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Susceptibility of Human Pathogenic Bacteria to Antimicrobial Peptides from Sesame Kernels

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Abstract. Hospital infection caused by Gram-negative bacteria is a serious and common problem, especially in developing countries. Aiming to reduce these infections, this report focuses on the identification and characterization of novel antimicrobial peptides from sesame (*Sesamum indicum*) kernel meals. Thus, sesame flour was extracted and precipitated with ammonium sulfate (100%). After dialysis, a rich fraction was applied to affinity red-Sepharose CL-6B chromatography, followed by reversed-phase high-performance liquid chromatography. Mass spectrometry analysis indicated the presence of a major peptide with molecular mass of ~5.8 kDa in both cultivars. The bactericidal activities of antimicrobial peptides were evaluated against several human pathogens that had been effective only against *Klebsiella* sp., a Gram-negative bacterium responsible for human urinary infection. These data indicate the biotechnological potential of sesame peptides as an alternative method for hospital infection control and also the decrease of bacterial resistance to synthetic antibiotics.

Sesame (*Sesamum indicum* L.) is an older oleaginous kernel that has been commonly used by several nations as a source of food and medicines [1–3]. Including sesame, all living organisms are able to develop self-defense mechanisms against pathogen attack. Among these defenses, were included important compounds such as phenols, secondary compounds, and antimicrobial peptides (AMPs) [4]. Antimicrobial peptides have been isolated from insects [5, 6], mammals [7], microorganisms [8], and several plant tissues such as leaves, flowers, and seeds [4]. In the large AMP class, several families are included, such as defensins [9], 2S albumins [10], vicilins [11], and several others. All of them contribute to plant innate host defense and represent an important source for antibiotics. Most of these peptides show the ability to bind on lipids of the cell surface, to induce cell permeability, and to cause enhanced lethality to pathogenic bacteria [12–14].

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On the other side, an uncontrolled increase of pathogens with antibiotic resistance, including several Gram-negative bacteria such as *Escherichia coli*, *Klebsiella* sp., and *Proteus* sp., is the cause of a serious problem known as hospital infection [15]. These lethal infections, commonly observed in developing countries, have led to a search for new strategies to control these infectious agents. A primary search has been focused on AMPs, since these peptides have shown high activity against pathogens but low toxicity against mammalian cells [14, 16]. In this report, cationic antimicrobial peptides from *S. indicum* kernels of black and white cultivars (AMP-SiB and AMP-SiW) were purified and characterized, and their ability to control human pathogenic Gram-negative was evaluated.

Materials and Methods

Extraction and Isolation of *S. indicum* Kernels Peptides. Kernels of two sesame (*S. indicum*) cultivars (black and white) were evaluated. Seeds were washed with sanitary water (30% solution) for 5 min

followed by distilled water for another 5 min. Sesame kernel meals were extracted with a solution containing 0.6M NaCl and 0.1% HCl, in a proportion of 1:3 (w/v), for 6 h at 4°C. This crude extract was centrifuged at 4000*g* for 2 h at 4°C. Proteins in the supernatant were precipitated with $(NH_4)_2SO_4$ at 100% saturation with constant stirring. After centrifugation (under equal conditions), the precipitate was resuspended and dialyzed (3.0 kDa cut off) against distilled water. A rich fraction was lyophilized and 5 mg of soluble protein was applied onto affinity red-Sepharose CL-6B chromatography, equilibrated with buffer A (0.1M Tris-HCl, pH 7.0, containing 0.05M $CaCl_2$). Nonretained proteins (NRP) were displaced with the same buffer. Retained proteins (RP) were eluted with buffer B (0.1M Tris-HCl, pH 7.0, containing 3.0M NaCl). Protein elution was analyzed at 280 nm. RP was dialyzed, lyophilized, resuspended with 0.1% trifluoroacetic acid (TFA), and applied to analytical reversed-phase high-performance liquid chromatography (HPLC) (Vydac C-18TP 522) where the retained fraction was eluted using a linear gradient of acetonitrile (0–100%) at a flow rate of 1.0 mL/min.

Molecular Mass Analyses. Protein fractions were analyzed by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) according Laemmli [17]. Gels were silver stained and bromophenol blue was used as the tracking dye. AMP-Si was also submitted to mass spectrometry analyses according Franco et al. [9].

Bioassays. *Klebsiella* sp., *Proteus* sp., and *E. coli* were challenged by use of 1.0 mL of Luria–Bertani (LB) broth (10 g/L NaCl, 5 g/L yeast extract, and 45 g/L bactopeptone). The bacteria were grown in LB broth for 18–24 h at 37°C before starting peptide evaluation. Distilled water was used as the negative control and 40 μg/mL chloranphenicol was used as the positive control. The protein fractions eluted from reversed-phase chromatography were incubated with Gram-negative bacteria on LB broth for 4 h at 37°C at standard concentrations of 10, 25, 50, and 100 μg/mL. Evaluation of bacteria growth was done by spectrophotometric analyses at 595 nm, for each experimental hour. Each experiment was carried out in triplicate.

Results

Purification of AMP-Sis. In order to identify novel antimicrobial peptides in sesame kernels, crude extracts from both cultivars were challenged against human pathogenic bacteria. After enrichment, these fractions were applied to affinity Red-Shepharose CL-6B chromatography (Fig. 1A), which is commonly utilized to purify cationic and hydrophobic peptides [10, 18, 19]. Only retained protein fractions from white and black cultivars were capable of reducing *Proteus* sp. and *Klebsiella* sp. growths (data not shown). After dialyses, RP was submitted to a reversed-phase HPLC (Figs. 1B and 1C). The white sesame cultivar showed a major peak at 18% acetonitrile, whereas the black sesame cultivar presented antibacterial proteins eluted in a major peak of 23% acetonitrile.

Molecular Mass Analysis. The SDS-PAGE analyses of HPLC major peaks showed single bands for both cultivars that correspond to proteins with ~5 kDa (data not shown). This result was confirmed by mass

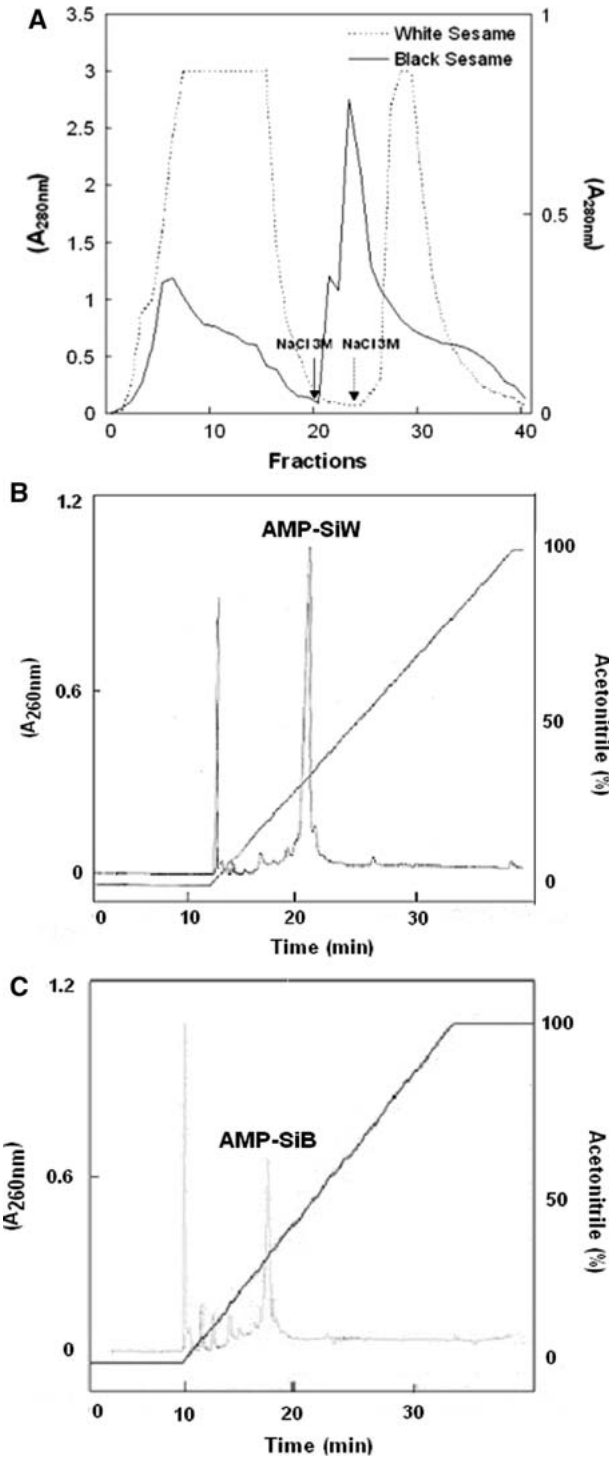

Fig. 1. **A** Red-Sepharose chromatographic profiles of white (dotted line) and black (solid line) sesame kernels. NRP corresponds to the nonretained peak and RP to the retained one. Black arrows indicate a single elution step of 3.0M NaCl. Therefore, white and black RPs were applied to a reversed-phase HPLC-generating chromatographic -profiles (**B**) and (**C**), respectively. Diagonal lines indicate acetonitrile gradient elution (0–100%).

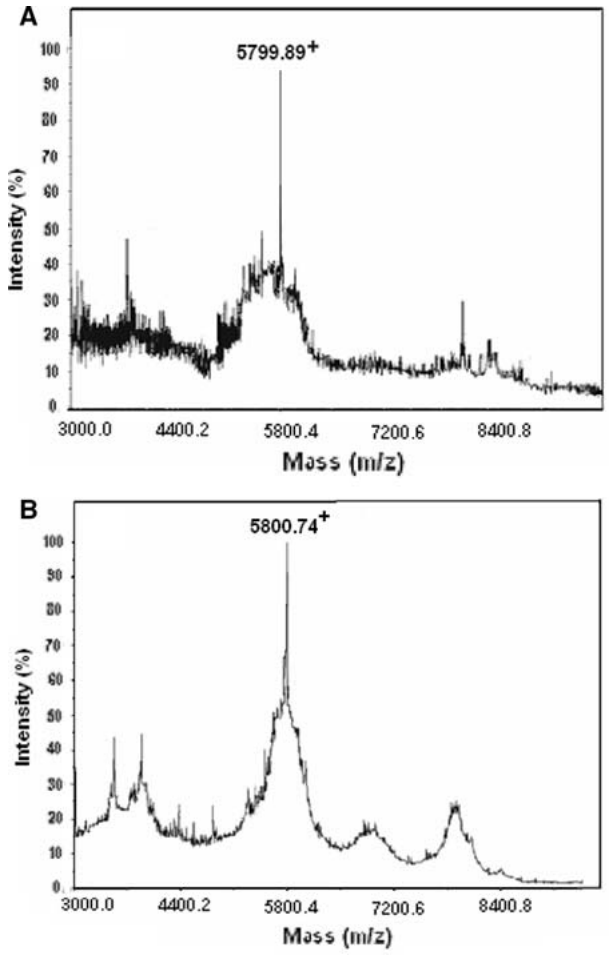

Fig. 2. Matrix assisted laser desorption/ionization time-flight spectra of AMP-Sis from white (**A**) and (**B**) black cultivars.

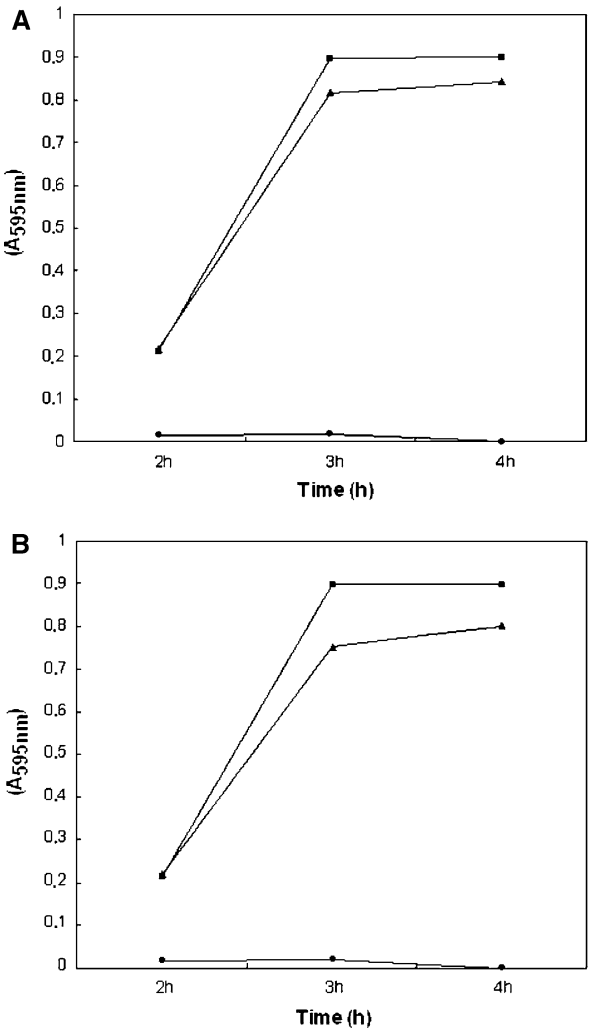

Fig. 3. Antimicrobial activities of AMP-Sis from white (AMP-SiW) (**A**) and black (AMP-SiB) (**B**) sesame kernels are indicated by black triangles. Black squares correspond to the negative control (distilled water) and black circles correspond to the positive control (chloranphenicol, 40 μg/mL). Vertical bars correspond to the standard deviation. Each assay was carried out in triplicate.

spectrometric analysis, where was observed two peptides of 5799.69 and 5800.74 Daltons, respectively, from black and white cultivars (Figs. 2A and 2B) were observed, denoted AMP-SiB and AMP-SiW, respectively.

Biological Activity. Crude extract was evaluated against the three Gram-negative bacteria previously described, at a standard concentration of 100 μg/mL. However, this extract was only active against *Klebsiella* sp., being capable of reducing their development by 50% (data not shown). Moreover, purified peptides obtained from HPLC fractions were compared against Gram-negative bacteria. AMP-SiW and AMP-SiB were capable of inhibiting ~25% of *Klebsiella* sp. growth (Figs. 3A and 3B) at concentration of 100 μg/mL. Nevertheless, at lower concentrations (10, 25, and 50 μg/mL) no activity was observed. Furthermore, no deleterious activity of purified peptides was observed against *Proteus* sp. and *E. coli* (data not shown).

Discussion

In spite of the sesame peptides reported here being unpublished, similar biochemical properties have been found in other bactericidal peptides. Among them we could observe similarities in the acetonitrile concentrations elution, as in the isolation of antimicrobial peptides from Japanese bamboo shoots (*Phyllostachys pubenscens*) [20], bulbs of the tulip (*Tulipa gesneriana* L.) [21], buckwheat seeds (*Fagopyrum esculentum* M.) [22], passion fruit seeds (*Passiflora edulis*) [10], cowpea seeds (*Vigna unguiculata*) [9–19], macadamia nut kernels (*Macadamia integrifolia*) [11], and radish seeds (*Raphanus sativus* L.) [23]. Also, molecular masses

were similar when compared to Cp-thionins from *V. unguiculata* seeds [9, 19] and 2S albumins from *P. edulis* [10]. Both classes show antimicrobial properties. AMP-Sis masses are similar to molecular weights observed in different antimicrobial peptide classes. For this reason, at this moment and using this parameter, it is impossible to classify them correctly. Further sequencing experiments will be conduced in order to elucidate this specific property.

Furthermore, AMP-SiB and AMP-SiW showed inhibitory activity against *Klebsiella* sp. Similar results were obtained by using antimicrobial peptides from different plant sources, such as cowpea seeds [9]. In this study, it was shown that cowpea peptide was active against both Gram-positive and Gram-negative bacteria [9]. Otherwise, several AMPs showed inhibitory activity against a wide variety of pathogenic micro-organisms, which included fungi, bacteria, and protozoa, indicating the enormous biotechnological potential of AMPs [10, 13, 21–23]. Moreover, it was observed that the expression of an antimicrobial peptide from motherwort (*Leonurus japonicus* Houtt) was able to promote disease resistance in tobacco [24].

Hence, our results showed severe growth inhibition of the human pathogen *Klebsiella* sp., a bacterium responsible for urinary and lung infections in weakened hospital patients. These data suggest that the development of new drugs from AMPs might be successful for the treatment of several infections, reducing hospital costs and also human mortality. The results observed here are quite encouraging, when compared with other AMPs with activities against Gram-negative bacteria. In addition, this research indicated the remarkable potential of the unexploited genetic resources of plant biodiversity. The discovery of valuable genes in plant biodiversity might also be an alternative for countries in development which have biotechnological research but have difficulties accessing novel drugs due to elevated costs and also patent restrictions.

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