Production of Cytokinin-like Substances by Planktonic Bacteria Isolated from Lake Jeziorak

W. Donderski, M. Głuchowska

Department of Water Microbiology and Biotechnology, Nicholas Copernicus University
Gagarina 9, 87-100 Torun, Poland

Received: April 10, 2000
Accepted: April 28, 2000

Abstract

Studies on cytokinin-like substances synthesized by planktonic bacteria isolated from littoral and pelagial zones of lake Jeziorak in spring and summer have been carried out. 62.5% of bacteria isolated in summer, and 12.5% of bacteria isolated in spring were able to produce cytokinin-like substances. Among synthesized substances we found isopentenyladenine (2iP, i6Ade), zeatin (Z, io6Ade) and zeatin riboside (ZR, io6A). No trace of isopentenyladenosine (2iPA, i6A) was detected. The amounts of cytokinin-like substances produced converted into 1 g dry mass of bacteria were as follows: 9.97 - 21.59 µg for isopentenyladenine; 3.08 - 35.08 µg for zeatin, and 0.35 - 18.69 µg for zeatin riboside. Various taxonomic groups of bacteria were capable of synthesising those compounds, such as Vibrio, Bacillus, Aeromonas, Achromobacter genera and Enterobacteriaceae family. Among the analyzed bacteria it was only the strain of Achromobacter spp that produced two compounds at the same time (zeatin and zeatin riboside).

Keywords: cytokinin substances, heterotrophic bacteria, planktonic bacteria, littoral, pelagial, identification.

Introduction

Cytokinins are cell division regulating plant hormones which are able to affect certain physiological responses in higher plants [38, 23, 27]. They make the main group of growth regulating substances [20, 21], and in co-operation with other plant hormones control plant development processes [23]. According to Richmond and Lang [35] and Maruyama et al. [24], they are responsible for retarding the aging processes; stimulate cell division, chlorophyll production, nucleic acid, protein, lipid and sugar syntheses; hamper root elongation; are likely to reduce the activities of ribonucleases, deoxyribonucleases, proteases and other enzymes; may stabilize cytoplasmatic membrane structure and that of ribosomes. Apart from free occurrence, cytokinins make components of tRNA particles in all organism types - they have been detected in tRNA hydrolyzates of bacterial, plant and animal origin [10, 20, 21, 24, 27, 43].

According to investigation results by Skoog and Armstrong [38] and Kampert and Strzelczyk [14], cytokinins also affect the growth of bacteria, unicellular algae and fungi. Unicellular marine algae examinations revealed that kinetin accelerated the growth of some phyto-flagellates known to cause red tides [23].

Many fungi species (mainly plant pathogens or mycorrhizal species) and bacteria species (mainly soil ones) have the ability of synthesizing cytokinin both as free occurring and as tRNA component [28]. Microorganisms produce various kinds of cytokinins, e.g. Corynebacterium fascians produce as many as seven different cytokinin types: cis-zeatin (c-io6Ade), trans-zeatin (t-io6Ade), zeatin riboside (ZR, io6A), metyltio-cis-zeatin (c-ms io6Ade), isopentenyladenine (2iP, i6Ade), isopen-
Examining Water Bacteria Ability to Synthesize Cytokinin

Among bacteria isolated from the lake areas under investigation in spring and summer, eight various strains were subjected to examination. The cytokinin-like substance production ability was tested by incubating on "A" mineral medium at 20°C. The aim of this investigation was to estimate the ability of planktonic bacteria isolated from eutrophic lake Jeziorak to produce substances of cytokinin type and their identification.

Materials and Methods

Study Area

The study was done in littoral and pelagial zones of the eutrophic lake Jeziorak. This lake is located in NE Poland and is a part of Itawa Lake District, coming within the Vistula-Drweca rivers basin. It is a post-glacial lake of meridional placement [19]. Lake Jeziorak is the fourth largest lake in Poland, with a surface of 3219.4 ha, max length 27.4 km, width 2.4 km, depth of 12.0 m, and mean depth 5 m [29].

Sampling

The sampling was carried out in spring (21st April 98) and summer (3rd August 98). Water was sampled at depths of 10 - 20 cm by means of automatic pipet pumps - Pipetboy (De Ville) into sterile pipettes and then transferred into sterile glass jars. The samples were immediately placed in ice-filled containers at max. +7°C and transported to the laboratory. The time between sampling and lab work did not exceed 6 hours.

Bacterial Strain Isolation

In order to isolate planktonic bacteria and determine their number, a Ferrer, Stapert and Sokolski [9] iron-peptone agar medium was used. Sterile buffered water was used as diluent [5]. The determination was done with the use of spread plates method. All inoculations were done in five simultaneous repetitions. Inoculated plates were incubated for 7 days at 20°C, then the bacterial colonies were counted (CFU). The results were converted into 1 ml of water. Next, 10 colonies from each sample were picked up and transferred into semi-solid iron-peptone agar medium (containing 5 g of agar/1). While isolating the bacteria, an effort was made to split off representative strains by observing such macroscopic features as the colony colour, surface type, shape, colony edges and the presence of substrat diffusing pigments. After 7 days incubation time at 20°C, bacterial culture purity was checked in Gram stained specimens. Those strains were stored at + 4°C in a fridge for further study. Every 2 months those strains were inoculated onto fresh medium.

Cytokinin Extraction

In order to extract cytokinin-like substances, the supernatant was adjusted to pH 2.5 - 3 by 1 N HCl and passed through a Dowex exchange column (Merck). Then the column was rinsed with 500 ml bidistilled water. Active material was eluted with 2N NH4OH (2 column capacities) and 5N NH4OH (4 column capacities). Thus obtained ammonia eluat was evaporated to dryness in vacuum at 60 - 70°C to remove ammonia. The dry remnants were dissolved in 2 ml 35% ethanol and then passed through a Sephadex LH-20 column (Pharmacia, Uppsala) only to rinse the active material with 35% ethanol, collecting 10 ml eluat fractions. The first 15 fractions (150 ml) were rejected, the next 24 fractions (240 ml) were collected in 100 ml Erlenmayer flasks, up to 40 ml each. After that, they were evaporated until nearly dry (leaving in about 2 drops) in a vacuum dryer at 50 - 60°C. Thus obtained material was tested for cytokinin by means of a Shimadzu GC - 14A gas chromatograph. In order to do so, the material was dissolved in 1 ml of 96% ethanol, next transferred into a vial to be dried under nitrogen until complete sample dryness. Thus obtained samples were stored in an P2O5 filled exiccator for 24 hours. Having done that, 50 - 100 ul BSA (N, O-bis (trimethylisyl)-acetamide; Sigma) were silledated and immediately placed in a sand bath for 1 hour at 80°C, and...
then the samples were placed in P_2O_5 exiccator for 24 hours. Thus prepared samples were passed through a gas chromatograph according to cytokinin standards (zeatin, zeatin riboside, isopentenyladenine, isopentenyladenosine). The amount of cytokinin-like substances produced by bacteria were converted into 1 g bacteria dry mass.

Strain Identification

The identification of planktonic bacteria capable of cytokinin-like substances producing was done on the basis of the scheme by Shewan, Hobbs and Hodgkins [36] and the data presented by Hugh & Leifson [12], Skerman [37], Collins and Taylor [4], Thomley [46], Hendrie [11], McMeekin [25, 26] and Bergey [3].

Results

Total Number of Heterotrophic Bacteria

Figure 1 present the results of a study on the total number of heterotrophic bacteria (CFU) inhabiting the littoral and pelagial zones of Jeziorak lake. According to those data, in spring fewer bacteria were recorded in the pelagial (20 x 10^3 cell/cm^3) than in the littoral zone (33.7 x 10^3 cell/cm^3), whereas in summer a reverse situation was found - more bacteria occurred in the pelagial (27 x 10^3 cell/cm^3) than in the littoral zone (20 x 10^3 cell/cm^3).

Selected Bacteria Strains Identification

Table 1 presents data on bacteria morphology isolated from lake Jeziorak investigated areas. As the results show, Gram-negative rods (62.5%) and Gram-positive bacilli (25%) were more abundant in the pelagial in spring than in the littoral (54.6% and 13.7% respectively). Gram-positive cocci (18.2%) and pleomorphic forms (13.5%) were more abundant in the littoral than in the pelagial (8.3% and 4.2% respectively). In summer, in the pelagial, there occurred more Gram-positive bacilli (24.0%) and Gram-positive cocci (16.0%) than in the littoral (3.7% and 3.7% respectively). In the same period of time, Gram-negative rods (81.5%) and pleomorphic forms (11.1%) were more abundant in the littoral than in the pelagial (52.0% and 8.0% respectively).

Table 1. Morphological types among bacteria isolated from littoral and pelagial zone of Jeziorak Lake (bacteria in percentage).

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Littoral zone</th>
<th>Pelagial zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>L</td>
</tr>
<tr>
<td>21.04.98</td>
<td>54.6</td>
<td>13.7</td>
</tr>
<tr>
<td>3.08.98</td>
<td>81.5</td>
<td>3.7</td>
</tr>
</tbody>
</table>


Fig. 1. Number of heterotrophic bacteria (CFU) isolated from water of Jeziorak Lake.

Fig. 2. Generic composition of bacteria isolated in spring from littoral and pelagial zone of Jeziorak Lake.
Fig. 3. Generic composition of bacteria isolated in the summer from littoral and pelagial zone of Jeziorak Lake.

Figure 2 a, b and 3 a, b present the bacteria generic composition isolated from lake Jeziorak that were used for a study on cytokinin-like substances synthesis. Among strains isolated from the littoral zone in spring (Fig. 2a) there occurred bacteria of *Achromobacter* (25%), *Alcaligenes* (25%), *Bacillus* (25%) and *Vibrio* (25%) genera. *Achromobacter* (25%), *Alcaligenes* (25%), *Flavobacterium-Cytophaga* (50%) bacteria were isolated from the pelagial zone (Fig. 2b) in this period. In summer the bacteria isolated from the littoral (Fig. 3a) were identified as *Aeromonas* (50%), *Vibrio* (25%) and *Enterobactriaceae* (25%); those isolated from the pelagial (Fig. 3b) were: *Achromobacter* (50%), *Bacillus* (25%) and *Enterobacteriaceae* (25%).

**Planktonic Bacteria Ability to Synthesize Cytokinin-Like Substances**

The study results on cytokinin-like substances production by planktonic bacteria in lake Jeziorak have been presented in Tabs. 2 and 3, and on chromatograms 1 - 10. The data indicate various bacteria being able to synthesise cytokinin-like substances, which is associated with the season rather than the lake zone from which they came.

Results in Tab. 2 show clearly that out of 16 studied strains that were isolated both in spring and summer, only six (37.5%) were able to synthesise cytokinin-like substances. Among those strains, only one was sampled in spring; the other five were summer samples. The spring isolates was isolated from the littoral and was determined as *Vibrio*. The strains isolated from the littoral in summer were identified as *Aeromonas*, *Vibrio* genera and of *Enterobacteriaceae* family; those coming from the pelagial were classified as *Achromobacter* and *Bacillus* genera.

The results listed in Tab. 3 clearly point to the fact that the substance most frequently synthesized was isopentenyladenine (2IP) treated by *Bacillus* and *Vibrio* genera bacteria and by those of *Enterobacteriaceae* family (Graph 1 - 4). A substance which was identified as zeatin (Z) was produced by strains of *Vibrio* and *Achromobacter* genera (Graph 5 - 7); whereas strains of *Achromobacter* and *Aeromonas* genera produced a substance the reten-

### Table 2. Production of cytokinin-like substances by planktonic bacteria isolated from Lake Jeziorak.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Cytokinin-like substances</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2IP</td>
</tr>
<tr>
<td><em>Achromobacter</em> (2L*)</td>
<td>-</td>
</tr>
<tr>
<td><em>Alcaligenes</em> (17L spring)</td>
<td>-</td>
</tr>
<tr>
<td><em>Bacillus</em> (14L spring)</td>
<td>-</td>
</tr>
<tr>
<td><em>Vibrio</em> (15L spring)</td>
<td>-</td>
</tr>
<tr>
<td><em>Achromobacter</em> (15P** spring)</td>
<td>-</td>
</tr>
<tr>
<td><em>Alcaligenes</em> (18P spring)</td>
<td>-</td>
</tr>
<tr>
<td><em>Flavobacterium</em>-Cytophaga (17P spring)</td>
<td>-</td>
</tr>
<tr>
<td><em>Flavobacterium</em>-Cytophaga (2IP spring)</td>
<td>-</td>
</tr>
<tr>
<td><em>Aeromonas</em> (16L summer)</td>
<td>-</td>
</tr>
<tr>
<td><em>Aeromonas</em> (8L summer)</td>
<td>-</td>
</tr>
<tr>
<td><em>Vibrio</em> (1L summer)</td>
<td>-</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em> (3L summer)</td>
<td>-</td>
</tr>
<tr>
<td><em>Achromobacter</em> (23P summer)</td>
<td>-</td>
</tr>
<tr>
<td><em>Achromobacter</em> (28P summer)</td>
<td>-</td>
</tr>
<tr>
<td><em>Bacillus</em> (13P summer)</td>
<td>-</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em> (9Psummer)</td>
<td>-</td>
</tr>
</tbody>
</table>

Explanations: L* - strains isolated from littoral zone, P** - strains isolated from pelagial zone, 2IP - isopentenyladenine, 2IPA - isopentenyladenosine, Z - zeatin, ZR - zeatin riboside.

### Table 3. Quantity of cytokinin-like substances production by bacteria isolated from water of Jeziorak Lake (in µg/g dry mass of bacteria).

<table>
<thead>
<tr>
<th>Strains</th>
<th>Quantity of cytokinin-like substances</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2IP</td>
</tr>
<tr>
<td><em>Vibrio</em> (15L spring)</td>
<td>9.97</td>
</tr>
<tr>
<td><em>Vibrio</em> (1L summer)</td>
<td>-</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em> (3L summer)</td>
<td>45.51</td>
</tr>
<tr>
<td><em>Aeromonas</em> (8L summer)</td>
<td>21.59</td>
</tr>
<tr>
<td><em>Bacillus</em> (13P summer)</td>
<td>-</td>
</tr>
<tr>
<td><em>Achromobacter</em> (28P summer)</td>
<td>-</td>
</tr>
</tbody>
</table>

Explanations: 2IP - isopentenyladenine, 2IPA - isopentenyladenosine, Z - zeatin, ZR - zeatin riboside.
Graph 1. Chromatogram of standard – isopentenyladenine (2IP); retention time: 5,557.

Graph 2. Chromatogram of cytokinin-like substance synthetize by strain from family Enterobacteriaceae (3L summer); retention time: 5,558.

Graph 4. Chromatogram of cytokinin-like substance synthetize by strain from genus Bacillus (13P summer); retention time: 5,532.

Graph 5 Chromatogram of standard – zeatin (Z); retention time: 9,073.

Graph 3. Chromatogram of cytokinin-like substance synthetize by strain from genus Vibrio (15L spring); retention time: 5,542.

Graph 6. Chromatogram of cytokinin-like substance synthetize by strain from genus Vibrio (1L summer); retention time: 9,098.
Data included in Tab. 3 point to isopenetenyladenine having been produced in greatest amount by a strain of the Enterobacteriaceae family (45.51 µg/g d.m.); a strain of Bacillus genus produced twice less (21.59 µg/g d.m.); and a Vibrio strain had a five times lesser result (9.97 µg/g d.m.). A Vibrio genus strain produced the greatest amount of a substance chromatographically identified as zeatin (35.08 µg/g d.m.) whereas a strain of Achromobacter genus was nearly ten times less productive (3.08 µg/g d.m.). Bacteria of Aeromonas and Achromobacter genera produced a substance which had its retention time very close to that of zeatin riboside: 18.69 µg/g d.m. and 0.35 µg/g d.m. respectively. The present study did not identify any bacteria capable of synthesizing isopenetenyladenosine.

Discussion

Studies on planktonic bacteria morphology isolated from lake Jeziorka have given evidence of a dominating occurrence of Gram-negative rods in that water body, regardless of season or the lake zone inhabited by those bacteria. These findings seem to confirm those by Taylor [44], Potter and Baker [33], Potter [34] and Donderski [6, 7] who have stated that those rods make the most common forms occurring in surface waters of eutrophic lakes. Wood [45] along with Strzelczyk et al. [39] unanimously maintain that pleomorphic bacteria are very likely to mass occur in surface waters, however the present study results are in no way in favour of that statement as very few pleomorphic forms have been found among the planktonic bacteria; cocci or rods alike.

While analyzing the planktonic bacteria generic composition, it was stated that they represented the genera of Achromobacter, Vibrio, Aeromonas; a group of Flavobacterium-Cytophaga and a family of Enterobacteriaceae, that is to say the genera and groups most abundantly occurring in the waters of Hawskie Lake District, which confirm earlier studies by Donderski [8].

Until now no studies have been done on the production of cytokinin-like type growth substances by bacteria...
inhabiting lake or river waters in that region. The issue that has mostly been dealt with was cytokinin production by marine and soil bacteria, or microbes that stayed in a tall plants-pathogene interaction. Therefore, it is impossible to provide material for a broader discussion on cytokinin-like substances which have been identified as isopentenyladenine (2iP), zeatin (Z) and zeatin riboside (ZR). Strains producing those substances belong to various groups and genera, which confirm earlier studies by Barea et al. [2]. Kampert and Strzelczyk [14, 15] and those by Maruyama et al. [23].

The present study makes an encouraging stimulus to continue research on cytokinin-like substances synthesis by bacteria inhabiting various freshwater bodies, with a simultaneous estimation of ecological factors affecting such synthesis and the importance those substances may have in the water bodies in question.

Conclusions

Based on the above described study it is possible to state that:

1. Planktonic bacteria inhabiting Jeziorak lake are able to synthesize cytokinin-like substances.
2. Strains bacteria isolated in summer seemed more likely to produce more cytokinin-like substances than those isolated in spring.
3. Among cytokinin-like substances synthesized by planktonic bacteria isolated from Jeziorak lake the following types were found: isopentenyladenine (2iP), zeatin (Z) and zeatin riboside (ZR).
4. Isopentenyladenine (2iP) production was most abundant, while zeatin riboside (ZR) was least abundant by planktonic bacteria.
5. The following bacterial genera were able to produce cytokinin-like substances: Vibrio, Bacillus, Aeromonas, Acinetobacter, and also belong in Enterobacteriaceae family.

References

27. MICHNIEWSICZ M. Fizjologia roslin. PWRiL. Warszawa. 1977
42. SZIRAKI J., BALAZS E., KIRALY Z. Increased levels of cytokinin and indoleacetic acid in peach leaves infected with Taphrinia deformans. Physio. Plant Pathol., 5, 45, 1975.
44. TAYLOR C.B. Bacteriology of fresh water. Ill. The types of bacteria present in lakes and streams and their relationships to the bacterial flora in soil. J. Hyg., 42, 284, 1942.