Synergistic Effect of Starch and Royal Jelly Against
*Staphylococcus aureus* and *Escherichia coli*

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Abstract

**Objectives:** To evaluate the synergistic action of starch on the antibacterial activity of royal jelly (RJ), a comparative method of adding RJ with and without starch to culture media was used.

**Methods:** Strains of *Staphylococcus aureus* and *Escherichia coli* have been used to determine the minimum inhibitory concentration (MIC) of a variety of freshly reaped RJ. In a second step, lower concentrations of RJ than the MIC were incubated with a set of concentrations of starch and then were added to media to determine the minimum synergistic inhibitory concentration.

**Results:** The MIC of RJ without starch was 1.7% (vol/vol) and 2% (vol/vol) against *S. aureus* and *E. coli*, respectively. When starch was incubated with RJ and then added to media, a significant MIC drop has been noticed against both strains. This MIC drop was 61% and 30% against *S. aureus* and *E. coli*, respectively.

**Conclusions:** The effectiveness of RJ against bacteria has been extensively reviewed, but this bee product remains unaffordable in most countries. Our findings suggest that combined mixture of RJ and starch could be used to treat infections that are resistant to conventional drugs, at a lower cost.

Introduction

R**oyal jelly** (RJ) consists of an emulsion of proteins, sugars, lipids, and some other substances in a water base. The antibacterial activity of RJ has been described, and one of the proteins in RJ called royalisin possesses antibiotic properties against gram-positive, but not gram-negative, bacteria. Lipids comprise about 5% of the substance. The potency of antibacterial properties of RJ could also be related to a particular fatty acid present in the ether-soluble fraction of royal jelly called *trans*-10-hydroxy decenoic acid. The characterization of novel antibacterial peptides isolated from the RJ (the jelleines; a series of short peptides presenting a broad spectrum of activity against gram-positive and gram-negative bacteria, and also against yeasts) has been reported. This study was carried out to evaluate the antibacterial properties of starch and RJ when used jointly to manage staphylococcal and colibacilli infections. In previous studies, we have shown that there is a synergistic action between honey and starch in terms of antibacterial and antifungal activity. We suggested that amylases present in honey originating from bees and pollen are responsible in the hydrolysis of starch chains to randomly produce dextrin and maltose that increase the osmotic effect of honey and consequently increase the antibacterial activity. Those results suggested that adding starch to media containing honey plays a synergistic role in the antibacterial activity. As RJ also contains amylases, we tried to extrapolate the same protocol we have used with honey to assess the additive action of RJ and starch against bacteria. On the other hand, we are exploring a novel concept that starch, normally a microbial nutrient, may actually enhance the antibacterial properties of RJ. The correlation between the additive action of starch and RJ and the minimum inhibitory concentration (MIC) decrease of both was statistically examined.

Materials and Methods

A variety of freshly reaped RJ was obtained directly from a beekeeper (who attested that no antibiotics had been used during the beekeeping) in central Algeria during 2007. Bacterial strains of *Staphylococcus aureus* and *Escherichia coli* were used as test organisms. *S. aureus* was isolated from a contaminated patisserie after an outbreak of food poisoning in western Algeria, while *E. coli* was isolated from a patient suffering from acute diarrhea. Both strains were maintained by subculture in broth media. Prior to the experiment, the strains were inoculated into nutrient agar media. The inoculum suspensions were obtained by taking five colonies from 24-hour-old cultures. The colonies were suspended in

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5 mL of sterile saline (0.85% NaCl). The inoculum suspensions were shaken for 15 seconds, and the density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to 1–5 × 10^6 colony-forming units/mL) with sterile saline. The suspensions were diluted 1:1000 in RPMI 1640 medium to give a final inoculum suspension equivalent to 0.5–2.5 × 10^3 colony-forming units/mL. Concentrations of RJ between 1% and 5% (vol/vol) were incorporated into Mueller-Hinton agar media to test its efficiency against S. aureus. The final volume of RJ and media in each plate was 5 mL. The plates were inoculated and incubated at 37 °C for 24 hours. The MIC was determined by finding the plates with the lowest concentration of RJ on which the strain would not grow. In a second step and to evaluate the effect of starch on the antibacterial action of RJ, a 10% starch solution was prepared using sterile water. Different volumes from the stock solution were added to a range of RJ concentrations below the MIC. The same volume of starch solution that gave inhibition with RJ is added alone to media as control. The final volume in each plate was 5 mL. Plates were inoculated and incubated at 37 °C for 24 hours. All inoculations were carried out in triplicates. Isobolographic analysis was carried out to measure the additive action of honey and RJ against the tested bacteria using Statistica® software (StatSoft, Tulsa, OK). To create the isobologram, the 50% effect level was used.

Results

RJ has been effective against both tested strains, and the MIC without starch was 1.7% (vol/vol) and 2% (vol/vol) against S. aureus and E. coli, respectively. When starch was incubated with RJ and then added to media, a significant MIC drop has been noticed against both strains. This MIC drop was 61% and 30% against S. aureus and E. coli, respectively. The isobolograms (Figs. 1 and 2) show an additive action between starch and RJ against both strains. No inhibition has been noticed on control media containing starch alone.

Discussion

Natural products are gaining interest in the medical field, and the emergence of antibiotic-resistant strains of bacteria has confounded the current use of antibiotic therapy, leading to the re-examination of former remedies. In the present study, RJ has shown a strong antibacterial activity. Adding starch to RJ had led to a significant MIC drop, especially with S. aureus. The mechanism of this additive action remains unclear. Further studies are needed to elucidate and optimize the effective combination of these natural products in clinical practice. The effectiveness of RJ against bacteria has been extensively reviewed, but this bee product remains unaffordable in most countries. Our findings suggest that a combined mixture of RJ and starch could be used to treat infections that are resistant to conventional drugs, at a lower cost. However, their use in professional care centers should be limited to those with safe and certified antibacterial activities.

Disclosure Statement

The authors state that no competing financial interests exist.

References


