Identifying Indigenous Bacteria Producing Antimicrobial Agents in Some Spices

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According to the recent scientific researches on chemical antimicrobial agents, it was reported that these agents have several negative effects. In addition to that, as a result of common use of synthetic antimicrobial agents, the resistance mechanisms of pathogenic bacteria were enhanced.
Also, the interest in natural foods and food products produced by using natural components increased among consumers. This situation gave acceleration in researces about new antimicrobial agents.
The present research was carried out with the aim of determining the microorganisms in spices being able to produce natural antimicrobial agents which can substitute for synthetic antimicrobial agents.
For this research, 33 different types of spices were supplied from a herbalist in Kayseri and they were analyzed for the microorganisms that produce antimicrobial agents. The spices were individually homogenized in sterile pepton water and diluted serially. Then, the samples were plated on four different agar medium (Nutrient Agar, Tryptic Soy Agar, Brain Heart Agar, Plate Count Agar) and incubated at 30±1°C for 48 hours.
Different colonies were chosen from the petri dishes for the isolation of microorganisms produce antimicrobial agents. The chosen colonies were plated on the soft Tryptic Soy Agar which contains the test microorganisms, *Listeria innocua*, *Micrococcus luteus*, *Escherichia coli* and *Saccharomyces cerevisiae*. They were incubated at 37±1°C for 24 hours. At the end of the incubation the zones and the activities against these microorganisms were determined. Then, the metabolites obtained from the isolates which show antimicrobial activity were inoculated on the soft TSA contains the test microorganisms and incubated at 37±1°C for 24 hours. The incubation area was observed and clear zone formation was accepted that the bacteria can synthesize antimicrobial agents.
Characterisation of the microorganisms produce antimicrobial agents was performed by using PCR based methods according to 16S rRNA gene region. For this purpose, first bacteria DNAs were isolated and amplified by using thermal cycler. Then the PCR products were observed through agarose gel The PCR products were purified and sent for sequence analysis. At the end of the analysis, the DNA sequences and the datas of genbank were compared the bakteria isolates were identified.
As a result of genotypic identification and antimicrobial tests, total of 19 bacteria [*Listeria innocua* (4), *Micrococcus luteus* (9), and *Escherichia coli* (6)] having antimicrobial effect were determined. As a result of confirming the antimicrobial effect by extraction method, the antimicrobial effect of total 14 samples on *L. innocua* (4 samples), and *M. luteus* (4 samples), *E.coli* (3 samples), and both *L. innocua*, and *M. luteus* (3 samples) were determined. In this study, a microorganism having antimicrobial effect on both Gram negative and Gram positive bacteria were not determined. Total 7 of bacteria isolates having the antimicrobial effect were genotypically determined. As a result of genotypic identification of the isolates, 6 isolates were forming endospores including *Bacillus* sp. (4 isolates) and *Paenibacillus* sp. (2 isolates), and remaining 1 isolate was determined as *Micrococcus* sp.
In this research, the most common isolates which show antimicrobial effects were determined

to be *Bacillus* sp., *Paenibacillus* sp. and *Micrococcus* sp. In the previous studies, it was already found that some strains of *Bacillus* sp., and *Paenibacillus* sp. have antimicrobial activity in harmony with the present study. Yet, there is no study encountered about *Micrococcus* sp. Therefore, the metabolite obtained from this bacteria is thought to be an antimicrobial agent which has not been identified before.
In conclusion, it might be suggested that the spices may have some bacteria that producing antimicrobial agents need to be studied in more detail.

Keywords: Antimicrobial, Bacillus sp., Micrococcus sp., Paenibacillus sp., PCR, Spices