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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

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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, i^6Ade), zeatin (Z, io^6Ade) and zeatin riboside (ZR, io^6A). No trace of isopentenyladenosine (2iPA, i^6A) 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, desoxyribonucleases, 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 pathogenes 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-io^6Ade), trans-zeatin (t-io^6Ade), zeatin riboside (ZR, io^6A), metyltio-cis-zeatin (c-ms^2io^6Ade), isopentenyladenine (2iP, i^6Ade), isopen-

Corespondence to: Donderski W. tel. 48-56 611-45-71, 611-44-33

tenyladeniosine (2iPA, i^6A) and methyloaminopurine (me^6Ade) [28, 14, 10] Kampert and Strzelczyk [14, 15]. revealed the following soil bacteria as producers of cytokinin: *Bacillus, Pseudomonas, Chromobacterium, Acinobacter, Flavobacterium* and also a *Corynebacterium-Achrobacter* group and unidentified Gram-negative rods. Barea et al. [2] and Maruyama et al. [23] found out that cytokinin was also produced by non-pathogenic and non-symbiotic bacteria.

There is little record of cytokinin production by water bacteria; the existing papers deal mainly with marine planktonic and benthic bacteria. According to Maruyama et al. [23], most marine cytokinin producing bacteria are benthos organisms (45-55%). Among planktonic bacteria only 5-15% strains had the facility. A taxonomic study carried out by Maruyama et al. [23] revealed that the following bacteria genera were capable of that production: *Flavobacterium, Acinetobacter, Vibrio* and *Pseudomonas.* Having identified the cytokinin synthesized by those bacteria, the author found out that it was mainly the 2iP and 2iPR substances [23, 24].

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.

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 ace. to Lochhead and Chase [22]. In order to achieve that, the treated bacteria were preincubated on iron-peptone agar medium slants for 5 days at 20°C and then washed by 5 ml of sterile buffered water, centrifuged and finally 200 ml "A" mineral medium doses were inoculated with washed sediment of bacteria. After 7 days of incubation at 20°C, the bacteria cultures were centrifuged at 15,000 RPM for 20 min. The post-cultivation liquid (supernatant) was used for cytokinin-like substances extraction while the bacterial sediment was dried at 105°C to stable weight in order to carry out bacteria dry mass determination.

Cytokinin Extraction

In order to extract cytokinin-like substances, the supernatant was adjusted to pH 2.5 - 3 by 1 N HC1 and passed through a Dowex exchange column (Merck). Then the column was rinsed with 500 ml bidistilled water. Active material was eluated with 2N NH_4OH (2 column capacities) and 5N NH_4OH (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 P_2O_5 filled exiccator for 24 hours. Having done that, 50 - 100 ul BSA (N, O-bis (trimethylisyl)-acetamide; Sigma) were sililated 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

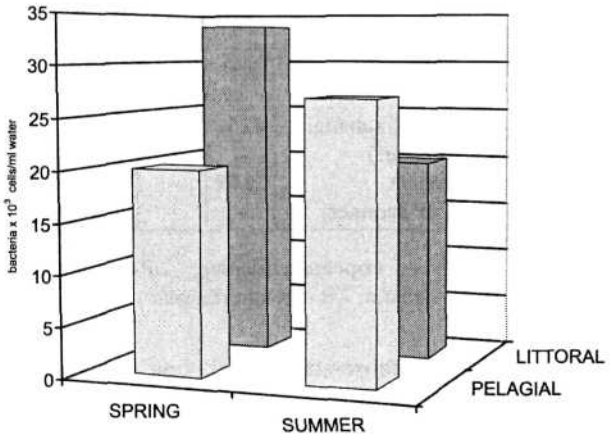
The identification of planktonic bacteria capable of cytokinin-like substances producing was done on the basis of the scheme by Shewan, Hobbs and Hodgkins [36] and the data presented by Hugh & Leifson [12], Skerman [37], Collins and Taylor [4], Thomley [46], Hendrie [11], McMeekin [25, 26] and Bergey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

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



water of Jeziorak Lake.

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).

Date of sampling	Place of origin							
	Littoral zone				Pelagial zone			
	P	L	Z	PF	P	L	Z	PF
21.04.98	54.6	13.7	18.2	13.5	62.5	25.0	8.3	4.2
3.08.98	81.5	3.7	3.7	11.1	52.0	24.0	16.0	8.0

Explanations: P – bacterium, L – bacilli, Z – cocci, PF – pleomorphic forms.

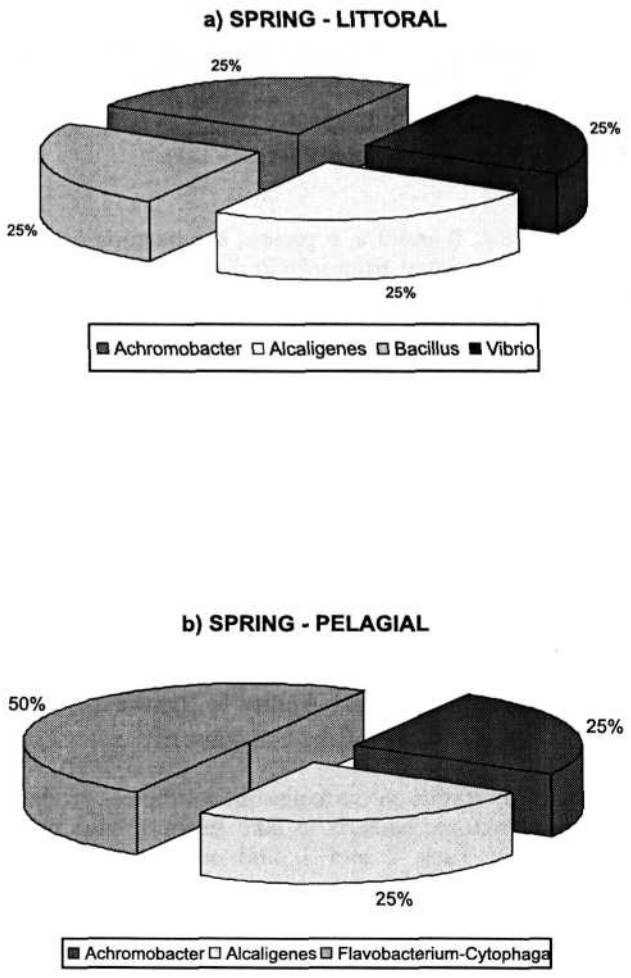


Fig. 2. Generic composition of bacteria isolated in spring from littoral and pelagial zone of Jeziorak Lake.

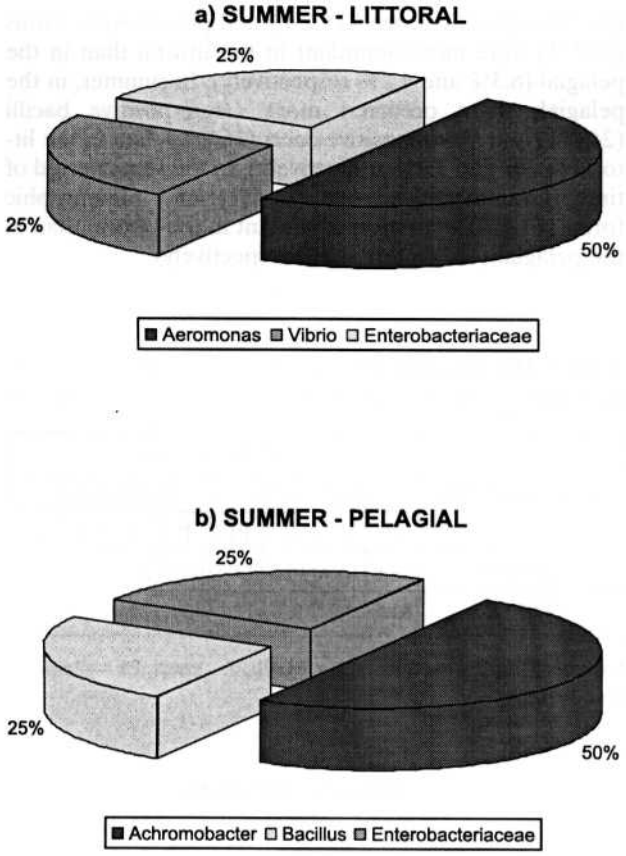


Fig. 3. Generic composition of bacteria isolated in the summer from littoral and pelagial zone of Jeziorak Lake.

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

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

Strains	Cytokinin-like substances			
	2iP	2iPA	Z	ZR
Achromobacter (2L* spring)	–	–	–	–
Alcaligenes (17L spring)	–	–	–	–
Bacillus (14L spring)	–	–	–	–
Vibrio (15L spring)	+	–	–	–
Achromobacter (15P** spring)	–	–	–	–
Alcaligenes (18P spring)	–	–	–	–
Flavobacterium--Cytophaga (17P spring)	–	–	–	–
Flavobacterium--Cytophaga (21P spring)	–	–	–	–
Aeromonas (16L summer)	–	–	–	–
Aeromonas (8L summer)	–	–	–	+
Vibrio (1L summer)	–	–	+	–
Enterobacteriaceae (3L summer)	+	–	–	–
Achromobacter (23P summer)	–	–	–	–
Achromobacter (28P summer)	–	–	+	+
Bacillus (13P summer)	+	–	–	–
Enterobacteriaceae (9Psummer)	–	–	–	–

Explanations: L* – strains isolated from littoral zone, P** – strains isolated from pelagial zone, 2iP – isopentenyladenine, 2iPA – isopentenyladenosine, Z – zeatin, ZR – zeatin riboside.

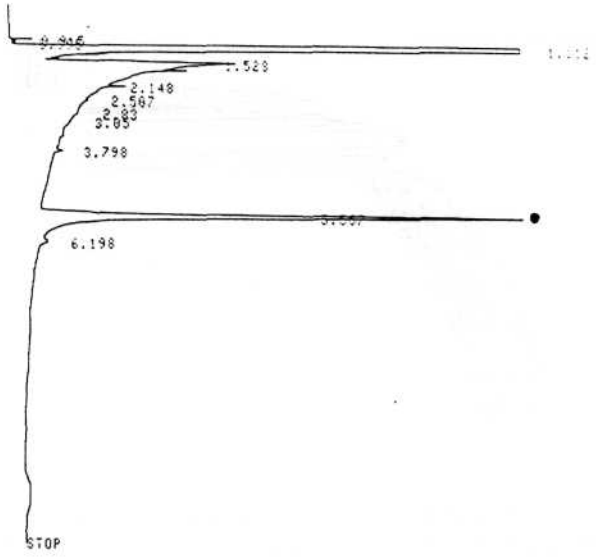
Table 3. Quantity of cytokinin-like substances production by bacteria isolated from water of Jeziorak Lake (in µg/g dry mass of bacteria).

Strains	Quantity of cytokinin-like substances			
	2iP	2iPA	Z	ZR
Vibrio (15L spring)	9.97	–	–	–
Vibrio (1L summer)	–	–	35.08	–
Enterobacteriaceae (3L summer)	45.51	–	–	–
Aeromonas (8L summer)	–	–	–	18.69
Bacillus (13P summer)	21.59	–	–	–
Achromobacter (28P summer)	–	–	3.08	0.35

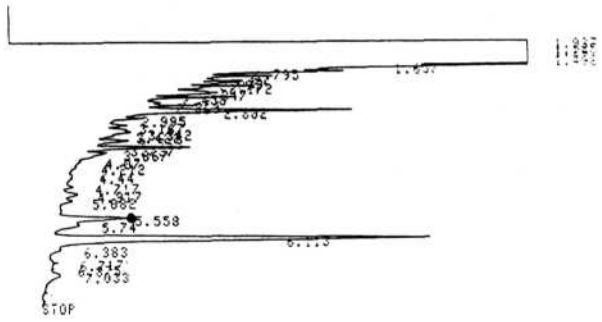
Explanations: 2iP – isopentenyladenine, 2iPA – isopentenyladenosine, Z – zeatin, ZR – zeatin riboside.

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.

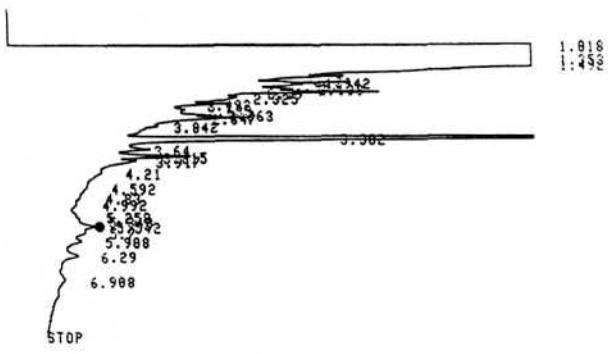
The results listed in Tab. 3 clearly point to the fact that the substance most frequently synthesized was isopentenyladenine (2iP) treated by *Bacillus* and *Vibrio* genera bacteria and by those of *Enterobacteriaceae* family (Graph 1 - 4). A substance which was identified as zeatin (Z) was produced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



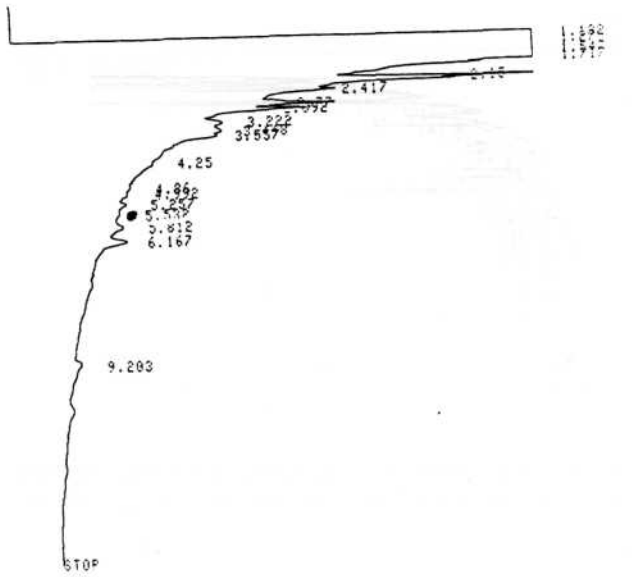
ıGraph 1. Chromatogram of standard – isopentenyladenine (2iP); retention time: 5,557.



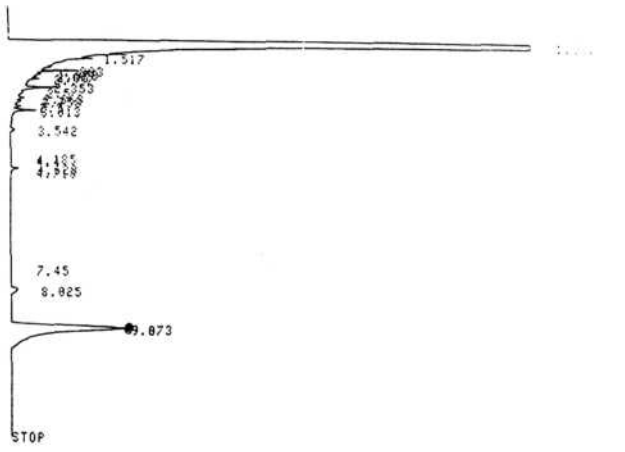
Graph 2. Chromatogram of cytokinin-like substance synthetize by strain from family Enterobacteriaceae (3L summer); retention time: 5,558.



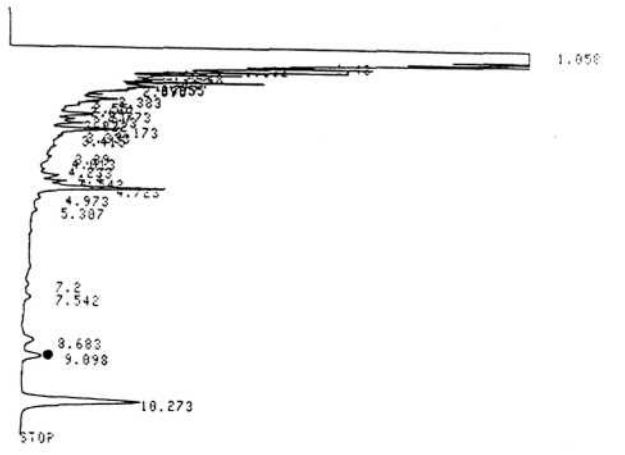
Graph 3. Chromatogram of cytokinin-like substance synthetize by strain from genus Vibrio (15L spring); retention time: 5,542.



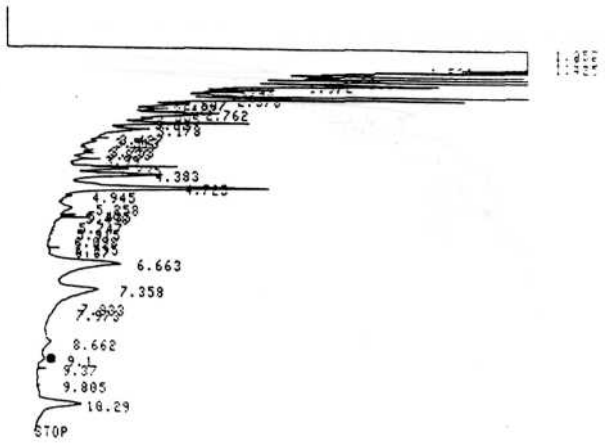
Graph 4. Chromatogram of cytokinin-like substance synthetize by strain from genus Bacillus (13P summer); retention time: 5,532.



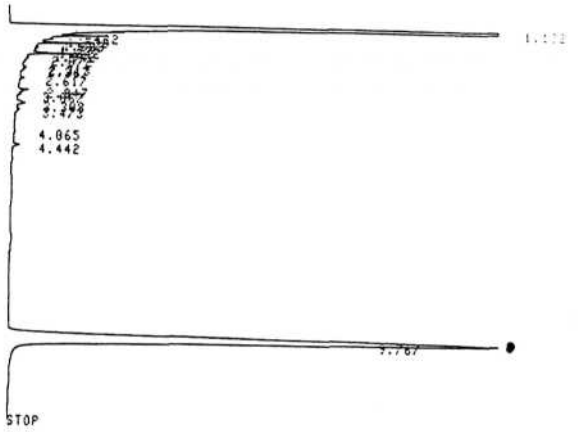
Graph 5 Chromatogram of standard – zeatin (Z); retention time: 9,073.



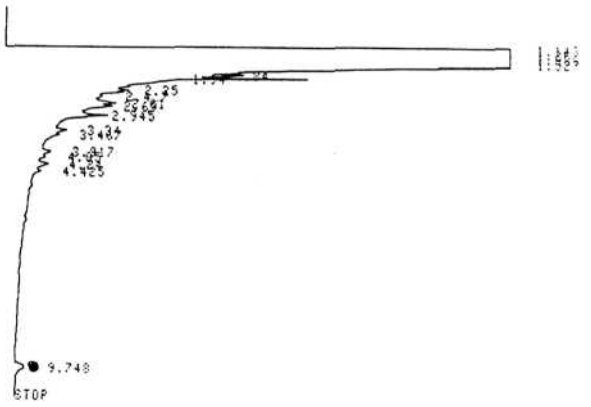
Graph 6. Chromatogram of cytokinin-like substance synthetize by strain from genus Vibrio (1L summer); retention time: 9,098.



Graph 7. Chromatogram of cytokinin-like substance synthetize by strain from genus Achromobacter (28P summer); retention time: 9,100.



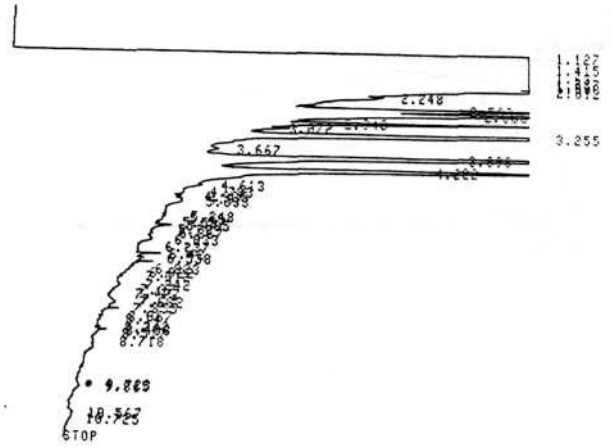
Graph 8. Chromatogram of standard - zeatinriboside (ZR); retention time: 9,767.



Graph 9. Chromatogram of cytokinin-like substance synthetize by strain from genus Aeromonas (8L summer); retention time: 9,748.

tion time of which much resembled that of zeatin riboside (ZR) (Graph 8 - 10).

It was only one strain of *Achromobacter* genus that was able to synthesise 2 types of cytokinin-like substances, namely: zeatin (Z) and zeatin riboside (ZR). The other strains produced only 1 cytokinin-like substance each.



Graph 10. Chromatogram of cytokinin-like substance synthetize by strain from genus Achromobacter (28P summer); retention time: 9,723.

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 isopentenyladenosine.

Discussion

Studies on planktonic bacteria morphology isolated from lake Jeziorak 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 leaving the results to be compared to other investigations carried out in different environments.

The present paper provides results obtained from a study on cytokinin-like substances synthesised by bacteria which give evidence on the Jeziorak lake bacteria producing isopentenyladenine (2iP), zeatin (Z) and zeatin riboside (ZR) in most cases.

Cytokinins of microbiological origin were found mainly in plant pathogenes, fungi and actinomecytes [45, 18, 48, 41, 42]. The ability to synthesise cytokinin was also recorded with mycorrhizal fungi [30, 13] and some marine algae [1],

According to some studies by Barea et al. [2] and by Kampert and Strzelczyk [15], some soil bacteria strains which are neither plant pathogenes nor symbionts were also able to produce a few cytokinin-like substances. Also marine bacteria are able to synthesise cytokinin, as was found out by Maruyama et al. [23]. Those findings point to the possibility of cytokinin being produced by various microorganism groups, regardless of their original environment.

Studies by Kentzer et al. [16, 17], Pedersen [31, **32]** and Maruyama et al. [24] on cytokinin-like substances produced in marine water give evidence of such production actually taking place. Pedersen [31, 32] detected isopentenyladenine in marine water, finding out at the same time that *Ascophyllum nodarum* and *Fucus vesiculosus* secrete growth substances into marine water, which stimulate the growth of *Ulva lactuca* and *Enteromorpha intestinalis.* Kentzer et al. [17] found isopentenyladenine (2iP) in the water embracing the thallus of *Fucus vesiculosus* and in the very thallus of the plant. However, it has not been clear so far whether 2iP is extracted by algae and water plants or provided by microorganisms staying on their surface. In her studies, Pedersen [32] showed that *Ulva lactuca, Monostroma oxysperum* and also *Ectocarpus fasiculatus* lose their regular morphology when grown in an environment deprived of microorganisms.

Maruyama et al. [23] found a *Vibrio* strain isolated from marine water being capable of synthesising cytokinin-like substances. Our study also provided evidence on isopentyneladenine (2iP) and zeatin (Z) being synthesised by two *Vibrio* determined strains.

Barea et al. [2] studied bacteria isolated from soil and found *Bacillus, Pseudomonas, Chromobacterium, Acinetobacter, Aerobacter* and *Flavobacterium* genera able to synthesise cytokinin. Such ability has also been recorded with bacteria of *Corynebacterium-Arthrobacter* group and with some Gram-negative rods isolated from soil by Kampert and Strzelczyk [15].

The presented data give evidence of the fact that egzoproduction of cytokinin type substances is not restricted to only one bacteria type, and that the compound synthesis is quite common among marine and soil microorganisms. Our studies provided data on freshwater bacteria also being capable of that process. Bacteria strains isolated from lake Jeziorak synthesised 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 iso lated in spring.
3. Among cytokinin-like substances synthesized by plank tonic bacteria isolated from Jeziorak lake the following types were found: isopentenyladenine (2iP), zeatin (Z) and zeatin riboside (ZR)
4. Isopentenyladenine (2iP) production was most abun dant, while zeatin riboside (ZR) was least abundant by planktonic bacteria.
5. The following bacteria genera were able to produce cytokinin-like substances: *Vibrio, Bacillus, Aeromonas, Achromobacter,* and also belong in *Enterobacteriaceae* family.

References

1. AUGIER H. Les hormones des algues. Etat actuel des connaissances. II - Recherche et tentatives d;identification des gibberellines, des cytokinines et de diverses autres substan ces de nature hormonale. Bot. Mar. **19,** 245, **1976.**
2. BAREA J.M, NAVARRO E., MONTOYA E. Production of plant growth regulators by rhizosphere phosphate-solubilizing bacteria. Journal of Applied Bacteri ology, **40,** 129, **1976**
3. BERGEY'S Manual of Determinative Bacteriology. Wiliams and Wilkins, Baltimore, Maryland, **1994.**
4. COLLINS V.C., TAYLOR C.E.D. Microbiological methods. Butterworths, London, wyd.II. **1967.**
5. DAUBNER I. Mikrobiologia vody. Slov. Acad. Vied. **2,** 268, **1967.**
6. DONDERSKI W. Incidence of physiological types among bacteria isolated from water and mud of the Lake Jeziorak. Zesz. Nauk. UMK Torun. Prace Limnol. **6,** 15, **1971**
7. DONDERSKI W. Nutritional requirements of bacteria iso lated from water and mud of the Lake Jeziorak. Zesz. Nauk. UMK Torun. Prace Limnol. 7, 13, 1972.
8. DONDERSKI W. Tlenowe bakterie heterotroficzne jezior o roznej trofii. UMK Torun, Rozprawy. **1983.**
9. FERRER E.B., STAPERT EM., SOKOLSKI W.T. A me dium for improved recovery of bacteria from water. Can. J. Microbial. 9, 420, **1963.**

10. GREEN E.M. Cytokinins production by microorganisms. The Botanical Review, **46,** 25, **1980.**
11. HENDRIE M.S., MITCHELL T.G, SHEW AN J.N. The identyfication of yellow-pigmented rods. In, Identyfication Method for Microbiologists. Acad. Press. London-New York. **1968.**
12. HUGH R., LEIFSON F. The taxonomic significance of fer mentative versus oxidative metabolisms of carbohydrates by various Gram-negative bacteria. Journal of Bacteriology, **66,** 24, **1953.**
13. KAMPERT M., STRZELCZYK E. Production of cytokinins by mycorrhizal fungi of pine *(Pinus silvestris* L.). Bull. Acad. Polon. Sci. XXVI, No **7,** 499, **1978.**
14. KAMPERT M., STRZELCZYK E. The synthesis of cytokinin-like substances by coryneform' bacteria isolated from the roots of pine seedlings *{Pinus silvestris* L.) Acta Microbiol. Pol., **29,** 117, **1980.**
15. KAMPERT M., STRZELCZYK E. Effect of pH on produc tion of cytokinin-like substances by bacteria isolated from soil, rhizosphere and mycorhizosphere of pine *{Pinus silves tris* L.). Acta Microbiol. Pol., **33,** 77, **1984.**
16. KENTZER T., KLUSZCZYNSKA K., RADZIWO-NOWSKA A, POTULSKA-KLEIN B. Zawartosc substancji typu cytokinin w wodzie morskiej i wptyw tych zwiazkow na procesy wzrostowe wybranych glonow battyckich. PAN KBM. Studia i Materiaty Oceanologiczne **19,** 164, **1977.**
17. KENTZER T., SYNAK R., BURKIEWICZ K., BANAS A. Cytokinin-like activity in sea water and *Fucus vesiculosus* L. Biologia Plantarum, **22,** 218, **1980.**
18. KLAMBT D. Nachweis eines Cytokinins aus *Agrobacterium tumefaciens* und sein Vergleich mit dem Cytokinin aus *Corynebacterium fascians.* Wiss. Z. Univ. Rostock, Math.-Naturwiss. Reihe, **16,** 623, **1967.**
19. LENCEWICZ S., KONDRACKI J. Geografia fizyczna Polski.PWN, Warszawa. **1959.**
20. LETHAM D.S. Chemistry and physiology of kinetin-like compounds. Ann. Review of Plant Physiology, **18,** 349, **1967.**
21. LETHAM D.S., PALNI L.M.S. The biosynthesis and metab olism of cytokinins. Ann. Rev. Plant Physiol., **34,** 163, **1983.**
22. LOCHHEAD A.G., CHASE F.E. Qualitative studies of soil microorganisms. Nutritional requirements of the predomi nant bacterial flora. Soil Sci., **55,** 185, **1943.**
23. MARUYAMA A., MAEDA M., SIMIDU U. Occurrence of plant hormone (cytokinin)-producing bacteria in the sea. Journal of Applied Bacteriology, **61,** 569, **1986.**
24. MARUYAMA A., YAMAGUCHI I., MAEDA M., SIMIDU U. Evidence of cytokinin production by a marine bacterium and its taxonomic characteristics. Canadian Jour nal of Microbiology, **34,** 829, **1988.**
25. MCMEEKIN T.A., PATERSON J.T., MURRAY J.G. An initial approach to the taxonomy of some Gram-negative yel low pigmented rods. Journal of Applied Bacteriology, **34,** 699, **1971.**
26. MCMEEKIN T.A., STEWARD D.B., MURRAY J.G. The adansonian taxonomy and the deoxyribonucleic acid base ra tio composition of some Gram-negative yellow pigmented rods. Journal of Applied Bacteriology, **35,** 129, **1972.**
27. MICHNIEWICZ M. Fizjologia roslin. PWRiL. Warszawa. **1977**
28. MICHNIEWICZ M. Rola regulatorow wzrostu w ukladzie roslina wyzsza - patogen. Postepy Nauk Rolniczych **5/82, 1982.**
29. MIKULSKI J.S. Biologia wod srodladowych. PWN.Warszawa. **1974.**
30. MILLER CO. Zeatin and zeatin riboside from mycorrhizal fungus. Science, **157,** 1055, **1967.**
31. PEDERSEN M., FRIDBORG G. Cytokinin-like activity in sea water from the *Fucus-Ascophyllum* zone. Experientia, **28,** 111, **1972.**
32. PEDERSEN M. Identyfication of a cytokinin, 6-(3-methyl-2-butenyloamino)purine, in sea water and the effect of cytokinins on brown algae. Physiologiae Plantarum, **28,101, 1973.**
33. POTTER L., BAKER C.E. The microbiology of Flathead and Rogers lakes Montana.II. Vertical distribution of the microbial population and chemical analyses of their environ ments. Ecology, **42,** 338, **1961.**
34. POTTER L. Planktonic and benthic bacteria of lakes and ponds. In, Principles and Aplications in Aquatic Microbiol ogy. Ed. Henkelkian H. Dondero N.C., New York - London - Sydney. **1964.**
35. RICHMOND A.E., LANG A. Effect of kinetin on protein content and survival of detached *Xantium* leaves. Science, **125,** 650, **1957.**
36. SHEWAN J.M., HOBBS G., HODGKINS W. A de-treminative schema for the identyfication of certain genera of Gram-negative bacteria with special reference to the *Pseudomonadaceae.* Journal of Applied Bacteriology, **23,** 379, **1960.**
37. SKERMANN V.B.O. A guide to the identification of the genera of bacteria. 2nd ed. Baltiomr, The Williams and Wilkins Comp. **1967.**
38. SKOOG F., ARMSTRONG D.J. Cytokinins. Annual Re view of Plant Physiology, **21,** 359, **1970.**
39. STRZELCZYK E., DONDERSKI W, MIELCZAREK A. Studies of cultural properties of planktonic, benthic and epiphytic bacteria. Zesz. Nauk. UMK Toruii, Prace Lim-**nol.,7,** 3, **1972.**
40. STRZELCZYK E., KAMPERT M. Production of cytokinin-like substances by *Cylindrocarpon deslructans* (Zins. Scholt.) isolates pathogenic and non-pathogenic to fir *(Abies alba* Mill.) seedlings. Phytopath., **106,** 90, **1983.**
41. SURICO G., SPARAPANO L., LERARIO P., DURBIN R.D., Iacobellis N. Cytokinin-like activity in extracts from culture filtrates of *Pseudomons savastanoi.* Experientia, **31,** 929, **1975.**
42. **SZIRAKI J.,** BALAZS E., KIRALY Z. Increased levels of cytokinin and indoleacetic acid in peach leaves infected with *Taphrina deformans.* Physio. Plant Pathol., **5,** 45, **1975.**
43. SZWEYKOWSKA A. Mechanizmy dziatania hormonow roslinnych. Badania i hipotezy. Wydawnictwo Naukowe UAM. Seria Biologia, **32,** 27, **1987.**
44. TAYLOR C.B. Bacteriology of fresh water. III. The types of bacteria present in lakes and streams and their realtionships to the bacterial flora in soil. J. Hyg., **42,** 284, **1942.**
45. THIMANN K.V, SACHS T. The role of cytokinins in the "fasciation" disease caused by *Corynebacterium fascians.* Amer. J. Bot., **53,** 731, **1966.**
46. THORNLEY M.J. A taxonomic study of *Acinetobacter* and related genera. J. Gen. Microbiol., **49,** 211, **1967.**
47. THORNLEY M.J. Properties of *Acinetobacter* and related genera. In, Identyfication Methods for Microbiologists. Acad. Press, London, New York. **1968.**
48. VIZAROVA G. Level of free cytokinins in susceptible and resistant cultivars of barley infected by powdery mildew. Phytopath. Z., **79,** 210, **1974.**
49. WOOD C.E. Marine microbiology ecology. Chapman and Holl Ltd, Reinhol, London, New York. **1965.**