International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

The Influence of Instant Coffee on the Survival Rate of Lactobacillus Acidophilus, Bifidobacterium Bifidum, Escherichia Coli and Staphylococcus Aureus

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ABSTRACT

Some studies show that the bioactive substances found in coffee may act as prebiotics while others may have an antibacterial effect, including on virulent genera for humans. This work verified the influence in vitro of instant coffee on the survival rate of two probiotic bacteria: Lactobacillus acidophilus and Bifidobacterium bifidum, and two potentially pathogenic species: Staphylococcus aureus and Escherichia coli. Commercial instant coffee was used as a reaction medium, adjusted to a pH of 6.0 and 7.5. The control tests were made in MRS broth for the probiotic bacteria and BHI broth for S. aureus and E. coli, medium components of PCA without agar. The survival rate (SR) was calculated through the relationship between the cell viability with the treatments and their respective controls. The experiments were done in aseptic conditions and in triplicate, and their data were submitted to statistical analysis. In pH 6.0, the instant coffee medium significantly decreased the survival rate (SR = 84%) of S. aureus compared to the other bacteria, but L. acidophilus (SR = 101%) showed a growth trend only in one hour of reaction. In pH 7.5, the instant coffee medium also showed a higher survival rate (99.5%) for L. acidophilus. B. bifidum and E. coli, which had a low sensibility to the coffee medium, not suffering significant interference with the pH variation. The instant coffee medium (in both pH tested) interfered positively or negatively with the SR, in different ways, for each bacterium under study.

Key words: Coffee – probiotic – gate – inhibition – stimulus

INTRODUCTION

Belonging to Coffee genus, the coffee has more than 2000 substances that can vary due to some factors such as species, farming and processing. The chemical compound of coffee beans is complex and diversified. Besides caffeine, it contains vitamins, mainly the B complex ones, and minerals such as potassium, phosphorus, magnesium and calcium among others. It also has nitrogen compounds, lipids, sugars and polysaccharides as well as phenolic compounds, from which stands out chloro genic acid with antioxidant power. [1,2]

The processing of coffee beans for brewed paper-filter and espresso coffee includes roasting and milling, and for instant ones, they are submitted to extraction and extract drying. During the roasting process, which classifies the final product in light, medium and dark, the coffee beans go through chemical and structural changes that result in the

formation or elimination of compounds that will interfere in the quality of the beverage. [3,4]

Some researches show that coffee has bioactive substances with antibacterial effect, including genus with virulence factors for humans. Positive results have been verified against Staphylococcus aureus, Escherichia coli, and Salmonella enterica, among others. [5,6] Additional results show the enhancement in growth of beneficial bacteria present in the gastrointestinal tract, mainly the ones from Bifidobacterium genus. [7,8] The complexes responsible for this effect were not identified; however, some authors suggest that the polysaccharides (arabinogalactans, galactomannans), the melanoidines and the phenolic compounds might act as prebiotics. [7,8,9]

Being an important source of antioxidant elements, coffee bean and its beverages have been the subject of many studies and the results show that a moderate consumption can help preventing degenerative chronical diseases such as: type II diabetes mellitus, obesity, neurological and cardiovascular diseases. However, there is few reports about relationships between bacteria and coffee. [10]

Usually, prebiotics are carbohydrates (oligosaccharides and polysaccharides) considered food ingredients not hydrolyzed by enzymes present in human gastrointestinal tract or by most microorganisms in the intestine. They benefit the host by selective growth and/or selective activity of only one or a limited group of bacteria in the colon, including the probiotic ones, mainly the lactobacilli and Bifidobacterium. [11]

According to nutritional point of view, both melanoidines and phenolic compounds do not fit the definition of dietary fiber. Nevertheless, in vitro evidences show that not being totally digested in the small intestine, both substances do not behave only as antioxidants, but they also serve as a substrate to the beneficial bacteria. At the same time, these results are still inconsistent to prove a prebiotic effect. [9]

The intestinal microbiota, described as a group of microorganisms that colonizes our gastrointestinal tract, has a huge impact on our health. Thus, feed assumes a fundamental role when food and beverages intake provides the growth of beneficial bacteria, preventing pathogenic microorganisms from causing any harm. [12,13, 14]

This study had as objective to verify in vitro the influence of instant coffee on the survival rate of two probiotic bacteria (Lactobacillus acidophilus and Bifidobacterium bifidum) and two potentially pathogenic ones (Staphylococcus aureus and Escherichia coli), in different pH.

METHODS

Microorganisms

The microorganisms used were: the probiotics Lactobacillus acidophilus LA 3 (Sacco®) and Bifidobacterium bifidum (Gama®), both commercially acquired; and Staphylococcus aureus and Escherichia coli, previously isolated from food, using Petrifilm Staph Express Count Plate 3M^{TM} and Petrifilm E. coli Count Plate 3M^{TM} methodology, respectively.

Coffee beverage

Traditional instant coffee was used to prepare the beverage. It was bought in a commercial establishment in the city of Marília/SP.

Reactivation of probiotic bacteria

All the experiments involving microorganisms in this study were performed under aseptic conditions.

The lyophilized probiotic cultures were reactivated following the methodology described by Oliveira with some modifications. Therefore, 0.1 g of each microorganism was added in tubes containing 7.9 ml of Molico reconstituted powdered milk at 12%, supplemented with L-cysteine (0.05%), glucose (1%) and yeast extract (1%). The tubes were incubated

under 37 °C, for 20 hours. Then, three successive transfers were performed with 0.1 ml of the culture in 7.9 ml of MRS broth (Man Rogosa and Sharpe Broth) supplemented with L-cysteine (0.05%), glucose (2%) and protease peptone (2%), incubated under 37 °C, for 38 horas.

Preparation of reaction media: Instant coffee beverage and positive controls

Sampling was executed in triplicate in the Microbiology Laboratory from Faculty of Food Technology in Marília/SP, Brasil (FATEC-Marília).

Instant coffee beverage, made according to the manufacturer's recommendation, was used as treatment. Simultaneously, appropriate culture media were used as control for each microorganism growth: MRS broth supplemented with L-cysteine (0.05%), glucose (2%) and protease peptone (2%), for the probiotic bacteria; BHI (Brain Heart Infusion) broth supplemented with glucose (3.8%) and yeast extract, for S. aureus; and a medium with PCA (Plate Count Agar) components without agar, for E. coli.

To avoid pH variation during the experiment, both the instant coffee beverage and the control media were prepared replacing water for phosphate buffered saline solution (0.8% of NaCl; 0.2% of KCl, 0.14% of Na_2HPO_4 and 0.024% of KH_2PO4). The pH was adjusted to 6.0 and 7.5, to simulate the small and large intestines pH, respectively. Then 7.9 mL of each media were transferred to test tubes. Control media were submitted to a sterilization treatment under 121°C for 15 minutes and the coffee beverage, under 100° C for 10 minutes.

Verifying the action of the instant coffee on the survival rate of L. acidophilus, B. bifidum, E. coli and S. aureus

Aliquots of 0.1 ml from each microbial culture under study were incubated in the test tubes (coffee pH 6.0 and 7.5) at 37 ºC/ 60 minutes and under the same conditions as the control media. The initial concentration of bacteria during the reactions was 10^7 UFC.mL^{-1}.

Counting viable microorganisms

After the microorganism's reaction time with the coffee medium and control media, the viable bacteria cell count was performed thought in-depth plating of the samples in MRS Agar, with L-cysteine (0.05%) and an anaerobic incubation at 37°C for 72 hours for the probiotic bacteria; in PCA agar with an incubation at 37 °C for 24 hours for E. coli; and in a BHI medium with agar (1.5%) with an incubation at 37 °C/24 hours for S. aureus.

Survival rate equation

The microorganisms' survival rate was calculated using the equation described by Tsai and Hwang. [15]

$$\%\ \text{Survival} = \frac{\text{Log UFC.ml}^{-1}\ \text{Treatment}}{\text{Log UFC.ml}^{-1}\ \text{Control}} \times 100$$

Analysis of the results

The data were submitted to an analysis of variance (ANOVA) and the means were compared through Tukey's test using the GRAPHPAD INSTAT (Rutgers University Camden, New Jersey) software. The t-test was applied using the same software when needed. The results were considered significant for $p<0.05$.

Results and discussion

Table 1 shows the mean survival rate of the microorganisms under study in relation to the instant coffee medium. (pH 6.0 and 7.5).

The results show that E. coli did not have a significant reduction of its survival rate when compared with the probiotic bacteria, both in pH 6.0 and 7.5. Moreover, in pH 6.0, these gram-negative bacteria had a significantly higher survival rate (2.5%) than the Bifidobacterium bifidum species. The pH of the treatment interfered significantly (t-test, $p = 0.038$) with E. coli, as the reaction in pH 7.5 resulted in a lower survival rate (3.3%) when compared to pH 6.0. It is worth mentioning that the time of contact between the antimicrobial agent and the microorganism is essential for its efficiency. Studies done with the coffee medium or with its antimicrobial compounds [5,6] have shown a higher inhibition potential on E. coli. These

studies, however, used reaction times of more than one hour, other strains of this species and different concentrations of the inhibitory compounds, which are often different than the real concentrations found in the beverage and could, explain such difference.

The most affected microorganism by the instant coffee medium was S. aureus, mainly in pH 6.0, with its SR being significantly reduced by 17% when compared to L. acidophilus. The best pH for the growth of these Gram-positive bacteria is 7.0, and this is one of the factors that might have contributed to its higher SR in pH 7.5. [16]

Table 1 – Mean Survival Rate (%) of Escherichia coli, Staphylococcus aureus, Bifidobacterium bifidum and Lactobacillus acidophilus in traditional instant coffee medium, pH 6 and 7.5

	E. coli	S. aureus	B. bifidum	L. acidophilus
pH 6.0	99.10 ± 1.88 BC[1]b[2]	84.00 ± 1.53 Aa	96.60 ± 1.87 Ba	100.1 ± 0.03 Ca
pH 7.5	95.80 ± 9.00 Aba	95.00 ± 1.56 Ab	97.00 ± 3.05 Aba	99.50 ± 0.12 Ba

(1) Means followed by the same capital letters, on the same line, do not differ from each other according to the Tukey test ($p < 0.05$).
(2) Means followed by the same lower case, in the same column, do not differ from each other according to the Student t-test ($p < 0.05$)

Almeida [5] evaluated the influence of pH on the antimicrobial effect of the isolated compounds found in coffee, observing that the inhibition percentage was significantly higher in acid pH, around 80-96%. The pH did not significantly influence the survival rates of B. bifidum and L. acidophilus (t-test, p >0.05) in the traditional instant coffee tested. L. acidophilus showed a higher SR, with a growth trend in pH 6.0 in only 60 minutes of reaction.

There is evidence that coffee might be a source of prebiotic compounds, acting as a substrate for the beneficial bacteria present in the colon, especially for the Bifidobacterium and Lactobacillus species. On the other hand, it also has compounds that have a detrimental effect on pathogenic bacteria.

Coffee is the subject of a lot of scientific research, mainly because of its elevated antioxidant activity (phenolic compounds), but there is little information about its impact on the intestinal microbiota and, consequently, on human health.

Jaquet et al. [7] evaluated healthy volunteers under a restricted diet and observed that the daily ingestion of three cups of coffee, during 3 weeks, increased the number of Bifidobacterium in subjects with lower levels of these bacteria beforehand. The authors did not identify which compounds were responsible for the effect, but based on other studies, they suggest that the chlorogenic acids (phenolic compounds) and the soluble portion of fibers (galactomannans and arabinogalactans) metabolized by the intestinal microbiota might act as prebiotics.

Gniechwitz et al. [17] observed that after 24 hours of fermentation in fecal samples of 4 healthy volunteers, 85% of the total carbohydrates in the sample of brewed coffee were degraded, resulting in an increase of 60% in the Bacteroides and Prevotella bacteria. The growth of Bifidobacterium and lactobacillus was not observed. The authors also concluded that the coffee beverage contributed to dietary fiber ingestion. Fibers are usually found in solid food, but beverages can also contribute, with coffee being an example. It has relatively high amounts of galactomannans and arabinogalactans, having its value as source of fibe. [18, 19]

Almeida [5] evaluated the antimicrobial activity of an aqueous coffee extract and some of its isolated chemical compounds (trigonelline, caffeine, chlorogenic acid, caffeic acid and protocateic acid) on Gram-positive and Gram-negative bacteria, filamentous fungi and yeasts. The author observed that the aqueous coffee extract and five of the tested compounds had an antimicrobial effect on Escherichia coli, Staphylococcus aureus, Streptococcus mutans and Salmonella enterica, with negative results for filamentous fungi and yeasts.

Daglia et al. [6] evaluated the antimicrobial activity of coffee for Gram-positive and Gram-negative bacteria, considering the beverage's preparation procedure, the coffee variety and coffee roast. The inhibition results were positive for E. coli and S. aureus and the authors concluded that the way the beverage was prepared didn't influence the antimicrobial activity. They also observed that Robusta coffee had a better antimicrobial activity than Arabica coffee, and that the dark roast had a superior activity when compared to medium and light roasts due to the formation of Maillard reaction products.

Almeida et al. [20] investigated the antimicrobial effect of some chemical compounds in coffee (Chlorogenic acid, caffeine, trigonellin and caffeic acid) on some enterobacteria, including E. coli. The authors concluded that the investigated compounds are potential natural antimicrobial agents against enterobacteria. Nakayama e Oishi [8] observed that the coffee beverage significantly reduced the numbers of E. coli. Clostridium spp, Enteroccus spp had a light decrease. In the same study, E. coli had its growth inhibited in an agar medium with 30 and 50% of coffee.

CONCLUSION

The traditional instant coffee beverage, in both pH tested, affected the survival rate of each bacterial species under study positively or negatively. Further studies are needed with more lineage variations, including different sources of the coffee beverage and longer contact times with the microorganisms, to reach more comprehensive conclusions.

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How to cite this article: Ribeiro MAC, Dorta C, Tanaka AY et al. The influence of instant coffee on the survival rate of lactobacillus acidophilus, bifidobacterium bifidum, escherichia coli and staphylococcus aureus. Int J Health Sci Res. 2017; 7(12):187-192.

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