

THE INHIBITORY EFFECTS OF PEDIOCIN 2292 AGAINST LISTERIA MONOCYTOGENES AND STAPHYLOCOCCUS AUREUS IN TOMATO SAUCE

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ABSTRACT

Pediocin 2292 produced by *Pediococcus acidilactici* NCIM 2292 has been applied separately on tomato sauce inoculated with *S. aureus* MTCC 7443 and *L. monocytogenes* MTCC 839. The initial count of the strains was maintained about 10^4 cfu/g in the food system. The efficacy of usage of pediocin has been examined and the experimental results has also been compared with a chemical preservative namely sodium benzoate. The concentrations of purified pediocin and sodium benzoate applied to tomato sauce were 456 AU/g and 100 µg/g respectively. The bacterial counts have been analyzed during two weeks at different time intervals.

Key words: pediocin 2292, *Pediococcus acidilactici* NCIM 2292, tomato sauce, *Listeria monocytogenes*, *Staphylococcus aureus*.

INTRODUCTION

The application of chemicals preservatives in foods is usually associated with toxicity and harmful effects on human health, immune system in particular [1, 2]. Health conscious consumers have been consistently concerned about possible adverse effects from the presence of chemical additives in their foods. They demand natural and “fresher” foods which are ready to eat, nutrient and vitamin rich, minimally-processed without chemical additives.

Natural antimicrobial substances like bacteriocins secreted by lactic acid bacteria have strong inhibitory activity against undesirable microorganisms include *Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas* and *Salmonella* sp. [3]. The control of *Listeria monocytogenes* and *Staphylococcus aureus* is quite difficult in contaminated vegetables food and canned fruits, especially in tomato products, with a pH of 4.1-5.0, since these organisms have the ability to grow in low pH [4,5].

In particular, pediocin-like bacteriocins have great potential as biopreservatives for food [6,7]. Pediocins produced by different strains of *Pediococcus acidilactici* have been studied and found to be effective against *L. monocytogenes* and *S. aureus* [8,9].

Under the investigation, the inhibitory effect of pediocin 2292 produced by *Pediococcus acidilactici* NCIM 2292 has been evaluated against *Staphylococcus aureus* and *Listeria monocytogenes* in cooked tomato sauce. The antimicrobial efficacy of pediocin 2292 has also been compared with sodium benzoate (Himedia, India).

MATERIALS AND METHODS

Bacterial strains and culture condition

Listeria monocytogenes MTCC 839 and *Staphylococcus aureus* MTCC 7443 used in this study were procured from Microbial Type Culture Collection (MTCC) at Institute of Microbial Technology, Chandigarh, India. Stock cultures of two strains were stored at -4°C on nutrient agar (Himedia, India) slants. Before use in experiments, both organisms were sub-cultured in nutrient broth and incubated at 37°C for 24 h.

Preparation of tomato sauce

Tomato procured from local market was homogenized and ground to a fine pulp in a mixture grinder. The pulp was cooked with appropriate spices and salt. The concentration of salt was 0.02 g/L in the pulp.

Purification of pediocin 2292

Pediocin 2292 produced by *Pediococcus acidilactici* NCIM 2292 was purified by ammonium sulfate precipitation followed by separation with a Superose 12 fast performance liquid chromatography (FPLC) as described in previous article [10].

Application of pediocin in tomato sauce

The sauce was autoclaved and divided into two parts, which were inoculated with 24 h culture of *S. aureus* MTCC 7443 and *L. monocytogenes* MTCC 839 separately. The initial count of two strains was about 10^4 cfu/g. Sodium benzoate and purified pediocin have been mixed up with each part of inoculated tomato sauce at 100 µg/g and 456 AU/g respectively. The sauce was packed in sterilized polyethylene-polyamide pouches and stored at room temperature (30°C). The amount of sauce was 50 g in each sample pouch. The pouches without preservatives have been used as control. The bacterial counts were analyzed at different time intervals (0, 1, 2, 4, 7, 10, 14 days). The sample pouches are shown in Figure 1.

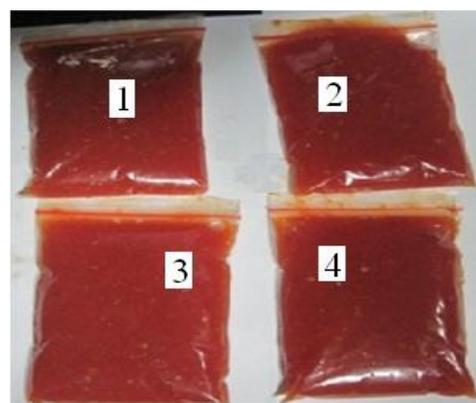


Figure 1: The sealed pouches of tomato sauce inoculated with *S. aureus* (1,2) and *L. monocytogenes* (3, 4).

Analytical Determination

Viable bacterial numbers were estimated by pour plate technique expressed in colony-forming unit (cfu). *L. monocytogenes* and *S. aureus* were grown on nutrient agar (NA) and incubated at 37°C for 24 h.

RESULTS AND DISCUSSION

Evaluation of inhibitory efficacy of pediocin in tomato sauce

Under the study, preservative property of purified pediocin has been compared with chemical additive (sodium benzoate) in tomato sauce using *S. aureus* and *L. monocytogenes* in two separate storage of 14 day. *S. aureus* of 3.64 log cfu/g has been inoculated in tomato sauce added with purified pediocin and sodium benzoate as shown in Figure 2. The sample without preservatives was used as control. After one day storage the log CFU/g of the tomato sauce samples for purified pediocin, sodium benzoate and control were observed to be 2.36, 3.46 and 3.72 respectively. The log CFU/g was found to be 2.11, 2.62 and 5.04 for purified pediocin, sodium benzoate and control respectively after 2 days storage. The values of log CFU/g were declined to 1.87, 2.16 and 5.91 for the similar preservatives and control counted after 4 days. At the end of 7 days the respective values were recorded as 3.15, 2.26 and 6.83. After 10 days storage, the values were 5.04, 3.34 and 7.93 respectively. Experimental results showed that the counts of log CFU/g for the preservatives (purified pediocin and sodium benzoate) were significantly declined during 4 days storage. Although, the counts were increased later, but the growth of *S. aureus* was well controlled comparing the results of control sample used in the study.

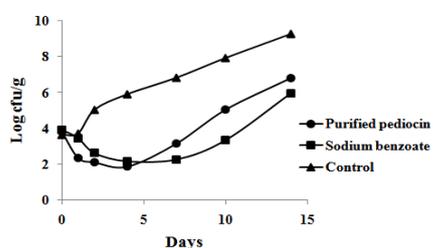


Fig. 2: Growth of *S. aureus* in tomato sauce treated with pediocin and sodium benzoate

To enhance the shelf life of tomato sauce, similar storage study has been conducted using *L. monocytogenes* shown in Figure 3. The initial log CFU/g of the samples has also been maintained about 3.64. Antimicrobial efficacy of purified pediocin and sodium benzoate have also been tested and compared with each other and control having no preservative. The counts of log CFU/g were reduced to 2.61 and 2.18 for purified pediocin and sodium benzoate, where it was increased to 5.42 for control after 4 days. At the end of the 14 days storage the growth of *L. monocytogenes* was significantly controlled comparing with tomato sample without preservative.

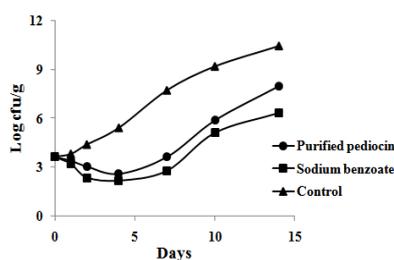


Figure 3: Growth of *L. monocytogenes* in tomato sauce treated with pediocin and sodium benzoate

CONCLUSION

The experimental results proved that pediocin produced by *P. acidilactici* NCIM 2292 is very much effective to control the growth *Staphylococcus aureus* and *Listeria monocytogenes* in cooked tomato sauce with low pH condition. The bacteriocin is able to enhance the shelf life of acidic food products with improving the quality and safety. The potential use of pediocin 2292 as biopreservatives in the food industry could be potential promising alternatives to chemical preservatives.

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