ANIBACTERIAL ACTIVITY OF TISSUES OF BIVALVE MOLLUSCS AVAILABLE ON POLISH MARKET

HANNA RÓŻAŃSKA, MIROSŁAW MICHALSKI, AND JACEK OSEK

Department of Hygiene of Food of Animal Origin, National Veterinary Research Institute, 24-100 Pulawy, Poland
bruna@piwet.pulawy.pl

Received: May 7, 2012 Accepted: November 5, 2012

Abstract

One hundred and ninety seven samples of molluscs representing different species were tested for the presence of antibacterial substances using a microbiological diffusion test – “4-plate” method. It was found that 58 samples (29.4%) were positive. The percentage of positive samples depended on species and varied from 0 (Ostrea edulis, Perna canaliculSus, Cardium edule, Myretrix lyrata, Mercenaria mercenaria) to 41.2 (Mytilus edulis) and 50.0 (Tapes semidecussatus and Ruditapes philippinarium). The randomly performed confirmatory analyses using HPLC –MS/MS method did not show the presence of any known antibiotics.

Key words: molluscs, antibacterial activity, screening method.

Frutti di mare”, including bivalve molluscs, have become more and more popular food in Poland. Due to delicate taste and high nutritional value (high content of proteins, vitamins, and trace elements) molluscs are very valuable foodstuffs (17, 23). As any other types of food, molluscs must be safe for consumers. Several factors, including pathogenic bacteria, viruses, marine biotoxins, and chemical contaminants may cause a threat for human health. The aim of the study was to evaluate the presence of antibacterial substances in molluscs offered on Polish market. The presence of these substances may have an influence on evaluation of microbiological quality of shellfish.

Material and Methods

One hundred and ninety seven bivalve molluscs of different species were tested for the presence of antibacterial substances. The molluscs were purchased directly from importers and were transported to the laboratory in controlled temperature conditions below 10°C. The following molluscs were examined: blue mussels (Mytilus edulis), manila clams (Tapes semidecussatus), Pacific cupper oyster (Crassostrea gigas), great scallops (Pecten maximus), European flat oyster (Ostrea edulis), Japanese carpet shell (Ruditapes philippinarium), hard clams (Myretrix lyrata), cockles (Cardium edule), New Zealand green lipped mussel (Perna canaliculSus), venus clams (Mercenaria mercenaria), and dog cockle (Glycymeris glycymeris). A microbiological screening method (“4-plate” method), with Bacillus subtilis BGA and Kocurìa varians ATCC 9341 as test strains, was used for the detection of antibacterial substances. Paper discs with diameter of 9 mm (Whatman No 3, Schleicher & Schuell) were soaked with the homogenised molluscs’ tissue for 30 min and then placed onto a surface of inoculated agar on Petri dishes. After overnight incubation at 30°C (Bacillus subtilis) and 37°C (Kocurìa varians) the zones of inhibition were measured using an electronic caliper. Each zone over 2 mm was evaluated as a positive result. Six of positive samples were retested using the liquid chromatography with mass spectrometry method (HPLC-MS/MS). This technique enables a simultaneous detection of 52 different antibiotics.

Results

The results of the investigation are summarised in Table 1. Antibacterial substances were detected in 58 out of 197 samples (29.4%). Among them, 28 were detected in blue mussels (41.2%), 16 in manila clams (50.0%), three in Pacific cupper oysters (12.5%), two in great scallops (9.5%), eight in Japanese carpet shell (50.0%), and one sample in dog cockle. The range of inhibition zones varied from 2.1 mm to 9.2 mm, depending on the species of molluscs and type of agar plates. The biggest inhibition zones were observed in samples from dog cockles and blue mussels. In the case of European flat oysters, hard clams, cockles, New Zealand green lipped mussels, and venus clams no
positive samples were observed. Furthermore, no positive results in the confirmatory analysis performed using HPLC-MS were noted.

Discussion

It is almost impossible to detect the residues of antibiotics in molluscs because these substances are not used for the treatment of diseases of these organisms due to economical and environmental reasons (23). Some authors (7, 10, 11, 18, 21) described trace amounts of antibiotics in molluscs, which live close to Atlantic salmon farms, where these substances are used therapeutically. However, these residues were detected only on a very small area. The results of the present study may indicate the presence of different natural antimicrobial substances in the molluscs tested, which gave inhibition zones on plates with different pH and test bacteria. Bivalve molluscs live in the environment, in which up to 10³ different bacteria and up to 10³ viruses per ml of water may exist (8, 15, 27). As filter-feeding organisms, molluscs are exposed to high concentrations of bacteria, including pathogens. For this reason their immune defence system must be based on non-specific, rapid cellular and humoral responses. Since 1980s, many different antimicrobial substances identified in molluscs were described and characterised (4, 9, 15, 16). It was demonstrated that small cysteine-rich peptides play a very important role in the defence system of molluscs (3, 4, 13, 14, 16, 24-27). The antimicrobial activity of marine molluscs was tested in many experiments. Annamalai et al. (2) described antibacterial activity of green mussel (Perna viridis) and edible oyster (Crassostrea madrasensis) against ten pathogenic bacteria, including Escherichia coli, Klebsiella oxytoca, K. pneumoniae, Pseudomonas aeruginosa, Salmonella Typhi, S. Paratyphi, and Staphylococcus aureus. The antimicrobial activity was different and depended on the species of bacteria and solvent used for extraction. Prem and Patterson (22) investigated the activity of tissue extracts from Cypraea sp. against 15 bacterial and three fungal pathogens. Chellaram et al. (6) studied antibacterial activity of the oysters (Pteria chinesis) against 12 human and 10 fish pathogens. The acetone extract was the most active and the range of inhibition zones varied between 1.5 and 6 mm (6). In the studies by Estari et al. (12), the activity of water, chloroform, and acetone extracts from freshwater mussels (Lamellidens marginalis) against nine species of bacteria were tested. The biggest zones of inhibition were created by chloroform and acetone extracts. In other studies, the various tissues of the horse mussels were active against Vibrio anguillarum, E. coli, Corynebacterium glutamicum, and S. aureus (15). In the investigations performed by Mercado et al. (19), the high activity of the gill tissues of the mussels (Mytilus edulis chilensis) against Micrococcus luteus was observed. Antibacterial activity of the plasma of oysters (Crassostrea virginica), and mussels (Mytilus edulis and Geucensia demissa) against Bacillus megaterium was also observed (1). Mytilin A, cysteine-rich peptide isolated from the blood of Mytilus edulis, was active against Gram-positive (Micrococcus luteus, Aerococcus viridans, Bacillus megaterium, Enterococcus faecalis, and S. aureus) and Gram-negative bacteria (E. coli, S. Typhimurium and others) (5). In the present study, the high antibacterial activity of Mytilus edulis tissues was also observed. The common prevalence of antimicrobial substances, especially antimicrobial peptides (AMPs) in marine organisms, including bivalve molluscs, and their high activity against bacteria may be taken into account as potential source of new therapeutic agents. In the present situation, one of the global problems is development of antimicrobial resistance of bacteria. For this reason, there is a growing need to look for new options of therapy (4, 13, 15, 20). On the other hand, a strong antibacterial activity of tissues of bivalve molluscs may cause some problems with interpretation of microbiological investigations of these organisms and potential risk for consumers.

<table>
<thead>
<tr>
<th>Molluscs</th>
<th>Analysed</th>
<th>Positive%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue mussels</td>
<td>68</td>
<td>28/41.2</td>
</tr>
<tr>
<td>Manila clams</td>
<td>32</td>
<td>16/50.0</td>
</tr>
<tr>
<td>Pacific cupper oyster</td>
<td>24</td>
<td>3/12.5</td>
</tr>
<tr>
<td>European flat oyster</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>Great scallop</td>
<td>21</td>
<td>2/9.5</td>
</tr>
<tr>
<td>Japanese carpet shell</td>
<td>16</td>
<td>8/50.0</td>
</tr>
<tr>
<td>Hard clam</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Cockle</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>New Zealand green lipped mussel</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Venus clam</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Dog cockle</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>197</td>
<td>58/29.4</td>
</tr>
</tbody>
</table>
References