STUDY ON ANALYSIS OF ANTIBIOTIC COMPOUNDS FROM ENTHOMOPATHOGENIC BACTERIA BY FT-IR

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Entomopathogenic bacteria produce antibiotic molecules effective against plant, animal and human plant pathogenic bacteria. They produce broad-spectrum antibiotics, which can be applied in several different fields where suppression of microbes is needed. These antibiotic molecules have different chemical structure such as peptides. Analysis and identification of these molecules provide useful ways in the research and development of drugs and agrochemicals.

Keywords: entomopathogenic bacteria, antimicrobial activity, peptides, analysis

Introduction

Insect pathogenic or entomopathogenic nematodes (EPN) and their symbiotic entomopathogenic bacteria (EPB) can be used as microbial control agents against agricultural insect pests [1]. These nematodes of *Heterorhabditis* and *Steinernema* species are symbiotically associated with the members of the bacteria family *Enterobacteriaceae* as *Photorhabdus* and *Xenorhabdus* species, respectively [2–3]. The EPB have several special functions in this symbiotic relationship. One of them is the production ability of broad-spectrum antibiotics, which keep monoxenic conditions in insect cadavers in soil [4].

Antibacterial resistance is increasing worldwide. The compounds produced by EPN symbiotic bacteria have showed a wide range of bioactivities of medicinal and agricultural interest, such as antibiotic, antymycotic and insecticidal effects [5]. These antimicrobial peptides have been successfully applied in pharmaceutics, plant disease control and many other fields [6]. Antimicrobial peptides have many beneficial characteristics, such as broad-spectrum antibiotic activity, thermal stability, and low molecular weight, and most significantly, compared with most antibiotics, they are not easy to lead to the development of resistance in the target [7]. These compounds were reported as showing in vitro activity against Gram-positive bacteria, including for example the multi-drug resistant strain of *Staphylococcus aureus*. They have diverse chemical structures including peptides as well [5].

Analytical study of these molecules can be achieved in different ways. Edman degradation, developed by Pehr Edman [8], is a method of sequencing amino acids in a peptide. In this method, the amino-terminal residue is labeled and cleaved from the peptide without disrupting the peptide bonds between other amino acid residues. A major drawback to this technique is that the peptides being sequenced in this manner cannot have more than 50 to 60 residues (and in practice, under 30). The peptide length is limited due to the cyclical derivatization not always going to completion. The infrared spectrum of a protein provides a wealth of information on structure and environment of the protein backbone and the amino acid side chains [9]. This makes infrared spectroscopy an extremely useful tool for the investigation of protein structure. The absorption of a side chain in a protein may deviate significantly from their absorption in solution or in crystal. The special environment provided by a protein is able to modulate the electron density and the polarity of bonds, thus changing the vibrational frequency and the absorption coefficient. Therefore, the band positions given in reviews should be regarded only as guidelines for interpretation of spectra [10].

In this study, we aimed the isolation and analysis of some peptide-type antibiotic compounds from two EPB strains.
Material and methods

Antibacterial peptide-producing entomopathogenic bacteria were *Xenorhabdus budapestensis* (EMA) and *Xenorhabdus szentirmaii* (EMC). They were cultured in Luria Broth (LB and LBA) liquid and solid media as previously described [11].

Cell-free culture media (CFCM) were prepared as follows: aliquots of the stock culture were added separately into 900 mL sterile medium. The flasks were incubated in a shaker at 200 rpm and 30°C for 24 h and centrifuged at 13 000 rpm (10 000 g for 30'). After centrifugation the supernatant was filtrated through Express Plus filter (0.22 µm) (Merck Millipore).

Purification of antimicrobial compounds: 1000 mL of CFCM was mixed with activated Amberlite XAD polymeric adsorbent in a 1:20 ratio and incubated for 24 h. The resin-CFCM mixture was filtered through Millipore Express Plus filter of 0.22 µm pore-size and washed subsequently with 200 ml of distilled water, 200-200 ml of 25 V/V%, 50 V/V% and 80 V/V% methanol (MeOH), removing all inactive compounds by this way. After this the resin was washed with 200 ml cc. MeOH:HCl (99:1) and 200 ml i-propanol (iPrOH) to eluate biologically active compounds. These samples were evaporated by vacuum distillation and resulted samples with MeOH and iPrOH, respectively.

For the analysis of the purified antimicrobial compounds a Thermo Nicolet AVATAR FT-IR-330 FT-IR apparatus was used to determine FT-IR spectra and a Hitachi U-2910 UV-Vis spectrophotometer was used to form the UV spectra. Solid samples were prepared for the IR: some quantity of samples were ground with purified KBr and this mixtures were pressed in a mechanical press to form a transparent pellet through which the beam of the spectrometer can pass. Resolution: 2.000 cm⁻¹, Scans: 16.

Results and discussion

The most precise method for the protein and peptide identification after their separation by high-performance liquid chromatography (HPLC) is mass spectrometry [12]. However, this method is quite expensive. Absorbance at low wavelength (<220 nm) detects peptide bonds and amino acid residues with detection limits between nanomoles and picomoles. Peptides having aromatic residues (phenylalanine (Phe), tyrosine (Tyr), and trypthophan (Trp)) can be detected at 254 or/and 280 nm. For this reason we made a UV spectra of the purified antibiotic activity compounds. Results of these measurements are shown in Table 1.

**Table 1:** UV absorbance (nm) of the antibiotic compounds

<table>
<thead>
<tr>
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<th>EMA</th>
<th>EMC</th>
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<tr>
<td>iPrOH</td>
<td>201</td>
<td>200</td>
</tr>
<tr>
<td>MeOH</td>
<td>203</td>
<td>202</td>
</tr>
<tr>
<td>iPrOH</td>
<td>213</td>
<td>-</td>
</tr>
<tr>
<td>MeOH</td>
<td>218</td>
<td>217</td>
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</table>

IR spectra were made with the above described method. Results of these measurements are shown in Figures 1–4.

![Figure 1: Antibiotic compounds purified with iPrOH from EMA](image1)

![Figure 2: Antibiotic compounds purified with MeOH from EMA](image2)

Figure 3: Antibiotic compounds purified with iPrOH from EMC

In the case of amino acids only two side chain moieties absorb in spectral regions that are free from overlapping absorption by other groups and thus allow the spectroscopists an unambiguous assignment without further experiments. These are the SH group of cysteine (2550–2600 cm⁻¹) and the carbonyl group of protonated carboxyl groups (1710–1790 cm⁻¹). These groups were obtained in none of the IR spectra.
All other side chain absorption overlap with the absorption of other side chains or of the polypeptide backbone and further experiments are needed to assign an absorption band to a specific side chain moiety.

Figure 4: Antibiotic compounds purified with MeOH from EMC

The ν(C=O) vibration of glutamine side chains near 1680 cm⁻¹ is a relative strong infrared absorber, the bands are sensitive to H-bonding and the band position is lower the stronger the H-bond is (Table 2, 3).

The ν(C=O) vibration of the deprotonated carboxylate group of the glutamate shows two strong bands near 1400 and 1570 cm⁻¹ for symmetric and antisymmetric stretching vibration, respectively (Tab. 2, 3).

The ν(CN) vibration of histidine is a useful band near 1100 cm⁻¹ (Tab. 2, 3).

Tyrosine is a relatively strong infrared absorber due to its polar character. The most intense bands originate from ν(CC), the ν(C-O) and the δ(COH) mode near 1517, at 1235–1270 and at 1169–1260 cm⁻¹ (Tab. 2, 3).

While the δ(CH₃), the δ(CH₂) and the δ(CH₃) vibration near 1465, 1450 and 1375 cm⁻¹ are relatively good group frequencies, the δ(CH) and γ(CH₂) vibrations are often coupled to other modes (Tab. 2, 3). The only tryptophan bands with considerable infrared intensity seem to be those at 1334 and 1455 cm⁻¹ (Tab. 2, 3).

Table 3: The IR spectra of EMC antibiotic compounds with different eluents

<table>
<thead>
<tr>
<th>Band position in cm⁻¹</th>
<th>Assignment</th>
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<tr>
<td>/PrOH</td>
<td>MeOH</td>
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“b” and “p” indicate vibrations of the benzene or pyrole moieties, respectively.

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REFERENCES


