British Microbiology Research Journal
13(2): 1-8, 2016, Article no.BMRJ.24074
ISSN: 2231-0886, NLM ID: 101608140

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Effect of Bacteriocinogenic *Pediococcus pentosaceus* IO1 Strain and Its Bacteriocin on Growth Performance and Intestinal Microbiota of Albino Rat

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Authors' contributions

This work was carried out in collaboration between all authors. Author IAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AOO and DJA managed the analyses of the study. Author IAA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/24074
Editor(s):
(1) Marcin Lukaszewicz, Department of Biotransformation, Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland and Division of Chemistry and Technology Fuels, Wroclaw University of Technology, Wroclaw, Poland.
Reviewers:
(1) Eliene Penha Rodrigues Pereira, State University of Campinas, Brazil.
(2) Tsong-Rong Yan, Tatung University, Taiwan.
(3) Wagner Loyola, Brazilian Corporation of Agricultural Research, Brazil.
Complete Peer review History: http://sciencedomain.org/review-history/13532

Original Research Article

Received 2nd January 2016
Accepted 9th February 2016
Published 3rd March 2016

ABSTRACT

Aims: To study the effect of bacteriocinogenic *Pediococcus pentosaceus* IO1 strain and its bacteriocin on the growth performance and intestinal microbiota composition of male albino rats, using faeces as a surrogate.
Place and Duration of Study: Department of Microbiology, Federal University of Technology, Akure, Nigeria and Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Nigeria between January, 2014 and April, 2014.
Methodology: A total of 16 rats were randomly assigned to four groups (A, B, C, and D) of four rats per group. Group A (control) was placed on the basal diet and orally dosed with 0.5 ml of MRS broth, while group B, C, and D were placed on the basal diet and also orally dosed with 0.5 ml of

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bacteriocin-containing cell-free culture supernatant (crude bacteriocin), 0.5 ml of 10^9 cfu/ml of viable bacteriocin producer (*P. pentosaceus* IO1), 0.5 ml of 10^9 cfu/ml of bacteriocin-negative producer respectively, for a period of 14 days. The weight of each rat was measured and faecal samples collected, at day 0, 7, and 14 of the experiment, from each rat was serially diluted and pour-plated on selective media for total viable bacteria count, lactic acid bacterial count, and enterobacteria count.

Results: Rat groups fed with *P. pentosaceus* IO1 strain and its bacteriocin showed better weight gain and decrease in enterobacteria count in the faeces as compared to control ($P<0.05$) on day 7 of experiment. Total viable bacteria count in the faeces was not significantly influenced. There was a slight significant increase in lactic acid bacterial count in the faeces of animals belonging to treatment group B, C, and D at day 14.

Conclusion: This study demonstrates that consumption of *P. pentosaceus* IO1 strain and its bacteriocin improve growth performance and modulate intestinal microbiota of the albino rat. Hence, *P. pentosaceus* IO1 strain may be used as probiotic or protective culture in food industry.

Keywords: Pediococcus pentosaceus; albino rat; probiotic; enterobacteria; lactic acid bacteria.

1. INTRODUCTION

Pediococcus spp. are lactic acid bacteria that occur in plant environments along with *Lactobacillus* and *Leuconostoc*. Consequently, they are often found in foods from fermentation processes such as beer, cider, silage, sauerkraut and other fermented vegetables. They are also found in other foods including cheese, cured meats, raw sausages, and fresh and marinated fish [1]. *Pediococcus* spp. are morphologically distinct from other lactic acid bacteria. They are more like micrococci in appearance as they divide in two planes at 90^o, forming tetrads. However, physiologically they have more in common with *Lactobacillus* spp. and *Leuconostoc* spp. than with streptococci [1]. Many lactic acid bacteria including *Pediococci* spp. are considered to have probiotic effects [2].

The Food and Agriculture Organization (FAO) and World Health Organization (WHO) [3] defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". The particularities of the use of probiotics in farm animals consist in their application not only for disease prevention but also for optimization of animal production. The colonization of the digestive tract in animals begins soon after birth, and the normal microbiota changes dramatically during the life of the host. The composition of the gastrointestinal microbiota differs between animal species [4].

The desirable property of a probiotic strain is the ability to produce antimicrobial substances such as bacteriocins that offer the potential to provide an advantage in competition and colonization of the gastrointestinal tract [5]. Bacteriocins are bacterially produced, small, heat-stable peptides that are active against other bacteria and to which the producer has a specific immunity mechanism. Bacteriocins can have a narrow or broad target spectrum [6]. *Pediococcus* spp. have been shown to produce a number of bacteriocins active against closely related bacteria and some Gram-positive pathogens [1].

Traditionally, bacteriocin production has been an important criterion in the selection of a probiotic strain, albeit that few studies have definitively demonstrated the impact of bacteriocin production on the ability of a strain to compete within the gastrointestinal tract and/or positively influence the health of the host [7].

The objectives of this study were therefore to evaluate the impact of oral administration of bacteriocinogenic *Pediococcus pentosaceus* IO1 strain and its bacteriocin on the growth performance and intestinal microbiota composition of healthy male albino rats.

2. MATERIALS AND METHODS

2.1 Bacterial Strain and Growth Condition

Pediococcus pentosaceus IO1, the bacteriocin producer strain, was isolated from "iru" (fermented African locust beans) using de Man Rogosa Sharpe (MRS, Oxoid Ltd) agar and broth. The strain was identified by API 50 CH test strip (BioMerieux, France). Before experimental use, the producer strain was subcultured twice in MRS broth at 30℃.

2.2 Experimental Animals

Sixteen (16) healthy male albino rats (*Rattus norvegicus*), each weighing 125–150 g, were used for the experiment. Rats were obtained from the animal house, University of Ibadan, Nigeria. The rats were acclimatized on basal diet for one week *ad libitum* before treatment.

2.3 Preparation of Bacteriocin-containing Cell-free Culture Supernatant

The bacteriocin-producing strain, *P. pentosaceus* IO1, was grown in MRS broth for 48 h at 30℃. The cell-free culture supernatant was obtained by centrifuging the culture at 4000 x g for 20 min, followed by adjusting to pH 6.5 with 1N NaOH and treatment with catalase before filtering the neutralized supernatant through a 0.45 µm pore-size membrane filter. The bacteriocin-containing cell-free culture supernatant (crude bacteriocin) was stored at 4℃ in the refrigerator until use [8].

2.4 *In vivo* Feeding Trial

The rats were randomly assigned to four groups (A, B, C, and D) of four rats per group. Group A (control) was placed on the basal diet and orally dosed with 0.5 ml of MRS broth, while group B, C, and D were placed on the basal diet and also orally dosed with 0.5 ml of bacteriocin-containing cell-free culture supernatant (crude bacteriocin), 0.5 ml of 10^9 cfu/ml of viable bacteriocin producer (*P. pentosaceus* IO1), 0.5 ml of 10^9 cfu/ml of bacteriocin-negative producer respectively. The treatment above was repeated every three days for a period of 14 days. The activity, behaviour and general health of the rats were monitored daily. The weight of each rat was measured on day 0, 7, and 14 of experiment. The total weight gain was calculated and expressed as the average weight gain (g) which was the difference in the weight of experimental rats at day 0 and 14 of the experiment. At the end of experiment (day 14), the rats were killed.

2.5 Isolation and Enumeration of Bacteria from Rat Faecal Samples

Samples of faeces were collected from each rat on day 0, 7, and 14 of the experiment. One gram of faeces was mixed with 9 ml of physiological saline (0.85 %) and homogenized. Samples were serially diluted and 1 ml of 10^{-3} and 10^{-6} dilutions was inoculated onto following media: Plate Count agar for enumeration of total viable bacteria, MacConkey agar for enumeration of Enterobacteria (*E. coli*) and de Man Rogosa Sharpe (MRS) agar for lactic acid bacteria. Total viable bacteria and Enterobacteria were incubated at 37℃ for 24 – 48 h. Lactic acid bacteria were incubated anaerobically at 30℃ for 48 h. Numbers of colony forming units (cfu) were expressed as $\log_{10}$ cfu/g [9].

2.6 Statistical Analysis

Statistical analyses of the data were performed with SPSS statistical software (SPSS for Windows v 16.0). Data recorded as mean± standard deviations were analysed by One-way ANOVA followed by Duncan's Multiple Range test ($P < 0.05$) to determine the significant differences between the mean values.

3. RESULTS

3.1 Growth Performance/ Total Weight Gain

Fig. 1 shows the total weight gain of the experimental animals from treatment groups (A – D). The total weight gain was significantly ($P<0.05$) higher in the rats orogastrically dosed with crude bacteriocin (B, 30.44 g) and its bacteriocin producer *P. pentosaceus* IO1 (C, 34.09 g) compared with the control (A, 12.66 g). The total weight gain of the rats was highest in group C. Throughout the experimental period, there was no noticeable change in activity, behaviour, or hair luster in any of the groups of rats. No diarrhea or other treatment-related sickness was recorded. At the end of the experimental period, all animals appeared healthy.

3.2 Bacterial Counts of Faeces of Rats in the Animal Experiment

3.2.1 Total viable count

Total viable cell count from rat faeces of experiment group A, B, C, and D were compared as shown in Fig. 2. On day 7, faeces of animals from groups A, B, C, and D had total viable cell count of 7.75, 7.72, 7.71, and 7.68 log cfu/g, respectively. After day 0, there was a general increase in total viable cell count of the faeces of animals from group A, B, and C, which was observed at day 7 and 14. The difference in total viable cell count between group B and C at day 7 and 14 were 0.01 and 0.12 log cfu/g respectively. No significant difference ($p>0.05$) was found in the total viable cell count at day 7 and 14 in all experiment groups.

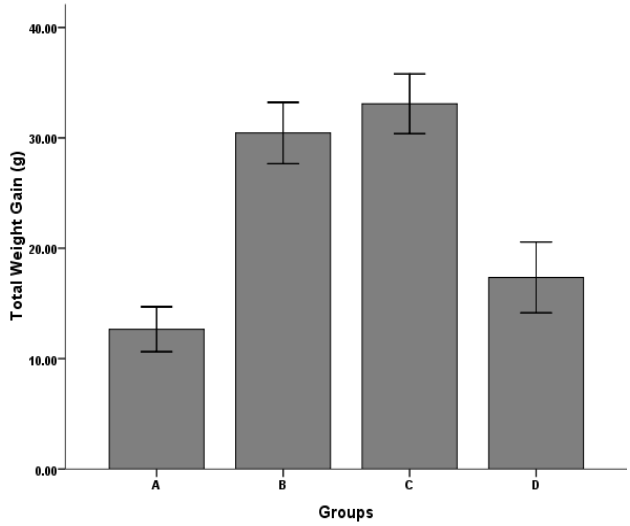

Fig. 1. Weight gain of experimental animals from treatment groups: A (Basal diet + MRS broth), B (Basal diet + Crude bacteriocin), C (Basal diet + Viable bacteriocin producer *P. pentosaceus* IO1), and D (Basal diet + Non-bacteriocin producer)

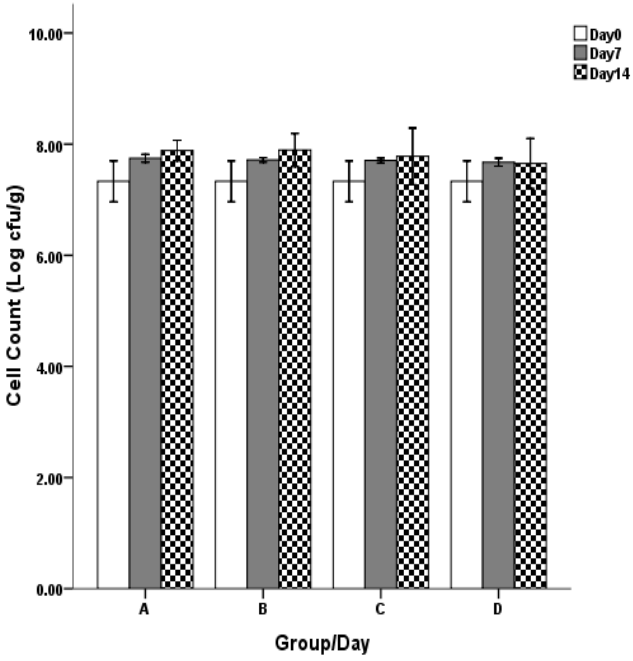

Fig. 2. Comparison of total viable bacteria cell count in faeces of experimental animals from treatment groups: A (Basal diet + MRS broth), B (Basal diet + Crude bacteriocin), C (Basal diet + Viable bacteriocin producer *P. pentosaceus* IO1), and D (Basal diet + Non-bacteriocin producer)

3.2.2 Enterobacteria count

Enterobacteria count from rat faeces of experiment group A, B. C, and D were compared as shown in Fig. 3. On day 7, faeces of animals from group A, B, C, and D had enterobacteria count of 7.72, 7.09, 6.24, and 7.66 log cfu/g, respectively. Enterobacteria cell count of treatment group B and C decreased significantly ($P<0.05$) at day 7. The decrease reached a maximum of 1.3 log cfu/g in group C. At day 14, however, the

enterobacteria count was shown to increase again.

3.2.3 Lactic acid bacterial count

Lactic acid bacterial count from rat faeces of experiment group A, B, C, and D were compared as shown in Fig. 4. On day 7, faeces of animals from group A, B, C, and D had lactic acid bacterial count of 7.80, 7.81, 7.84, and 7.85 log cfu/g, respectively. At day 14, there was a slight increase in cell count in group B, C, and D when compared with the control group (A). Therefore, group B, C, and D differ significantly ($P<0.05$) from the control group (A) at day 14.

4. DISCUSSION

Bacteriocin production has been described as a functional trait of probiotic bacteria, and probiotic strains that produce bacteriocins have found application for human consumption [10].

In this study, the bacteriocin-producing *Pediococcus pentosaceus* IO1 strain and its bacteriocin was used in an animal study to determine their influence on the growth performance and composition of the intestinal microbiota of male albino rat.

The total weight gain in rats that consumed bacteriocinogenic *Pediococcus pentosaceus* IO1 and its bacteriocin was higher and significantly different ($P<0.05$) from the control. This is an indication that bacteriocinogenic *P. pentosaceus* IO1 and its bacteriocin could be used as growth enhancer/ promoter. Probiotic LAB had been used as growth promoters due to their ability to suppress the growth and activities of growth depressing microflora and their ability in enhancing absorption of nutrients through the production of digestive enzymes [11].

In order to ensure optimal growth, production, and health of farm animals, the beneficial microbiota of the gastrointestinal ecosystem can be supported by manipulation of the diet and application of probiotic microorganisms. Probiotics could represent an effective and safe alternative to the use of synthetic substances, for example, antibiotics, in nutrition and medicine [12,13].

The wide use of antibiotics as growth promoters stimulated the emergence of antibiotic-resistant pathogenic bacteria and contamination of the food chain with residues of antibiotics [14,15]. Human health can either be affected directly

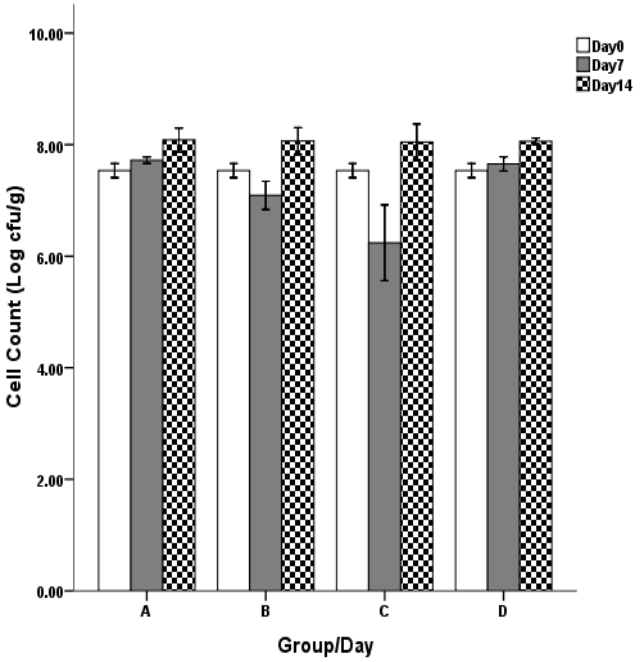

Fig. 3. Comparison of enterobacteria cell count in faeces of experimental animals from treatment groups: A (Basal diet + MRS broth), B (Basal diet + Crude bacteriocin), C (Basal diet + Viable bacteriocin producer *P. pentosaceus* IO1), and D (Basal diet + Non-bacteriocin producer)

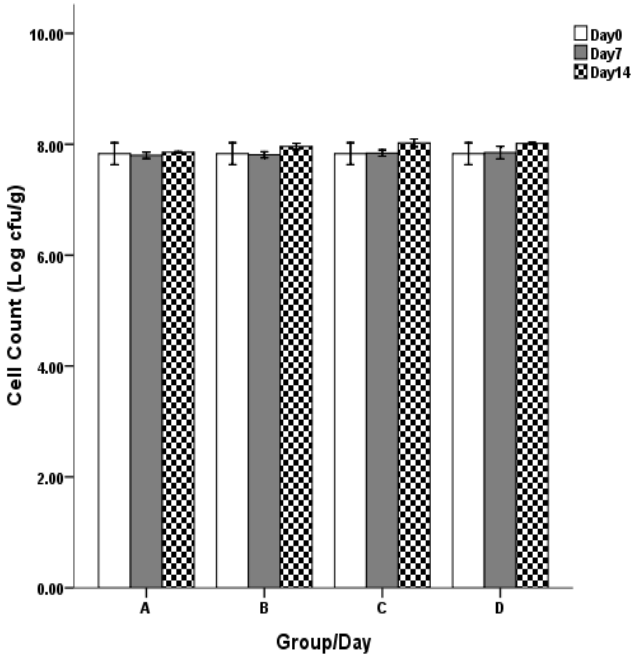

Fig. 4. Comparison of lactic acid bacteria cell count in faeces of experimental animals from treatment groups: A (Basal diet + MRS broth), B (Basal diet + Crude bacteriocin), C (Basal diet + Viable bacteriocin producer *P. pentosaceus* IO1) and D (Basal diet + Non-bacteriocin producer)

through residues of antibiotics in food of animal origin, or indirectly through the selection of antibiotic resistance determinants that may spread to human pathogens and limit the therapeutic potency of antibiotic [4].

Overall, there was no considerable effect of oral administration of the *P. pentosaceus* IO1 and its bacteriocin on the numbers of bacteria in the rat faeces. However, a slight significant increase in lactic acid bacterial count was noticed in the rat faeces of animals belonging to treatment group B, C, and D at day 14. A significant decrease in numbers of enterobacteria in faeces from animals treated with the bacteriocin-producer and its bacteriocin was noticed at day 7, which may be explained by the antimicrobial activity of the bacteriocin produced by *P. pentosaceus* IO1. However, at day 14 enterobacteria counts increased again, which may imply that enterobacteria became resistant to the bacteriocin. A reduction effect of bacteriocin-producing probiotic bacteria on enterobacteriaceae in the gastrointestinal tract of animals is well known [9].

Effect of bacteriocinogenic/ probiotic cultures of *Lactobacillus* sp. [2], *Lactococcus* sp. [16], or *Enterococcus* sp. [10,17] on different bacterial cells in faeces has been previously studied. So far, limited literature is available, on the effect of *Pediococcus* spp. on different bacterial cells in the faeces of the experimental animals. The findings in this study are in accordance with those reported earlier by Bhardwaj et al. [17]. They observed the effect of bacteriocin-producing *Enterococcus faecium* KH 24 on the faecal microflora of mice and reported a decrease in coliform count and increase in the count of *Lactobacillus* during the 12 day experimental period. They concluded that the increase in lactobacilli numbers may be a stimulatory effect of the bacteriocinogenic enterococci.

O'Toole and Cooney [18] have reported that probiotic bacteria affect the composition and function of intestinal microbial population. The predominant presence of the probiotic in the gut prevents the pathogens access to the ecological niche, interfering with the attachment of pathogens to the gut and subverting the eventual infection process [19]. Besides physical displacement of pathogens, several probiotics also produce bacteriocins, which are antimicrobial peptides that inactivate pathogens.

Additionally, probiotics stimulate the immune system and help in mounting protective response against pathogen interaction with host cells [20]. Mechanisms via which bacteriocins could contribute to probiotic functionality have been reported by Dobson et al. [21]. The bacteriocins may act as colonizing peptides, facilitating the competition of a probiotic with the resident microbiota. They may function as killing peptides, directly eliminating pathogens; or they may serve as signaling peptides, signaling other bacteria or the immune system [21].

5. CONCLUSION

The oral administration of bacteriocinogenic *P. pentosaceus* IO1 strain and its bacteriocin improve the growth performance and modulate intestinal microbiota of the albino rats. Reduction in the enterobacteria count may provide valuable alternatives to the antibiotics for the treatment of animal or human infections. Thus, *P. pentosaceus* IO1 strain may be exploited as a probiotic and protective culture in food industry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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