Lactobacillus Acidophilus and *Lactobacillus Casei* Exposed to Wi-Fi Radiofrequency Electromagnetic Radiation Show Enhanced Growth and Lactic Acid Production

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ABSTRACT

Background: *Lactobacillus acidophilus* and *Lactobacillus casei* are gram-positive probiotics and members of the genus Lactobacillus. These bacteria are widely applicable in food and dairy industries. Increasing bacterial load and decreasing fermentation time make them more profitable for manufacturers.

Objective: This study was aimed at assessing the biological effects of short-term exposure of *L. acidophilus* and *L. casei* to 2.4 GHz Wi-Fi radiofrequency electromagnetic fields (RF-EMF) generated by a Wi-Fi router on the lactic acid production and proliferation of these probiotic bacteria.

Material and Methods: This experimental study was performed on pure culture strains of *L. acidophilus* and *L. casei*, first direct vat sets (DVS) were activated in MRS broth for 24 hours then transferred to new culture mediums. Afterward, these mediums were exposed to RF-EMF for 15, 30, 45 and 60 minutes. The control samples were sham-exposed. After 72 hours of incubation on MRS agar cell counts were enumerated.

Results: Exposure for 30, 45 and 60 minutes significantly increased the growth of *L. acidophilus* and *L. casei*. No difference was found between the growth of the samples exposed to RF-EMF for 15 minutes compared to that of sham-exposed bacteria. In addition, lactic acid concentration in *L. acidophilus* medium was amplified after 15, 30 and 45 minutes of exposure. However, in *L. casei* samples, only 30 and 60 min exposures could stimulate the production of lactic acid.

Conclusion: *L. acidophilus* and *L. casei* probiotic bacteria exposed for a short time to radiofrequency electromagnetic radiation (RF-EMF) generated by a widely used commercial Wi-Fi router show significantly increased proliferation and lactic acid production.

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Keywords

Radiation; Electromagnetic; Probiotics; Lactobacillus acidophilus; Lactobacillus Casei

Introduction

Probiotics, a Greek term meaning "for life", can be defined as microorganisms that provide specific health advantages for the host when administered in adequate amounts [1]. As a major limitation, products containing probiotic bacteria must contain >10^7 living microorganisms/gram. Over the last decade, an enormous number of studies have observed beneficial effects of probiotics supplements and foods containing these bacteria on a vast spectrum of disease; from immune function and infection to gastrointestinal disorders and metabolic diseases [2-4]. It is believed that one of the potential health benefits of these bacteria is due to lactic acid production. Lactic acid decreases the level of pH in the digestive system and precludes the detrimental bacteria growth [5]. Moreover, fermentation process of probiotics and its byproducts like lactic acid is highly desirable for food and chemical industries. Therefore, they apply well-known approaches such as temperature regulation and oxygen exposure to accelerate bacterial reproduction and fermentation.

Non-ionizing electromagnetic radiation at frequencies ranging 300 MHz to 300 GHz are known as microwaves. Microwaves are widely used in mobile phones, Wi-Fi, radars, etc. Over the past several years, studies have shown microwave irradiation can alter microorganism subcellular metabolism. Some studies have shown that microwave exposure can decrease the growth of pathogen bacteria and stop urease activity [6]. Kushwah *et al*., showed that exposing Bacillus subtitles to low power microwave resulted in amylase and pectinase activity alteration along with an increase in exopolysaccharide production [7]. Depending on radiation period and intensity, microwave may either stimulate or suppress the fungal growth [8, 9]. These results indicate that only if microorganisms are treated with microwave exposure at right frequency and duration, desirable changes in growth and metabolism would be induced.

Lactobacillus acidophilus and *Lactobacillus casei* are gram-positive probiotics which belong to the genus Lactobacillus. These bacteria are widely applicable in food and dairy industries. Increasing bacterial load and decreasing fermentation time make these industries more profitable. Therefore, this study was aimed at assessing the biological effects of short-term exposures of *L. acidophilus* and *L. casei* probiotic bacteria to 2.4 GHz RF-EMF generated by a commercial Wi-Fi router on the lactic acid production and proliferation and of these microorganisms.

Material and Methods

In this experimental study, pure culture strains of *L. acidophilus* and *L. casei* (DVS, Chris- Hansen Denmark) were cultured in MRS broth medium (Merck, Darmstadt, Germany) at 37 °C for 18 hours. Then 500 µL of the mixed suspension was transferred to MRS broth again and incubated at 37 °C for 6 hours. After incubation, samples were exposed to the RF-EMF emitted from a 2.4 GHz Wi-Fi router (D-Link, D-Link Corporation, Taiwan) for 15, 30, 45 and 60 minutes at the distance of 5 cm from the router antenna. The control samples were sham-exposed to RF-EMF. The specific absorption rate (SAR) level was computed by an expert. After exposure, mixed suspensions were diluted up to 10^{-6} and 100 µL of diluted samples was transferred to the MRS agar medium (Merck, Darmstadt, Germany) and incubated for 72 hours at 37 °C in an anaerobic jar (AnaerocultA gas pack, MerckDarmstadt, Germany). Bacterial counts were enumerated after 72 hours of incubation. These counts were reported as colony forming unit (CFU) per ml. After 6 hours of incubation at 37 °C, 1 ml of each suspension was used for the lactic acid assay.

Lactic acid measurement

In order to estimate the level of lactic acid, Kimberley and Taylor's method was used [10] with some modifications. The medium was centrifuged (Sigma 3K30, Germany) at 7500 rpm for 15 min. One ml of the supernatant after ten-fold dilution was added to 1 ml of 20% $CuSO_4$ (Merck, Darmstadt, Germany) solution followed by 8 ml water and 1 g calcium hydroxide. After 30 minutes rest at room temperature, the mixture was centrifuged for 15 min at 7500 rpm. One ml of remaining liquid was added to 0.05 ml of 4% $CuSO_4$ solution and after that 6 ml concentrated sulfuric acid was added to the mixture (Merck, Darmstadt, Germany). After complete mixing on a vortex mixer for 5 min, the solution was incubated in boiling water for 5 min followed by a cooling bath phase. Next step was adding 0.1 ml of 1.5% p-hydroxydiphenyl (Sigma, Germany) dissolved in 95% ethanol and 30 min incubation at room temperature. The mixture was then placed in boiling water bath for 90 seconds. After cooling, absorbance was read at 410 nm (Apel PD303, Japan). Then the same method was used to obtain a standard curve for pure lactic acid (Sigma, Germany) and lactic acid concentration of the samples was measured.

Statistical analysis

The means and standard deviation (SD) were compared using the non-parametric Mann-Whitney test and comparison between groups were done by using Kruskal-Wallis test. Data were log-transformed and effect of radiation on lactic acid concentration was assessed by ANCOVA with a number of CFU as the co-variate. $P<0.05$ was considered as statistically significant.

Results

The growth of *L. acidophilus* after exposure to RF-EMF for 30, 45 and 60 min significantly increased ($P<0.01$, $P<0.01$, $P<0.01$, respectively) compared to bacteria received a sham-exposure. Moreover, no difference was found between the growth of samples exposed to RF-EMF for 15 min and bacteria that were sham-exposed (Table 1). In a similar pattern, while no difference was observed between the growth of samples exposed to RF-EMF for 15 min, the *L. casei* proliferation in samples irradiated for 30, 45 and 60 minutes significantly increased ($P<0.05$, $P<0.01$ and $P<0.01$, respectively) compared to bacteria that were sham-exposed (Table 1).

After exposure to RF-EMF for 15, 30 and 45 min, lactic acid concentrations in the medium of *L. acidophilus* were significantly higher than the shame-exposed bacteria ($P=0.003$, $P=0.002$, and $P=0.001$, respectively). Moreover, the difference between these three groups was not statistically significant ($P\geq0.05$) (Table 2). The concentration of lactic acid in *L. casei* mediums were significantly higher after 30 and 60 min exposure to RF-EMF ($P=0.006$ and $P=0.004$, respectively). Though, 15 and 45 min exposure to RF-EMF failed to increase lactic acid levels significantly ($P\geq0.05$) (Table 2). Independent effect of RF radiation on a lactic acid production of *L. acidophilus*

Table 1: Colony forming unit (per ml × 10^5) in the control samples and those exposed for 15, 30, 45 and 60 min to 2.4 GHz Wi-Fi radiofrequency radiation.

	L. acidophilus CFU (mean ± S.D)			*L. casei* CFU (mean ± S.D)		
Exp. Time	**Sham-exposed**	**RF-EMF Exposure**	***p-value**	**Sham-exposed**	**RF-EMF Exposure**	***p-value**
15 min	8.25 ± 0.41	8.48 ± 0.11	0.151	7.40 ± 0.80	8.07 ± 0.40	0.097
30 min	7.48 ± 0.26	8.15 ± 0.15	0.001	7.34 ± 0.66	8.11 ± 0.48	0.041
45 min	7.69 ± 0.30	8.62 ± 0.15	0.002	7.5 ± 0.53	8.46 ± 0.41	0.008
60 min	7.55 ± 0.20	8.67 ± 0.09	0.002	7.57 ± 0.48	8.80 ± 0.09	0.002

*Mann-Whitney test

Table 2: Effect of low power microwaves (MW) on lactic acid production in *L. acidophilus* and *L. casei*

	L. acidophilus		*L. casei*	
Duration of RF-EMF Exposure (min)	**Lactic acid (µg/ml) (Mean ± SD)**	**Magnitude of the change (compared to sham) (%)**	**Lactic acid (µg/ml) (Mean ± SD)**	**Magnitude of the change (compared to sham) (%)**
Sham (0 min)	291.12 ±16.88	0.00	176.37 ± 12.74	0.00
15	352.97 ± 9.87	*21.24	210.46 ± 24.59	19.32
30	359.12 ± 43.69	*23.35	229.51 ± 33.43	*30.12
45	359.42 ± 16.76	**23.46	174.53 ± 44.02	-1.04
60	332.40 ± 30.36	14.17	231.04 ± 26.49	*30.99

P-value compared to those of the sham-exposed group, *P<0.05; **P<0.001.

and *L. casei* were confirmed by ANCOVA test (P<0.001 and P=0.012 respectively). Alterations of lactic acid production in L. acidophilus and L. casei exposed to 2.4 GHz Wi-Fi radiofrequency radiation for 15, 30, 45 and 60 min are shown in Figure 1.

Discussion

Altogether, the findings of present study revealed that both *Lactobacillus acidophilus* and *Lactobacillus casei* exposed to 2.4 GHz RF-EMF produced by a commercial Wi-Fi router showed significantly increased lactic acid production and proliferation. Using RF-EMFs to alter the growth of various fungi and bacteria have been studied before. However, these studies showed different results based on species and intervention traits [11-13]. In this light, Kushwah *et al*., reported while 2 min exposure to 2450 MHz MW radiation enhanced *Streptococcus mutans* growth, four minutes of the same exposure reduced the growth of *Pectobacteriumcarotovorum* [7]. Even in the same species, e.g. *Saccharomyce-*

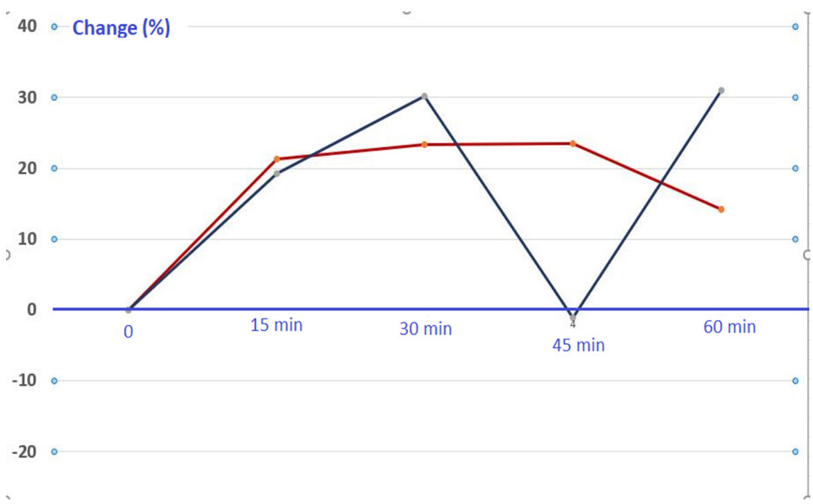

Figure 1: Alterations of lactic acid production in L. acidophilus (red) and *L. casei (green)* exposed to 2.4 GHz Wi-Fi radiofrequency radiation for 15, 30, 45 and 60 min. The magnitudes of the changes compared to those of the sham (%) are shown in this figure.

tes cerevisiae, changing exposure frequency between 41.8 to 42.0 GHz caused increased or suppressed growth by 15% and 29%, respectively [14]. The reason behind declined cell viability after MW radiation was addressed in many studies. Growth limitation effects of RF-EMF have been mostly linked to changes in enzymatic activity and DNA damage caused by an increased level of reactive oxygen species (ROS) [15, 16]. Moreover, Vrhovac *et al.*, have stated that colony growth reduction after exposure to 905 MHz RF-EMF generated by Global System for Mobile Communication (GSM) pulses (at a SAR of 0.12 W/kg) at least in a part was due to the impairment of DNA repairing mechanisms [9]. On the other hand, the exact mechanisms which RF-EMF stimulatestheproliferation of microorganisms are notfully known, yet. It has been suggested that microwaves radiation increase life forms accessibility to energy sources like glucose by enhancing membrane permeability [17]. Another possible mechanism may be the effect of microwave radiation on the reduction of activity and expression of certain proteins involved in the apoptosis [18, 19].

Our results showed that RF radiation increased lactic acid production in both strains. This was independent of an increase in growth rate which was confirmed by ANCOVA test. Lin *et al.,* stated similar results when *L. rhamnosus* strains were exposed to microwave radiation (2450 MHz) for 3 min, in some strains lactic acid production was amplified even after 9 generations [20]. Based on polymorphism analysis, it has been suggested that mutation in malate/lactate dehydrogenase and pyruvate kinase genes were the possible causes of the elevated lactic acid production.

We have previously investigated the effects of exposure to RF-EMFs of Wi-Fi routers on the sensitivity of different bacteria to widely used antibiotics. The results of our current study confirm the findings obtained in our previous study which showed that exposure to RF-EMFs only between a specific upper and lower level of irradiation (what is usually called an "exposure window") affects microorganisms [21]. A similar pattern was also reported by Carta and Desogus previously [22].

Altogether these findings may help scientists accelerate the proliferation of some probiotic bacteria and lactic acid production by short-term exposure of RF-EMF. These results open new horizons to health and food industries to manipulate manufacturing processes time, increase production efficiency and profit margin. However, further experiments are still necessary to determine the optimum frequency, exposure time and power. Moreover, the effect of these exposure levels on the growth of pathogenic bacteria should be evaluated.

Conclusion

In this study we investigated the biological effects of the exposure of *L. acidophilus* and *L. casei* probiotic bacteria to 2.4 GHz RF-EMF generated by a commercial Wi-Fi router on the proliferation and lactic acid production of these probiotic bacteria. Altogether our findings showed that these short-term exposures increase the proliferation and lactic acid production of *L. acidophilus* and *L. casei* probiotic bacteria. Considering the promising findings of our study, more research in this challenging area can expand the borders of our knowledge about probiotics and its growing applications in food industry.

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Conflict of Interest

None

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