology* 32: 42 – 46.

Rezac, S., Kok, C.R., Heermann, M and Hutkins, R. (2018). Fermented foods as a dietary source of live organisms. *Frontier in Microbiology* 9: 1785 – 1792

Szajewska, H. and Horvath, A. (2018). "*Lactobacillus rhamnosus* GG in the Primary Prevention of Eczema in Children: A Systematic Review and Meta-Analysis". *Nutrients **10** (9): 1319.*

Segers, M. E. and Lebeer, S. (2014). Towards a better understanding of *Lactobacillus rhamnosus* GG–host interactions. *Microbial cell factories* 1: 7 – 10

Shahbazi, R., Sharifzad, F., Bagheri, R., Alsadi, N., Yasavoli-Sharahi, H. and Matar, C. (2021). Anti-inflammatory and immunomodulatory properties of fermented plant foods. *Nutrients* 3 (5): 1516 – 1535

Singh, B.,. Singh, J.P., Singh, N. and Kaur, A. (2017). Saponins in pulses and their health promoting activities: A review. *Food Chemistry* 233: 540 – 549.

Smith, S.B. and Ravel, J. (2017). The vaginal microbiota, host defence and reproductive physiology. *Physiological Journal* 595: 451–463.

Tachedjian, G., Aldunate, M., Bradshaw, C.S. and Cone, R.A. (2017). The role of lactic acid production by probiotic Lactobacillus species in vaginal health. *Microbiological Research* 168: 782–792.

Tang, C., Sun, J., Zhou, B., Jin, C., Liu, J., Kan, J. and Zhang, N. (2018). Effects of

polysaccharides from purple sweet potatoes on immune response and gut microbiota composition in normal and cyclophosphamide treated mice. *Food and Function* 9(2): 937– 950

Thursby, E, E. and Juge, N. (2017). Introduction to the human and gut microbiota. *Biochnemistgry Journal* 474: 1823 – 1836

van Baarlen, P., Wells, J. M. and Kleerebezem, M. (2013). Regulation of intestinal homeostasis and immunity with probiotic lactobacilli. *Trends Immunology* 34: 208 – 215

Veljović, K., Dinić, M., Lukić, J., Mihajlović, S., Tolinački, M., Živković, M., Begović, J. and Mrvaljević, I. (2017). Promotion of early gut colonization by probiotic intervention on microbiota diversity in pregnant sows. *Frontier in Microbiology* 8: 1–12

Villanueva-Suárez, M.-J., Pérez-Cózar, M.L., Mateos-Aparicio, I. and Redondo-Cuenca, A. (2016). Potential fat-lowering and prebiotic effects of enzymatically treated okara in high-cholesterol-fed Wistar rats. *International Journal of Food Science Nutrition* 67: 828 – 833

Wang, X.Q. Zhang, A.H. Miao, J.H., Sun, H., Yan, G.L.; Wu, F.F., Begović, J., Mrvaljević, I.,

Golić, N. and Terzić-Vidojević, A. (2018). Gut microbiota as important modulator of metabolism in health and disease. *RSC Advances* 8: 42380–42389

Yahfoufi, N., Mallet, J., Graham, E. and Matar, C. (2018). Role of probiotics and prebiotics in immunomodulation. *Current Opinion in Food Sciences* 20: 82 – 91

Zulkani, N., Ng, K.H., Zamberi, R., Yeap, S.K., Satharasinghe, D., Jaganath, I.B., Jamaluddin, A.B., Tan, S.W., Ho, W.Y. and Alitheen, N.B. (2017). In vitro characterization and in vivo toxicity, antioxidant and immunomodulatory effect of fermented foodxs. *BMC Complementary Alternative Medicine* 17: 344 – 355

Zhang, S. and Chen, D.C. (2019). Facing a new challenge: The adverse effects of antibiotics on gutmicrobiota and host immunity. *Clinical Medicine Journal* 132: 1135 – 1138

Zhang, Z.H., Fan, S.T., Huang, D.F., Yu, Q., Liu, X.Z., Li, C. and Xie, M.Y. (2018). Effect of *Lactobacillus plantarum* NCU116 Fermentation on *Asparagus officinalis* Polysaccharide: Characterization, Antioxidative, and Immunoregulatory Activities. *Journal of Agricultural and Food Chemistry* 66(41): 10703 –10711

Zheng, N., Guo, R., Wang, J., Zhou, W. and Ling, Z. (2021). Contribution of *Lactobacillus iners* to vaginal health and diseases: A systematic review. *Frontiers Cellular Infection in Microbiology* 11: 1177

Zeng, M. Y., Cisalpino, D., Varadarajan, S., Hellman, J., Warren, H. S., Cascalho, M. and

Nunez, G. (2016). Gut Microbiota-Induced Immunoglobulin G Controls Systemic Infection by Symbiotic Bacteria and Pathogens. *Immunity* 44(3): 647 – 658

Zheng, J., Wittouck, S., Salvetti, E., Franz, C.M.A.P., Harris, H.M.B. and Mattarelli, P. (2020). "A taxonomic note on the genus *Lactobacillus:* Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901*, and* union of *Lactobacillaceae and Leuconostocaceae*". *International Journal of Systematic and Evolutionary Microbiolog* ***70*** *(4): 2782–2858*

Zhong-Hua, L., Hong-Lian, G., Rui-Ling, L. and Jin-Hui, Z. (2015). Extraction and antioxidant activity of soybean saponins from low temperature soybean meal by MTEH. *Open Biotechnology Journal* 9: 178–184