

The effect of oat products fermented by probiotic bacteria on the blood biochemical parameters in experimental rats

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Abstract

Fermentation of oat by probiotic bacteria enhances its nutritional value and can be considered as a significant source of bioactive compounds and alive probiotics for the human body. Moringa leaves powder (MLP) at the levels of 0.25 and 0.50% were used as an additional prebiotic source to supply the oat fermentation by *Lactobacillus plantarum* ATCC 14917 and *Lactobacillus delbrueckii ssp. Bulgaricus* EMCC 11102 and produce healthier oat products. The results indicated that oat products supplemented with MLP at a level of 0.50% and fermented by *L. plantarum* ATCC 14917 and *L. delbrueckii ssp. Bulgaricus* EMCC 11102 showed the highest probiotic counts (10.61 and 10.11 log cfu/g, respectively) and exhibited more survival of probiotics than oat fermented products without MLP ($p > 0.05$) throughout the storage period. Moreover, fermented oat products supplemented with MLP at the same level showed higher activity of reducing both fasting and postprandial blood glucose levels in the experimental rats. These products may have a potential role as effective vehicles for probiotics and control of type 2 diabetes mellitus (DM) in the future.

1. Introduction

Oat is the sixth most important cereal in the world and consumed as a part of daily diet in many countries. Among approximately seventy species of oat, the common oat (*Avena sativa L.*) is one of the most oat species consumed around the world [1]. It contains good amounts of dietary fibers (55% soluble fiber and 45% insoluble fiber), proteins (high level of lysine), unsaturated fatty acids (linolenic, linoleic and oleic acids), vitamins (A, E, D and B12), minerals (Ca, P and Fe) and bioactive compounds [2-4]. Due to its significant content of these bioactive components such as phenolic compounds and β -glucan, oat consumption has been related to several healthy benefits, such as antioxidant, anti-inflammatory, anticancer, reducing blood cholesterol and blood glucose levels [5, 6].

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host such as alleviation of gastrointestinal infections, stimulation of immune system, serum cholesterol lowering, prevention and treatment of allergies, antimutagenic effects and stabilization of the gut mucosal barrier [7, 8]. Lactobacilli strains are one of the most generally used probiotics in the functional foods [9]. Prebiotic constituents are nondigestible ingredients of food that selectively stimulate the growth of beneficial microorganisms (probiotics) in the gastrointestinal tract of the host [10]. Synbiotics are foods combined probiotics and prebiotics and might be more efficient than the individual components in the host colon [11].

Probiotic bacteria are mainly used in dairy products and show the biggest share of the probiotic food market [12]. However, some disadvantages of dairy products containing probiotics

have been recorded for many consumers worldwide such as lactose intolerance; allergy to β -caseine in cow's milk; high content of cholesterol and saturated fatty acids in dairy products as well as high costs of milk [13]. Probiotic cereal foods may be produced as a good alternative to avoid drawbacks of fermented dairy products [12]. It has been shown that oat can promote the growth of probiotic bacteria due to its content of prebiotics such as β -glucan [14].

MLP produce numerous healthy benefits and have a good amount of crude protein, crude fiber, extract ether, carbohydrates, energy, minerals, vitamins, β -carotene and polysaccharides [15, 16]. Also, MLP displayed a prebiotic effect may be due to its significant content of prebiotic compounds such as oligosaccharides [17, 18]. This study aimed to evaluate the effects of supplementation with MLP as a source of prebiotics to produce healthier fermented oat products.

2. Materials and methods

2.1. Materials

Oat (*Avena sativa L.*) seeds was purchased from Agricultural Research Center, Giza, Egypt. Moringa (*Moringa oleifera*) leaves were obtained from a local farm located in Albalyana City, Sohag, Egypt, and sugar was purchased from a local market in Assiut City, Egypt. The reagents used in the biological experiment were purchased from Spectrum Company in Assiut City, Egypt. *Lactobacillus plantarum* ATCC 14917 and *L. delbrueckii ssp. Bulgaricus* EMCC 11102 were purchased from Microbiological Resources center (Cairo MIRCEN) Ain Shams University, Cairo, Egypt. Forty adult male white albino rats were obtained from the animal house of Faculty of Medicine, Assiut University, Assiut, Egypt.

2.2. Methods

2.2.1. Preparation of oat fermentation

2.2.1.1. Preparation of raw materials and oat blends

Grain oat seeds was washed and dried at 60 °C for 8 h and milled to get the whole oat flour (WOF). The moringa leaves were dried and milled to produce MLP, which stored in a cool dry place until the experimental work. The blends of WOF, MLP and sugar were prepared to produce the final formulas as follow in Table 1:

Table (1): Formulas of fermented oat products

	Oat %	Sugar %	MLP %	Water %
Control	10	2	---	88
FOP	10	2	---	88
FOP₁	10	2	0.25	87.75
FOP₂	10	2	0.50	87.5
FOD	10	2	---	88
FOD₁	10	2	0.25	87.75
FOD₂	10	2	0.50	87.5

Control, Non-fermented oat; **FOP**, Fermented oat by *L. plantarum* ATCC 14917; **FOP₁**, Fermented oat with 0.25% MLP by *L. plantarum* ATCC 14917; **FOP₂**, Fermented oat with 0.5% MLP by *L. plantarum* ATCC 14917; **FOD**, Fermented oat by *L. delbrueckii ssp. Bulgaricus* EMCC 11102; **FOD₁**, Fermented oat with 0.25% MLP by *L. delbrueckii ssp. Bulgaricus* EMCC 11102; **FOD₂**, Fermented oat with 0.5% MLP by *L. delbrueckii ssp. Bulgaricus* EMCC 11102.

In the next step, the mixtures were gelatinized using the water bath and autoclaved at 121 °C for 15 min.

2.2.1.2. Inoculant strains and oat fermentation

conditions

The *Lactobacillus* bacteria strains were activated by inoculating in sterile MRS broth (9 ml) and incubation at 37 °C for 24 h. The cells were separated from the broth by centrifuging at 3000 rpm for 20 min, and re-suspended in sterile saline solution (9 ml) with final concentration 10⁸ CFU/mL [19].

After 24 h, activated culture of probiotic bacteria (10⁸ CFU/mL) were added to the previous oat mixtures by a concentration of 1%. A control sample without inoculation of bacteria was prepared. All treatments were incubated at 37 °C for 24 h for *L. plantarum* ATCC 14917 fermentation and 8 h for *L. delbrueckii ssp. Bulgaricus* EMCC 11102 fermentation. The fermented oat products were stored after fermentation at 4 ± 1 °C for 21 days. Chemical and microbiological properties of fermented oat products were estimated at 0, 7, 14 and 21 days.

2.2.2. Determination of total acidity

Total acidity was determined by titration method (10 g of fermented products with 90 ml distilled water, using 0.1 N

NaOH solution and phenolphthalein as indicator) according to [20].

2.2.3. Determination of *Lactobacillus* bacteria counts

Probiotic viable counts were determined in fermented oat products using serial dilution technique and de Man-Rogosa-Sharpe (MRS) agar medium, the plates were anaerobically incubated at 37 °C for 48 h [21].

2.2.4. Biological experiment

2.2.4.1. Adaptation and distributing of experimental

animals

The animals were housed as groups in wire cages under the normal laboratory conditions and fed on basal diets for 10 days as adaptation period. The rats were distributed to 4 groups, each group was contained 10 rats containing control group and fed during experimental period (30 days) (Table 2).

Table (2): experimental groups and feeding diets

Groups	The experimental diets
Group 1 (control)	Fed on basal diet
Group 2	Fed on basal diet containing of 30% of non-fermented oat
Group 3	Fed on basal diet containing of 30% of FOP ₂
Group 4	Fed on basal diet containing of 30% of FOD ₂

2.2.4.2. Blood sampling

At the day of blood sampling collection, rats were fasted overnight and anesthetized. Blood samples were collected from the retro-orbital plexus from all animals of each group into clean, dry and labeled tubes. The tubes were centrifuged at 3500 rpm for 15 min to separate the blood serum, which used to estimation of biochemical parameters [22].

2.2.4.3. Determination of fasting and postprandial

blood glucose

The concentrations of fasting and postprandial blood glucose were determined according to [23] using enzymatic kits (SPECTRUM diagnosis, Germany).

2.2.4.4. Determination of serum aminotransferases

(ASAT and ALAT) activity (U/L)

Serum alanine aminotransferases (ALAT) and aspartate aminotransferases (ASAT) activities were determined according to the kinetic method described by [24]. The assay was performed according to the instruction manual of reagent kits purchased from SPECTRUM diagnosis, Germany.

2.2.4.5. Determination of creatinine (mg/dl):

Serum creatinine activity was determined according to the kinetic method described by [25]. The assay was performed according to the instruction manual of reagent kits purchased from SPECTRUM diagnosis, Germany.

2.2.4.6. Determination of blood urea (mg/dl):

Serum urea activity was determined according to the enzymatic colorimetric method described by [25]. The assay was performed according to the instruction manual of reagent kits purchased from SPECTRUM diagnosis, Germany.

2.2.5. Statistical analysis

Basic statistics and analysis of variance (ANOVA) were performed to data analysis and test the significance within replications and between treatments by using IBM SPSS software version 22 [26]. Duncan test was used to determine the differences among the means at the significance level of 0.05%.

3. Results and discussions

3.1. changes in total acidity of fermented oat products

Changes in total acidity of fermented oat products during storage at $4\text{ }^{\circ}\text{C} \pm 1$ for 21 days were illustrated in Table 3. After fermentation process by *L. plantarum* and *L. delbrueckii ssp. Bulgaricus*, the acidity increased from 0.274 % to values ranged between 0.392 and 0.418%. The treatment of FOD₂ showed the highest acidity value (0.418%) followed by FOP₂ (0.410%) may be due to the highest count of probiotic bacteria in these treatments, while FOP treatment presented the lowest acidity value (0.392%) after control. Lactate produced by lactic acid bacteria is responsible for the acidity increasing in the fermented oat products. Furthermore, the acidity values were continuously increased during storage period and the highest values were recorded at the end of storage for all treatments by 0.506, 0.515, 0.513, 0.510, 0.503, 0.515 and 0.323 % for FOP, FOP₁, FOP₂, FOD, FOD₁, FOD₂ and control, respectively.

3.2. Changes in probiotic bacteria counts (cfu/g) of fermented oat products

To obtain required population of probiotic bacteria, the oat mixtures were inoculated with *L. plantarum* and *L. delbrueckii ssp. Bulgaricus* and incubated at 37 °C. The results indicated that, the adding of MLP especially at the highest level (0.50%) to fermented oat products led to encourage the growth and significantly increase ($p > 0.05$) the populations of probiotic bacteria. After the end of fermentation time, FOP₂ showed 10.61 Log CFU/g in contrast of 9.15 Log CFU/g for FOP, while FOD₂ showed 10.11 in contrast of 8.92 Log CFU/g for FOD. Moreover, oat fermented products supplemented with MLP exhibited more survival of probiotics than oat fermented products without MLP ($p > 0.05$) throughout the storage period as presented in Table 4. At the end of storage period, *L. plantarum* count was 9.64, 9.35 and 7.75 Log CFU/g for FOP₂, FOP₁ and FOP, respectively, while it recorded 9.42, 8.95 and 7.80 Log CFU/g for FOD₂, FOD₁ and FOD, respectively. The prebiotic effect of MLP may be due to its significant content of prebiotic compounds such as oligosaccharides, including fructo-

oligosaccharides (FOS) and mannan-oligosaccharides (MOS) [18, 27]. These results are agree with those obtained [28, 29].

3.3. Determination of blood parameters

3.3.1. Determination of fasting and postprandial

blood glucose

The type 2 diabetes mellitus (DM) is one of the most prevalent chronic diseases around the world. It is be characterized by high level of blood glucose, resistance to insulin and relative insulin deficiency, accompanying with multiple systemic complications. According to International Diabetes Federation (IDF), DM has affected 415 million adults, and this number can increase to more than 600 million up to 2040 [30]. Data showed in Tables 5 and 6 illustrated the effect of feeding with fermented oat products by probiotic bacteria on fasting and postprandial blood glucose levels of the experimental rats. The results indicated that the feeding with fermented oat products by probiotic bacteria significantly decreased ($P > 0.05$) fasting blood glucose levels. Group 3 (Fed with oat FOP₂) showed the highest decrement of fasting blood glucose level (17.18%) followed by group 4 (Fed with FOD₂). Moreover, Group 2 (fed with non-fermented oat) exhibited significant decrease ($P > 0.05$) in the fasting blood glucose level (10.17%), but lower than groups fed with fermented oat products supplemented with MLP. Similar results were observed in the postprandial glucose levels, groups 3 and 4 presented higher decrement in postprandial glucose levels (17.18 and 15.25%, respectively) than group 2 (10.17%). The higher activity of reducing blood glucose which was recorded in the groups 3 and 4 may be duo to the presence of probiotic bacteria and prebiotic effect of MLP. These results are in the same line with [31, 32], they reported that probiotic bacteria showed reducing blood glucose activity in bath *in vitro* and *in vivo* studies.

3.3.2. Liver enzymes

Determination of serum alanine aminotransferase (ALT) is a readily available, common and inexpensive laboratory test. The activity of ALT is measured to detect the liver diseases as well as it used to monitor overall health [33]. Also, AST activity is commonly used in the differential diagnosis of icteric and non-icteric hepatic disorders [34].

Fig. 1 and **2** presented the levels of liver enzymes of experimental rats during the feeding period. ALT enzyme activity showed levels ranged between 29.50 to 35.14, 27.23 to 35.32, 25.11 to 38.87 and 30.15 to 38.00 U/I for group 1, 2, 3 and 4, respectively through the feeding period for 30 days (**Fig. 1**). On the other hand, the AST enzyme also displayed values ranged between 21.66 and 35.41 U/I during the feeding period (**Fig. 2**). It has been observed that the feeding of experimental rats with fermented oat products did not increase the liver enzymes levels throughout the feeding period.

3.3.3. Kidney functions

The serum creatinine and blood urea concentrations are widely interpreted as a measure of the glomerular filtration rate (GFR) and is used as an index of renal function in clinical practice [35].

The effects of feeding with fermented oat products on the kidney functions were exhibited in **Fig 3** and **4**. Data presented that serum creatinine values of experimental rats were less than 1.40 mg/dL until the end of feeding period (**Fig. 3**). The lowest value of creatinine was recorded in group 4 at the tenth day (0.83 mg/dL), while the highest value was detected in group 1 at the tenth day (1.30 mg/dL). Also, blood urea levels showed to be in normal levels ranged between 32.23 and 48.46 mg/dL during the feeding period (**Fig. 4**). The feeding with fermented oat products did not negatively effect on the kidney function levels of experimental rats during the feeding period for 30 days.

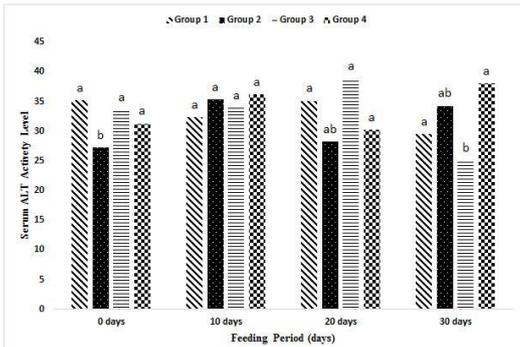


Figure 1: Changes in ALT enzyme activity of experimental rats groups during feeding period. **Group (1)**, Control group; **Group (2)**, Fed with non-fermented oat; **Group (3)**, Fed with FOP₂; **Group (4)**, Fed with FOD₂. Columns with different superscript small letters are significantly different (P > 0.05)

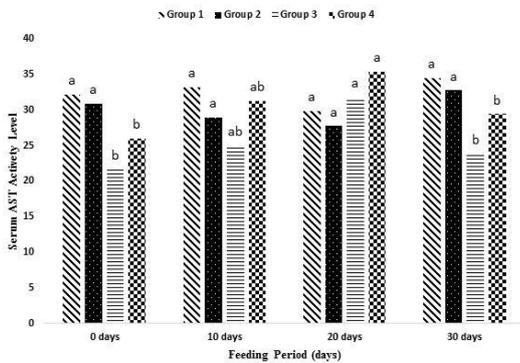


Figure2: Changes in AST enzyme activity of experimental rats groups during feeding period. **Group (1)**, Control group; **Group (2)**, Fed with non-fermented oat; **Group (3)**, Fed with FOP₂; **Group (4)**, Fed with FOD₂. Columns with different superscript small letters are significantly different (P > 0.05)

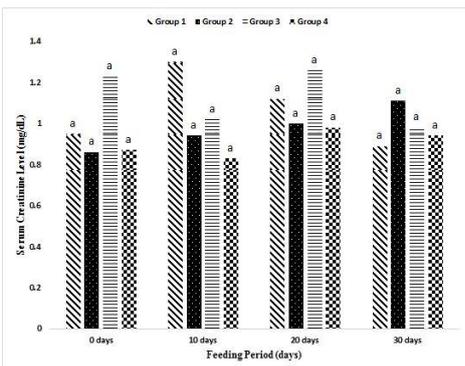


Figure 3: Changes in serum creatinine level of experimental rats groups during feeding period. **Group (1)**, Control group; **Group (2)**, Fed with non-fermented oat; **Group (3)**, Fed with FOP₂; **Group (4)**, Fed with FOD₂. Columns with different superscript small letters are significantly different (P > 0.05).

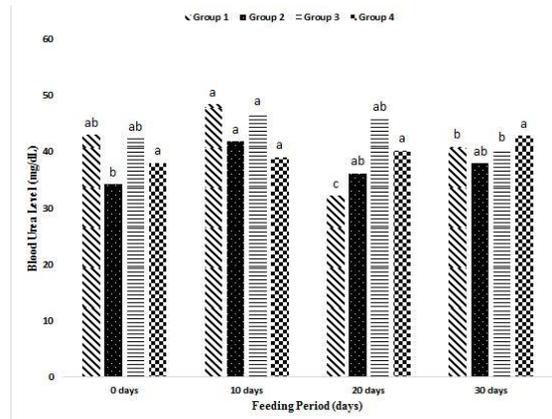


Figure 4: Changes in blood urea level of experimental rats groups during feeding period. **Group (1)**, Control group; **Group (2)**, Fed with non-fermented oat; **Group (3)**, Fed with FOP₂; **Group (4)**, Fed with FOD₂. Columns with different superscript small letters are significantly different (P > 0.05).

Table (3): changes in titrable acidity % of fermented oat products during storage at 4 °C ± 1 for 21 days

Treatments	Storage periods (days)			
	0	7	14	21
Control	0.274 ^{Bb}	0.272 ^{Bb}	0.278 ^{Bb}	0.323 ^{Ba}
FOP	0.392 ^{Ab}	0.412 ^{Ab}	0.418 ^{Ab}	0.506 ^{Aa}
FOP ₁	0.404 ^{Ac}	0.415 ^{Abc}	0.435 ^{Ab}	0.515 ^{Aa}
FOP ₂	0.412 ^{Ac}	0.418 ^{Abc}	0.442 ^{Ab}	0.513 ^{Aa}
FOD	0.395 ^{Ac}	0.409 ^{Abc}	0.425 ^{Ab}	0.510 ^{Aa}
FOD ₁	0.410 ^{Ab}	0.424 ^{Ab}	0.435 ^{Ab}	0.503 ^{Aa}
FOD ₂	0.418 ^{Ab}	0.426 ^{Ab}	0.443 ^{Ab}	0.515 ^{Aa}

Control, Non-fermented oat; **FOP**, Fermented oat by *L. plantarum* ATCC 14917; **FOP₁**, Fermented oat with 0.25% MLP by *L. plantarum* ATCC 14917; **FOP₂**, Fermented oat with 0.5% MLP by *L. plantarum* ATCC 14917; **FOD**, Fermented oat by *L. delbrueckii ssp. Bulgaricus* EMCC 11102; **FOD₁**, Fermented oat with 0.25% MLP by *L. delbrueckii ssp. Bulgaricus* EMCC 11102; **FOD₂**, Fermented oat with 0.5% MLP by *L. delbrueckii ssp. Bulgaricus* EMCC 11102.

*Means within a column with different superscript capital letters are significantly different (P > 0.05); means within a row with different superscript small letters are significantly different (P > 0.05).

Table (4): Changes in probiotic bacteria count (log cfu/g) of fermented oat products during storage at 4 °C ± 1 for 21 days

Treatments	Storage periods (days)			
	0	7	14	21
Control	>1 ^{Ea}	>1 ^{Fa}	>1 ^{Ea}	>1 ^{Ea}
FOP	9.15 ^{Ca}	9.00 ^{Da}	8.52 ^{Db}	7.75 ^{Dc}
FOP ₁	10.16 ^{Ba}	10.21 ^{Ba}	9.90 ^{Bb}	9.35 ^{Bc}
FOP ₂	10.61 ^{Aa}	10.40 ^{Ab}	10.07 ^{Ac}	9.64 ^{Ad}
FOD	8.92 ^{Da}	8.76 ^{Ea}	8.45 ^{Db}	7.80 ^{Dc}
FOD ₁	10.05 ^{Ba}	10.10 ^{Ba}	9.55 ^{Cb}	8.95 ^{Cc}
FOD ₂	10.11 ^{Ba}	9.90 ^{Cb}	9.81 ^{Bb}	9.42 ^{Bc}

Control, Non-fermented oat; **FOP**, Fermented oat by *L. plantarum* ATCC 14917; **FOP₁**, Fermented oat with 0.25% MLP by *L. plantarum* ATCC 14917; **FOP₂**, Fermented oat with 0.5% MLP by *L. plantarum* ATCC 14917; **FOD**, Fermented oat by *L. delbrueckii ssp. Bulgaricus* EMCC 11102; **FOD₁**, Fermented oat with 0.25% MLP by *L. delbrueckii ssp. Bulgaricus* EMCC 11102; **FOD₂**, Fermented oat with 0.5% MLP by *L. delbrueckii ssp. Bulgaricus* EMCC 11102.

*Means within a column with different superscript capital letters are significantly different ($P > 0.05$); means within a row with different superscript small letters are significantly different ($P > 0.05$).

Table (5): Changes in serum fasting glucose (mg/dl) in the studied groups of the experimental rats

Treatments	Experimental periods (days)				Decrement %
	0	10	20	30	
Group ₁	81.74 ^b	84.78 ^b	96.73 ^a	85.33 ^b	---
Group ₂	90.14 ^a	83.33 ^{ab}	79.14 ^b	80.97 ^b	10.17
Group ₃	84.78 ^a	76.44 ^{ab}	77.36 ^{ab}	70.20 ^b	17.18
Group ₄	86.30 ^a	87.69 ^a	71.15 ^b	73.14 ^b	15.25

Group (1), Control group; **Group (2)**, Fed with non-fermented oat; **Group (3)**, Fed with FOP₂; **Group (4)**, Fed with FOD₂. Means within a row with different superscript small letters are significantly different ($P > 0.05$).

Table (6): Changes in serum postprandial glucose (mg/dl) in the studied groups of the experimental rats

Treatments	Experimental periods (days)				Decrement %
	0	10	20	30	
Group ₁	138.25 ^a	124.33 ^b	142.20 ^a	136.36 ^a	---
Group ₂	129.35 ^a	133.41 ^a	129.91 ^a	115.78 ^b	10.49
Group ₃	141.01 ^a	135.08 ^a	120.15 ^b	113.95 ^b	19.19
Group ₄	137.14 ^a	128.86 ^a	129.54 ^a	117.22 ^b	14.53

Group (1), Control group; **Group (2)**, Fed with non-fermented oat; **Group (3)**, Fed with FOP₂; **Group (4)**, Fed with FOD₂. Means within a row with different superscript small letters are significantly different ($P > 0.05$).

4. Conclusion

Symbiotic foods based on oat fermented by probiotic bacteria were produced as a significant source of bioactive compounds and alive probiotics for the human body. Moringa leaves powder (MLP) was added at the concentrations of 0.25 and 0.50% to stimulate the growth and survival of *Lactobacillus plantarum* ATCC 14917 and *Lactobacillus delbrueckii ssp. Bulgaricus* EMCC 11102 in fermented oat products. Fermented oat products supplemented with the higher level of MLP

(0.50%) displayed the highest count for both selected probiotic bacteria (10.61 and 10.11 log cfu/g, respectively) with more survival of probiotics during storage period than oat fermented products without MLP ($p > 0.05$). Moreover, fermented oat products supplemented with MLP at the same level presented higher activity of reducing both fasting and postprandial blood glucose in the tested experimental rats. These products may have an effective role as a proper vehicle for probiotics and control of type 2 diabetes mellitus (DM). More studies are needed in the future to test more kinds of probiotic bacteria with different concentration of MLP in oat fermentation.

Conflict of Interest

No conflict of interest exists in this paper.

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