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Antimicrobial Activity of Bacterial Isolates from Honey

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Abstract: The antimicrobial properties of honey have been known for thousands of years. In Egypt, the honey has been consumed as a medicinal product due to its high level of antimicrobial activity. The objective of this study was to examine the honey microflora for the production of antimicrobial substances that may be responsible for the unidentified antimicrobial activity observed with select honeys. Many bacterial strains isolated from six domestic honeys were screened for production of antimicrobial compounds against some Gram positive and Gram negative bacteria. Some of the isolated bacteria exhibited antimicrobial activity against at least one of the tested microorganisms (*Staphylococcus aureus*, *Micrococcus luteus*, *Bordetella bronchiseptica* and *Klebsiella pneumoniae*). The most effective strain of the all isolated bacteria from honey was identified as *Bacillus brevis*. The high rate of antimicrobial activity exhibited by the bacterial strains isolated from different honey sources could provide potential sources of novel antimicrobial compounds.

Key words: *Bacillus* · Antibiotic · Microorganisms

INTRODUCTION

Honey is the natural sweet substance produced by honey bees from nectar or blossoms or from the secretion of living parts of plants or excretions of plants, which honey bees collect, transform and combine with specific substances of their own to ripen and mature [1].

The antimicrobial properties of honey have been known for thousands of years [2]. Honey has been studied to clarify which components are responsible for antagonistic activity against pathogenic microorganisms including *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [3, 4]. The antimicrobial activity of honey has been attributed to hydrogen peroxide, osmolarity, acidity, aromatic acids and phenolic compounds [5, 6]. The high osmolarity of honey is due to the high content of sugar (average over 85% of honey) including fructose, glucose, maltose, sucrose and other types of carbohydrates [7]. Hydrogen peroxide produced by the oxidation of glucose by the enzyme glucose-oxidase, which is activated by successive dilutions of honey [8].

These intrinsic properties of honey affect the growth and survival of microorganisms by bacteriostatic or bactericidal action and, in particular, the low pH and high sugar content of undiluted honeys prevent the growth of many species of microorganisms. In consequence, honey can be expected to contain a small number and a limited variety of microorganisms [9].

Microorganisms that survive in honey are those that withstand the concentrated sugar, acidity and other antimicrobial characters of honey. The primary sources of microbial contamination are likely to include pollen, the digestive tracts of honeybees, dirt, dust, air and flowers. Microbes found in honeycomb are principally bacteria and yeast and come from the bees, the raw materials (nectar) or from external sources [10]. Larvae may be sterile initially, but they are fed nectar and pollen by workers and therefore subject to inoculation by the nectar, pollen and workers flora before pupation [11].

Microorganisms commonly existing in honey have been investigated and shown to contain mold, yeast and spore-forming bacteria, but vegetative cells of pathogenic bacteria were not detected [10].

Many bacterial isolates from honeybee larvae were identified and evaluated to determine their antibacterial activity against *Paenibacillus larvae* subsp. *larvae*, the causative agent of American Foulbrood disease, which kills honeybee larvae and pupae, but does not affect adult honeybees [12, 13].

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The present investigation was carried out to isolate bacterial strains from honey which exhibiting antimicrobial activity against some Gram positive and Gram negative microorganisms.

MATERIALS AND METHODS

Honey Samples: The study was carried out with 10 unpasteurized polyfloral honey samples [Egyptian clover (Berseem)] harvested directly from apiaries from different geographical locations. All of the collected honeys were preserved at -20°C upon arrival and thawed completely at room temperature prior to testing.

Isolation of Bacterial Strains from Honeys: One gram of each honey sample was added to 1ml of sterile deionized water to make a 50% (w/v) honey solution. One hundred microliters of each 50% (w/v) honey solution was spread on tryptic soy agar (TSA; Oxoid) plates and incubated at 37°C for 24 h. The bacterial isolates were streaked on TSA plates and preserved at 4°C for further study.

Antimicrobial Activity Assay of Bacterial Isolates: The antimicrobial activity of bacterial isolates was carried out by the well-diffusion assay [14]. Cell-free supernatants (CFSs) corresponding to the different isolated bacteria were analyzed. CFSs were obtained from these bacteria cultured in tryptic soy broth (TSB, Oxoid) for 24 h at 37°C without shaking. They were centrifuged (10,000 xg for 10 min at 4°C), filter-sterilized (0.45 μm pore size cellulose acetate filter) and kept at 4°C until use.

Cultures of *Staphylococcus aureus* (ATCC 29737), *Micrococcus luteus* (ATCC 10240), *Bordetella bronchiseptica* (ATCC 4617) and *Klebsiella pneumoniae* (ATCC 10031) grown in TSB at 37°C for 24 h were added to TSB and immediately poured on plates. The plates were stored at 4°C for 2 h before being used and wells (8 mm diameter) were cut in the agar.

A 100 μl aliquot of each CFS sample was added to each well. Cultures were incubated at 37°C for 24 h. Antibacterial activity was assessed by measuring the size of the zones of inhibition surrounding wells.

Identification of the Most Producer Strain: The following tests were performed to identify the strains: Gram and spore stains, light microscopy observation, mobility, Voges-Proskauer test and catalase tests, nitrate reduction, growth at different incubation temperatures and NaCl concentrations, starch hydrolysis and use of glucose, xylose and mannitol [15].

RESULTS AND DISCUSSION

In this study we attempted to isolate bacterial strains from honey that produce a broad-spectrum antimicrobial activity, which would be highly applicable to the industry.

Total of 27 bacterial strains were isolated from 10 unpasteurized polyfloral honey samples harvested directly from apiaries from different geographical locations (Table 1). All these strains were tested for their capability to produce antimicrobial activity.

Screening for antimicrobial activity-producing bacteria isolated from honey samples allowed the selection of BH3c strain. In fact, this strain exhibited a broad antagonistic activity spectrum by the agar diffusion assay and showed highest level of antimicrobial activity against clinical isolates of *Staphylococcus aureus*, *Micrococcus luteus*, *Bordetella bronchiseptica* and *Klebsiella pneumoniae* (Table 2).

The identification of this strain through phenotypical and biochemical characteristics showed that this isolate is identified as *Bacillus brevis*. It is a Gram-positive bacilli, aerobic, grow at 7% concentration of NaCl, motile spore-forming organism, hydrolyze starch, catalase positive, oxidase-negative, amylase negative, casein negative, gelatinase positives, indole negative, Voges-Proskauer negative and utilize glucose, xylose and mannitol.

The genus *Bacillus* species are ubiquitous, endosporeforming Gram-positive bacteria that are harmless to mammalians, with the exception of Bacillus cereus and Bacillus anthracis. Their primary habitat is the soil and, due to their extreme heat-resistant spores, they can colonize different environments such as honey, food, animals, insects, etc. [16, 17].

Table 1: Number of bacteria isolated from different honey samples

Honey samples	Number of isolates
BH1	4
BH2	2
BH3	5
BH4	3
BH5	3
BH6	2
BH7	1
BH8	2
BH9	1
BH10	4

Table 2: Antimicrobial activity assay of bacterial isolates from honey against some pathogenic bacteria

	Diameter of inhibition zone in mm			
Bacterial isolates	*Micrococcus luteus*	*Staphylococcus aureus*	*Bordetella bronchiseptica*	*Klebsiella pneumoniae*
BH1a	12	13	11	11
BH1b	-	-	-	-
BH1c	-	-	-	-
BH1d	-	-	-	-
BH2a	12	13	12	13
BH2b	11	11	12	12
BH3a	-	-	-	-
BH3b	-	-	-	-
BH3c	20	25	28	27
BH3d	-	-	-	-
BH3e	17	17	19	18
BH4a	14	13	16	16
BH4b	12	11	13	15
BH4c	12	11	14	15
BH5a	-	-	-	-
BH5b	15	11	12	13
BH5c	12	12	12	13
BH6a	13	16	17	20
BH6b	18	20	18	21
BH7a	-	-	-	-
BH8a	13	13	11	12
BH8b	14	17	12	11
BH9a	12	11	15	15
BH10a	18	18	17	18
BH10b	-	-	-	-
BH10c	14	15	16	17
BH10d	15	14	16	12

Bacillus species have been frequently isolated from apiarian sources and have been reported to be effective biocontrol agents [18-21] by producing a wide variety of secondary metabolites such as antibiotics, bioinsecticides, enzymes and lipopeptides with anti-metabolic and pharmacological activities [22-25].

In conclusion, the bacterial strain BH3c was isolated and identified as *Bacillus brevis* from honey and exhibited large antimicrobial activity against some Gram positive and Gram negative bacteria. Additional investigations should be performed to purify at homogeneity the bioactive compounds of this bacterial strain in order to establish their exact chemical structures with special interest in optimizing their production. In addition, further screening of the antimicrobial activity produced by bacterial strains from honey against multi-drug resistant human pathogens may expand the application of these antimicrobial compounds to clinical applications.

REFERENCES

1. Codex A limentarius Commission, 1989. Codex Standards for sugar (honey). Supplement 2 to codex Alimentarius Volume 111. Food and Agriculture Organisation of the United Nations and World Health Organisation, Rome.
2. Zumla, A. and A. Lulat, 1989. Honey: a remedy rediscovered. J. the Royal Society of Medicine, 82: 384-385.
3. Cooper, R.A., P.C. Molan and K.G. Harding, 2002. The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. J. Appl. Microbiol., 93: 857-863.
4. Willix, D.J., P.C. Molan and C.G. Harfoot, 1992. A comparison of the sensitivity of wound infecting species of bacteria to the antibacterial activity of manuka honey and other honey. J. Appl. Bacteriol., 73: 388-394.

5. Molan, P.C., 1992a. The antibacterial activity of honey: 1. The nature of the antibacterial activity. Bee World, 73: 5-28.
6. Molan, P.C., 1992b. The antibacterial activity of honey: 2. Variation in the potency of the antibacterial activity. Bee World, 73: 59-76.
7. White, Jr. J.W., 1975. Composition of honey. In: E. Crane, (Ed.), Honey: a Comprehensive Survey. Crane, Russak and Company, Inc., New York, pp: 157-206.
8. White, Jr. J.W., M.H. Subers and A.I. Schepartz, 1963. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. Biochimica et Biophysica Acta, 73: 57-70.
9. Peter, B.O., E.A. Olufemi and O.O. Iyabo, 2007. Honey: a reservoir for microorganisms and an inhibitory agent for microbes. African Health Sci., 7(3): 159-165.
10. Snowdon, J.A. and D.O. Cliver, 1996. Microorganisms in honey. International J. Food Microbiol., 31: 1-26.
11. Lee, C.Y. and R.W. Kime, 1984. The use of honey for clarifying apple juice. J. Apic. Res., 23: 45-49.
12. Evans, J.D. and T.N. Armstrong, 2005. Inhibition of the American foulbrood bacterium, *Paenibacillus larvae* subsp. *larvae*, by bacteria isolated from honey bees. J. Apic. Res., 44: 168-171.
13. Evans, J.D. and T.N. Armstrong, 2006. Antagonistic interactions between honey bee bacterial symbionts and implications for disease. BMC Ecol., 6: 4.
14. Audisio, M.C., H.R. Terzolo and M.C. Apella, 2005. Bacteriocin from honeybee beebread *Enterococcus avium* active against *Listeria monocytogenes*. Applied and Environmental Microbiol., 71: 3373-3375.
15. Gibson, T. and R.E. Gordon, 1986. Bergey's Manual of Determinative Bacteriology. Baltimore: The Williams and Wilkins Co.
16. Sanders, M.E., L. Morelli and T.A. Tompkins, 2003. Sporeformers as human probiotics: *Bacillus*, *Sporolactobacillus* and *Brevibacillus*. Comprehensive Reviews in Food Science and Food Safety, 2: 101-110.
17. Urdaci, M.C., P. Bressollier and I. Pinchuk, 2004. *Bacillus clausii* Probiotic Strains: Antimicrobial and immunomodulatory activities. J. Clinical Gastroenterol., 38: S86-s90.
18. El-Ghaouth, A., 1997. Biologically-based alternatives to synthetic fungicides for the control of postharvest diseases. J. Industrial Microbiol. and Biotechnol., 19: 160-162.
19. Tsuge, K., T. Akiyama and M. Shoda, 2001. Cloning, sequencing and characterization of the iturin a operon. J. Bacteriol., 183: 6265-6273.
20. Reva, O.N., C. Dixelius, J. Meijer and F.G. Priest, 2004. Taxonomic characterization and plant colonizing abilities of some bacteria related to *Bacillus amyloliquefaciens* and *Bacillus subtilis*. FEMS Microbiol. Ecol., 48: 249-259.
21. Romero, D., M.E. Pe´rez-Garcı´a Rivera, F.M. Cazoela and A. Vicente, 2004. Isolation and evaluation of antagonistic bacteria towards the cucurbit powdery milder fungus *Podospheara fusca*. Applied Microbiol. and Biotechnol., 64: 263-269.
22. Desai, J.D. and I.M. Banat, 1997. Microbial production of surfactant and their commercial potential. Microbiology and Molecular Biology Reviews, 61: 47-64.
23. Healy, M.G., C.M. Devine and R. Murphy, 1996. Microbial production of biosurfactants. Resources, Conservation and Recycling, 18: 41-57.
24. Katz, E. and A.L. Demain, 1977. The peptide antibiotics of *Bacillus*: chemistry, biogenesis and possible functions. Bacteriological Reviews, 41: 449-474.
25. Phister, T.G., D.J. O'Sullivan and L.L. McKay, 2004. Identification of bacilysin, chlorotetaine and iturin a produced by *Bacillus* sp. Strain CS93 isolated from pozol, a Mexican fermented maize dough. Applied and Environmental Microbiol., 70: 631-634.