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Biodegradation of DNA and nucleotides to nucleosides and free bases

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Abstract

Thirty-two different microorganisms were examined in order to check their ability to degrade an exogenous DNA. Bacteria from species: *Stenotrophomonas maltophilia, Brevundimonas diminuta, Bacillus subtilis, Mycobacterium butyricum* and fungus *Fusarium moniliforme* were capable to degrade DNA to nucleic bases or their derivatives. Degradation of DNA by *S. maltophilia* resulted in formation of free bases, such as hypoxanthine, thymine, uracil and xanthine. The optimum concentration of DNA seemed to be 3 mg ml^{-1}. The mode of degradation of DNA nucleotides depended on the type of nucleotide and its concentration, but nucleic bases or their derivatives were always formed at the end of the reaction process.

Keywords: DNA; Nucleotides; Degradation; *Stenotrophomonas maltophilia*; *Brevundimonas diminuta*

1. Introduction

Nucleosides and free bases are commonly used for synthesis of various drugs, such as antitumor [1], antiviral [2,3] and antibacterial drugs [4] or immunomodulators [5]. Usually they are obtained either by chemical or biological ways. For example, a Japanese Patent [6] discloses a method of hydrolysis of pyridinium nucleotides in the presence of 5′-nucleotidase yielding the respective nucleosides. Intramolecular coupling of bases with 2′-deoxyribosides to obtain nucleosides was described by Lipshuts et al. [7]. Takami et al. [8] described hydrolysis of 5′-nucleotides (AMP, GMP, CMP, UMP) in formate buffer solution yielding nucleosides with 88% efficiency. Friedman [9] described a controlled degradation of phosphate bonds in DNA by the treatment with alkylating against, such as diazomethane. During the reaction secondary esters of phosphoric acid are transformed to the corresponding tertiary alkylesters that weaken DNA backbone and enable its hydrolysis to oligonucleotides under mild conditions (2 M NaOH, 35 °C). A method of specific degradation of DNA by methylation of nucleobases and subsequent treatment with piperidine was described by Maxam and Gilbert [10] and used for DNA sequencing. In addition to the chemical methods of DNA degradation, several non-chemical methods were also described. DNA degradation was obtained with preparations of pure enzymes, for instance 5′-nucleotide phosphodiesterase from snake venom [11], endonuclease from wheat chloroplasts [12] or nucleases from tea leaves [13]. It was also found that some bacteria, such as *B. subtilis* [14] or *Prevotella* sp. [15] secrete DNA- or RNA-nucleases extracellularly.

Chmielowiec et al. [16,17] described hydrolysis of nucleotides to nucleosides and free bases by environmental bacteria–*Stenotrophomonas (Xanthomonas) maltophilia*. The authors were able to obtain all nucleosides and guanine and thymine from nucleotides. The aim of the present work was to find whether it is possible to obtain adenine and cytosine in similar experimental condition and to find microorganism, which efficiently degrades an exogenous DNA and analyse the progress of reaction to find an easy-to-use and cost effective alternative of production of nucleic bases.

The second aspect of this study refers to environmental protection. The amount of free DNA derived from human

Abbreviations: dAMP, 2′-deoxyadenosine-5′-monophosphoric acid; dCMP, 2′-deoxycytidine-5′-monophosphoric acid; dGMP, 2′-deoxyguanosine-5′-monophosphoric acid; dTMP, 2′-deoxythymidine-5′-monophosphoric acid; dA, 2′-deoxyadenosine; dC, 2′-deoxycytidine; dG, 2′-deoxyguanosine; dT, -2′-deoxythymidine; dI, -2′-deoxyinosine.

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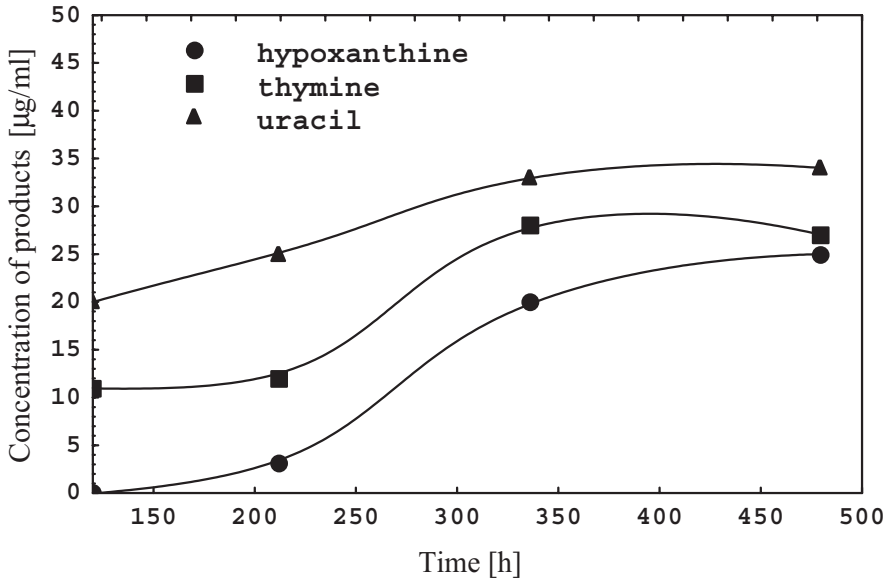


Fig. 1. The yields of hypoxanthine, thymine and uracil during the DNA (1 mg ml^{-1}) degradation by *S. maltophilia.*

environment in biological sewage plants or domestic setting tanks may be significant [18]. Even small amount of DNA, originating from the lysis of bacterial, plant or animal cells (likely genetically modified) or human/animal viruses, present in our environment may transform bacteria. A natural genetic transformation of bacteria encompasses an active uptake of free (extracellular) DNA and a heritable incorporation of the genetic information into bacterial genome [19]. It may cause bacterial resistance to antibiotics or acquisition of another new feature [20,21]. With the respect to this aspect of the present work, a discovery of microorganism, which efficiently degrades an exogenous DNA and can be included in wastewater treatment, would prevent DNA transformation in environment. Therefore, the additional aim of this study was to find DNA degrading microorganisms which could be easy included into the biological products used for the sewage treatment in biological wastewater plants or domestic setting tanks.

2. Experimental

2.1. Materials

The following microorganisms were obtained from the American Type Culture Collection: *Escherichia coli*—ATCC 8739, *Pseudomonas aeruginosa*—ATCC 15442, *B. diminuta*—ATCC 19146, *S. maltophilia* (previous name

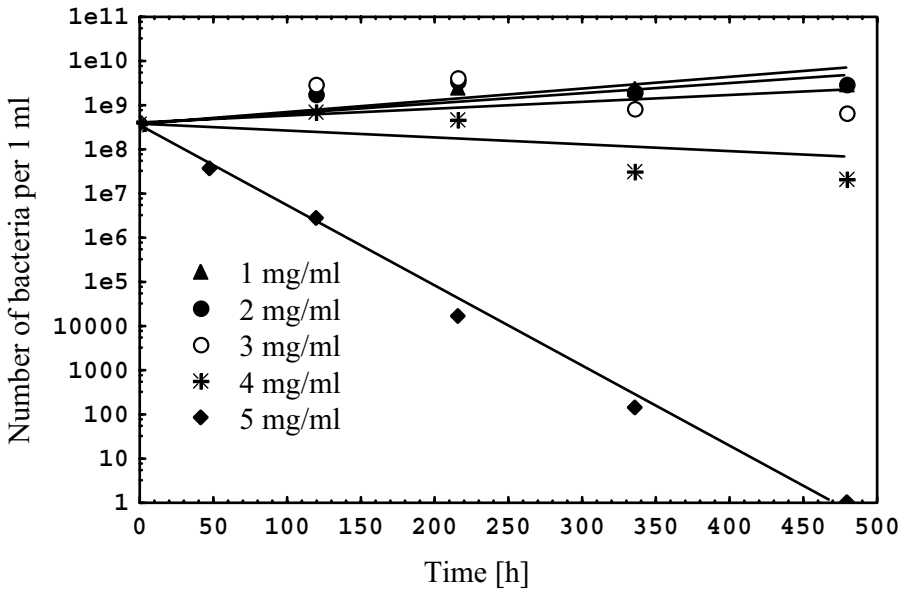


Fig. 2. The growth of *S. maltophilia* in different concentrations of DNA in reaction mixture.

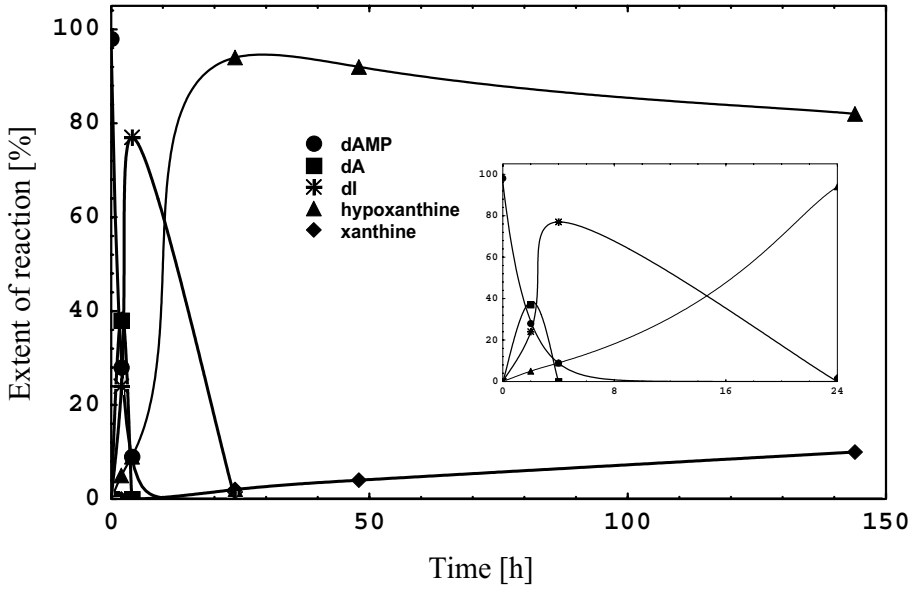


Fig. 3. Hydrolysis of dAMP (1 mg ml^{-1}) by *S. maltophilia.*

Xanthomonas maltophilia [22])—ATCC 12714, *B. subtilis* ATCC 6633, *Candida albicans* ATCC 2091, *Nocardia convoluta* ATCC 4273. *S. maltophilia* PCM 1942, *B. diminuta* PCM 2381 and *Pseudomonas saccharophila* PCM 2119 were obtained from the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences, Wrocław. *Rhodotorula rubra* 4, 7, 82, *Rhodotorula glutinis* 242, *Aphanocladium album* G 417, *Rhodotorula rosea* 4 were obtained from Wroclaw Medical Academy. The following microorganisms were obtained from the industrial strains collection of Pharmaceutical Research Institute in Warsaw: *Saccharomyces cerevisiae* TV-105, *Saccharomyces cerevisiae* YE-3, *M. butyricum* 7166, *Mycobacterium phlei, Mycobacterium rabinowitch* 636, *Mycobacterium frienburg, F. moniliforme* 1, *Nocardia* sp. 10554, *Schisosaccaromyces pombe* Y-658, *Saccharomyces chevalieri* 10-8-1, *Rodococcus equi* 4, *Arthrobacter simplex* 2232 MG. *Lactobacillus acidophilus* was obtained from the strains collection of Biomed, Kraków. The following bacteria were obtained from different sources in Warsaw area: *S. maltophilia*—from tap water, *Enterobacter sakazaki*—from pharmaceutical product and *Salmonella* sp.—from food product. Biochemical Api tests (BioMerieux) were used for bacteria identification.

All chemicals were of HPLC or analytical grade (J.T. Baker Co.). High molecular weight DNA from salmon milt, DNA nucleotides (dAMP, dCMP, dGMP, dTMP), respective nucleosides and bases were obtained from Pharma Waldhof GmbH (Dusseldorf, Germany).

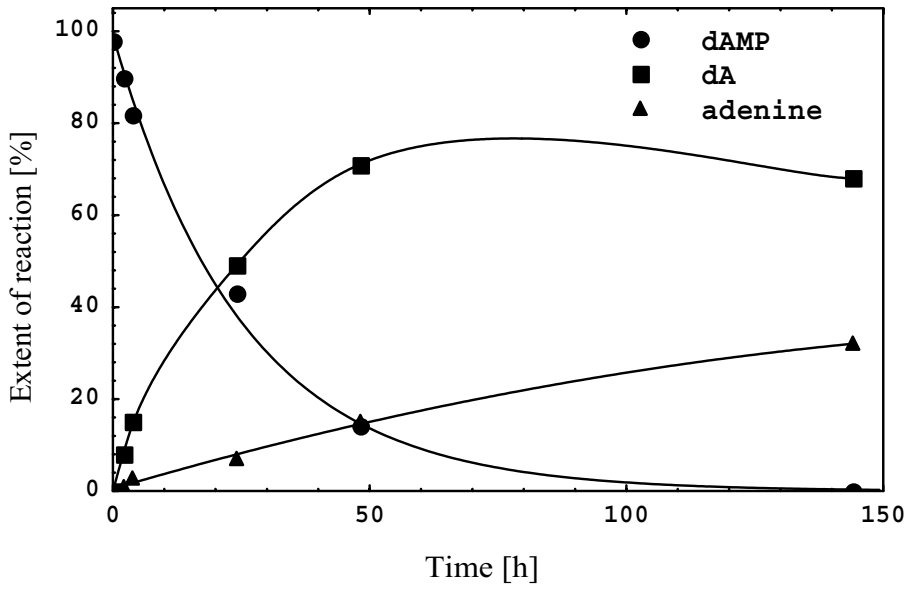


Fig. 4. Hydrolysis of dAMP (5 mg ml^{-1}) by *S. maltophilia.*

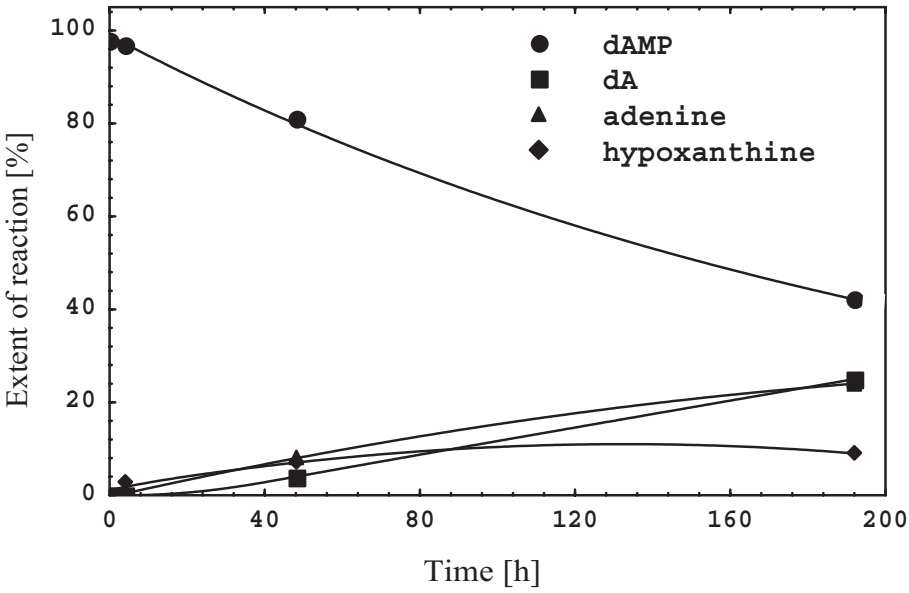


Fig. 5. Hydrolysis of dAMP (1 mg ml^{-1}) by *E. coli.*

2.2. Hydrolysis

An overnight culture of tested strain was used to inoculate 100 ml of the culture medium in 500 ml flask containing: peptone 0.6%, glucose 0.5%, pH 7.0. The culture was incubated at 30 °C, in a rotary shaker at 150 rpm for 24 h, than centrifuged at 1200 *g* for 30 min, and the appropriate amount of bacterial pellet resuspended in 50 ml of 0.08 M phosphate buffer (pH 7.0 ± 0.2) supplemented with 0.5% glucose or maltose. Bacterial colony forming units were estimated by plate-count method.

The reaction was started by addition of 50 mg of DNA or appropriate nucleotide to the 50 ml of bacteria suspension. The mixture was incubated at 30 °C in a rotary shaker at 100 rpm and samples were taken in indicated time intervals for HPLC analysis.

2.3. Method of analysis

Two hundred microlitres of the sample were centrifuged (1600 *g*, 15 min), diluted 40 times in a 0.02 M phosphate buffer (pH 4) and passed through the membrane filter (0.45 μm, ∅ = 4 mm, Cole-Palmer). Twenty microlitres of the sample were injected into Hypersil ODS column (25 cm, 4.6 mm × 5 μm) and HPLC analysis was performed on Shimadzu apparatus with spectrophotometric detector UV/vis (λ = 254 nm). An aqueous buffer (0.02 M NaH_2PO_4, pH 4) was used as a mobile phase with the flow rate 1 ml/min. For TLC analysis, 0.2 ml of the sample was centrifuged as described above and loaded on silica gel 60 F_{254} plates (Merck). The plates were developed with a 1 M NaCl as a mobile phase and read under UV light (λ = 254 nm). 'Extent of reaction' is the percentage of the particular component in

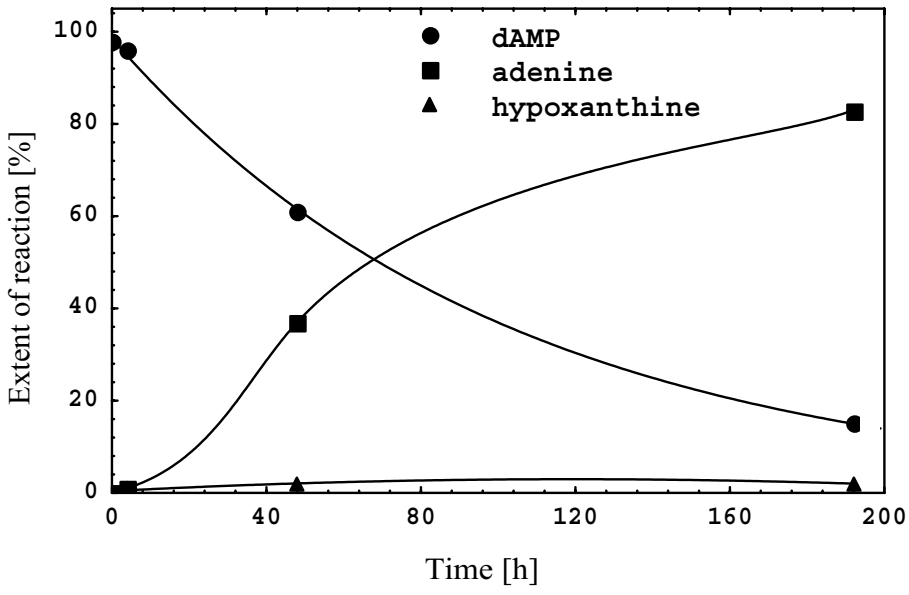


Fig. 6. Hydrolysis of dAMP (1 mg ml^{-1}) by *Lactobacillus acidophilus.*

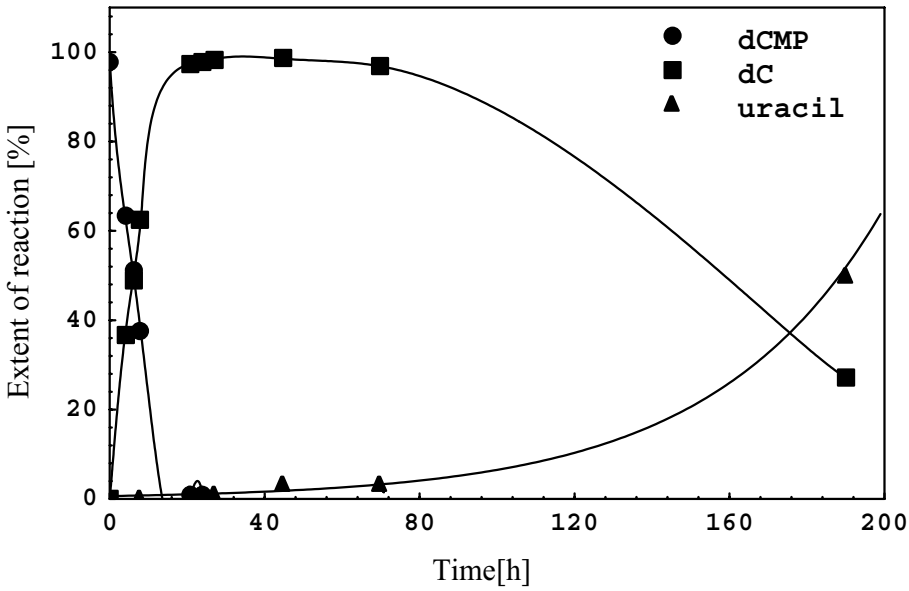


Fig. 7. Hydrolysis of dCMP (1 mg ml^{-1}) by *S. maltophilia.*

the reaction mixture (Figs. 3–7). The quantitive determination of reaction product was done by the external standard calibration method (Fig. 1 and Tables 1 and 2).

3. Results and discussion

Thirty two microorganisms (bacteria, yeast and moulds) were analysed for their ability to degrade an exogenous DNA. Only five species of tested microorganisms were able to degrade 1 mg ml^{-1} DNA (bacteria *B. subtilis, B. diminuta, M. utyricum, S. maltophilia* and fungus *F. moniliforme*). DNA in this concentration did not influence growth of any tested strains. The highest rate of degradation of high molecular weight DNA to short oligonucleotides was found for *B. diminuta* and *S. maltophilia* (96 h). After 480 h of hydrolysis only nucleic bases or its derivatives, usually thymine, hypoxanthine, xanthine, uracil and cytosine accumulated as the product of degradation (Table 1).

Among the bacterial strains that exhibited the highest rate of DNA degradation, the strain of *S. maltophilia* isolated from tap water was chosen for further study (all three tested strains of *S maltophilia* degraded 1 mg ml^{-1} DNA in the same manner). To elaborate the ability of *S. maltophilia* to degrade DNA in higher concentration, the reaction was performed at five different concentrations of DNA (1–5 mg ml^{-1}). Interestingly, mode of degradation depended on the DNA concentration. DNA in concentration 1 mg ml^{-1} was degraded to nucleic bases or its derivatives after 480 h of incubation. The amount of nucleic bases increased gradually and accumulated, that might be due to the lack of ability of studied bacteria to degrade these compounds (Fig. 1). However, when reaction mixture contained 2 mg ml^{-1} DNA, in addition to nucleic bases in the reaction mixture, we also found nucleosides (dT and dA). When reaction mixture contained 3 mg ml^{-1} DNA, additional nucleosides (dA, dT, dG, dC and dI) were found. It seems that at low DNA concentrations,

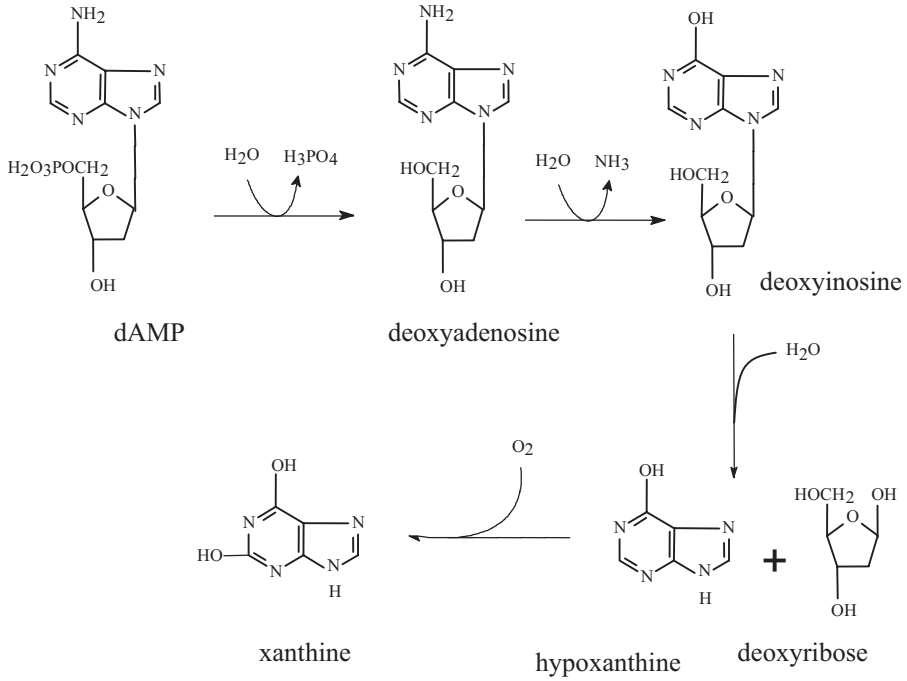
Table 1
Products of DNA (1 mg ml^{-1}) degradation by microorganisms after 480 h of reaction

Strain	Products of degradation (μg ml^{-1})				
	Thymine	Hypoxanthine	Xantine	Cytosine	Uracil
B. diminuta [a]	17	24	–	6	42
B. subtilis	56	58	47	3	27
F. monilifirme	49	–	–	–	40
M. butyricum	–	11	24	–	–
S. maltophilia	32	29	30	–	33

[a] It was found 14 μg ml^{-1}guanine.

Table 2
Products of DNA degradation by *S. maltophilia* after 480 h of reaction in different DNA concentration

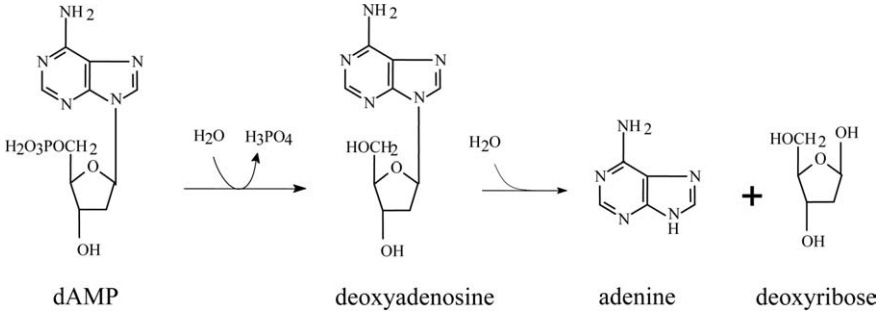
DNA concentration (mg ml^{-1})	Products of degradation (μg ml^{-1})								
	dA	dG	dT	dC	dI	Hyp	Thy	Xan	Ura
1	0	0	0	0	0	25	27	34	34
2	7	0	29	0	0	92	66	85	60
3	30	23	163	3	3	205	105	167	83



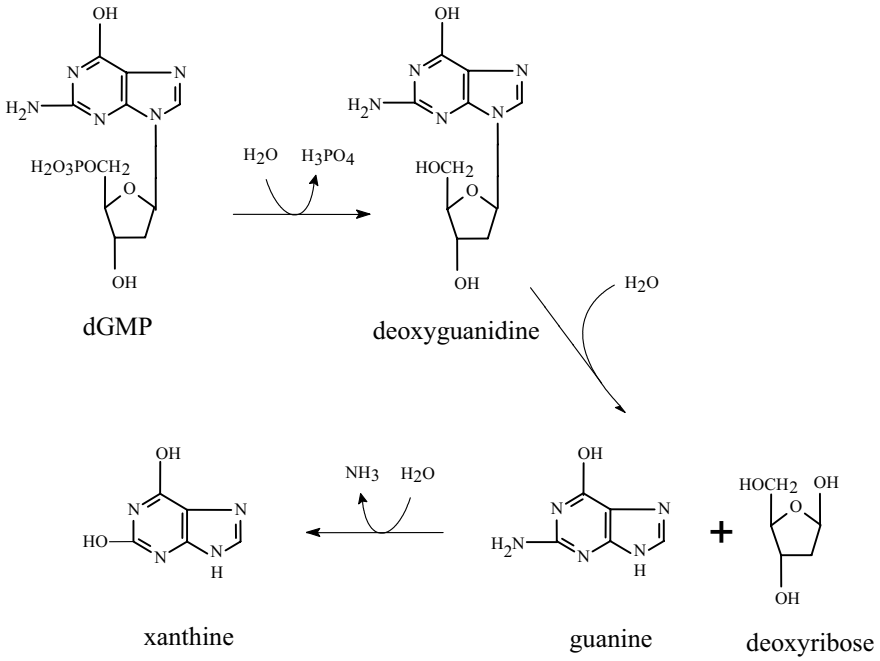
Scheme 1

arising nucleosides were immediately degraded do the corresponding bases, hence, no accumulation of nucleosides was observed. At the higher concentrations of DNA, production of nucleosides was more intense than their degradation, resulting in their accumulation in the reaction mixture. Interestingly, some nucleosides were degraded preferentially, since only dA and dT were found after 480 h of incubation, when the reaction mixture contained 2 mg ml^{-1} DNA (Table 2). In the sample containing 4 mg ml^{-1} DNA, oligonucleotides were still present in the reaction mixture. Interestingly, an exogenous DNA in concentration 5 mg ml^{-1} inhibited growth of bacteria and caused a total lysis at 480th h of reaction. For comparison, when DNA was added in concentration 4 mg ml^{-1} only small inhibition of growth was observed (Fig. 2).

To obtain some insight on DNA degradation process and to explain why adenine and cytosine do not accumulate during the DNA hydrolysis, degradation of dAMP and dCMP by the same bacterial strain was studied. We found that similarly to DNA, dAMP and dCMP in concentration 5 mg ml^{-1} inhibited growth of *S. maltophilia*. The total lysis of bacteria was observed 144 h after the addition of nucleotide. The progress of degradation of dAMP in concentration 1 and 5 mg ml^{-1} is shown in Figs. 3 and 4. The proposed hydrolysis reaction is shown in Scheme 1 for concentration dAMP equal and below 2 mg ml^{-1} and in Scheme 2 for concentration of dAMP 5 mg ml^{-1}. For the dAMP concentrations below 2 mg ml^{-1} reaction run in four steps. Initially formed deoxyadenosine was subsequently deaminated to deoxyinosine then hydrolysed to hypoxanthine and oxidised to xanthine. Deamination of adenosine to inosine was also found by Elshafei et al. [23] during the degradation of adenosine by extracts of *Aspergillus terricola*. When the initial concentration of dAMP was 5 mg ml^{-1}, the degradation of dAMP by *S. maltophilia* proceeded to adenosine and than to adenine. We hypothesise that in this case lysis of bacteria caused by 5 mg ml^{-1} dAMP resulted in the release of constitutive, intracellular nucleases to the reaction medium. Interestingly, another Gram (–) rod—*E. coli*, hydrolysed dAMP (1 mg ml^{-1}) very slowly; at 216 h of reaction only 60% of



Scheme 2



Scheme 3

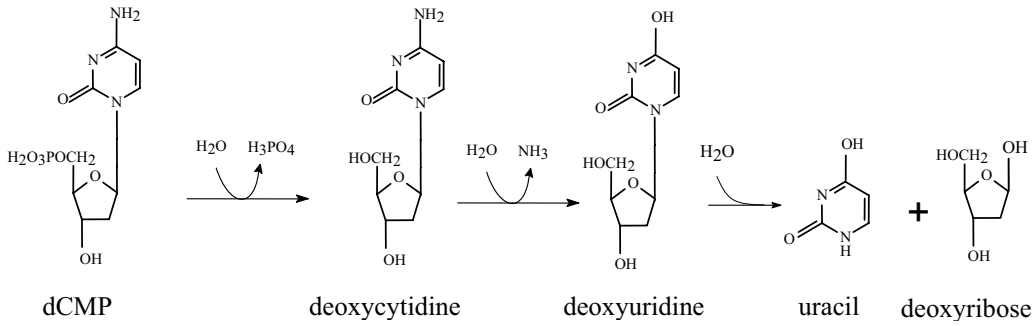
dAMP was hydrolysed, forming small amounts of adenosine (25%), hypoxanthine (10%) and adenine (24 %) (Fig. 5). On the contrary, Gram (+) rods—*L. acidophilus* hydrolysed dAMP directly to adenine (Fig. 6).

As previously described another purine nucleotide – dGMP was degraded by *S. maltophilia* directly to guanine [16], but after 192 h of reaction guanine was deaminated to xanthine, and after 240 h of reaction 70 % of obtained product was xanthine (Scheme 3). The nucleotide dCMP was degraded to deoxycytidine, but unexpectedly in the reaction mixture instead of cytosine–uracil was found (Fig. 7). During the reaction very small amount of deoxyuridine was found (1.5–3.4%). By the parallel to the degradation of dAMP, we suggest that *S. maltophilia* degrades dCMP through a deamination step, i.e. initially formed deoxycytidine is subsequently deaminated to deoxyuridine and then hydrolysed to uracil. The proposed degradation reaction is shown in Scheme 4. The pyrimidine nucleotide dTMP was hydrolysed to deoxythymidine and then to a free thymine [16]. Thus, the analysis of hydrolysis of nucleotides by *S. maltophilia* supported the results obtained for hydrolysis of DNA.

Since 4 mg ml^{-1} of DNA inhibited growth of *S. maltophilia* (Fig. 2), thus influenced the degradation process, the optimal concentration of DNA to obtain free bases, such as hypoxanthine, thymine, uracil and xanthine, seemed to be 3 mg ml^{-1}.

In the given experimental condition, it is not possible to obtain adenine and cytosine during the hydrolysis of 1 mg ml^{-1} dAMP and dCMP by *S. maltophilia*, as dAMP was finally degraded to hypoxanthine and xanthine and dCMP was finally degraded to uracil.

Concerning the environmental problems, we found that *S. maltophilia* and *B. diminuta*, which grow well in different environmental conditions, degraded DNA reasonably quickly. Typical biological products used for sewage treatment predominantly contain only protein, fat and carbohydrates degrading microorganisms. Incorporation of DNA degrading bacteria such as *S. maltophilia* and *B. diminuta* into the products used for the sewage treatment in biological wastewater plants or domestic setting tanks may be beneficial and could eliminate the risk of acquisition of drug resistance by pathogenic bacteria in course of transformation process.



Scheme 4

4. Conclusions

The concentration 3 mg ml^{-1} DNA seems to be optimal to obtain free bases like hypoxanthine, thymine, uracil and xanthine in quite mild condition, during the degradation process of DNA by *S. maltophilia*.

The manner of degradation of DNA nucleotides depended on the type of nucleotide and its concentration, but nucleic bases or their derivatives were always formed at the end of the reaction process.

S. maltophilia and *B. diminuta* degraded DNA to nucleic bases or their derivatives in the shortest time, thus they could be useful for the sewage treatment.

References

[1] A. Matsuda, A. Don, N. Mikanawa, S.J. Tregear, S. Okazaki, Y. Sugimato, et al., Synthesis of 1-(2-deoxy-2-isocyano β-D-arabinofuranosyl)-cytosine and related nucleosides as a potential antitumor agents, J. Med. Chem 36 (1993) 4190–4194.

[2] W.S. Mungall, L.J. Lemmen, K.L. Lemmen, J.K. Dethmers, L.L. Norling, Nucleoside 5′-monophosphate analogues, J. Med. Chem 21 (1978) 704–706.

[3] K.G. Nicholson, Antiviral agents, in: F. Grady, H.P. Lambert, R.G. Finch, D. Greenwood (Eds.) New York, Edinburgh, London, Antibiotics and Chemotherapy, Churchill-Livingstone, 1997.

[4] H. Osada, K. Isono, Mechanism of action and selective toxicity of ascamycin, a nucleoside antibiotic, Antimicrob. Agents Chemother 27 (1985) 230–233.

[5] G.E. Hardart, G.W. Sullivan, H.T. Carper, G.L. Mandell, Adenosine and 2-phenylaminoadenosine (CV-1808) inhibit human neutrophil bactericidal function, Infect. Immun 59 (1991) 885–889.

[6] K. Shichiro, T. Shuichi, S. Akio, Pharmaceutical pyridine nucleoside derivatives—prepared by treating pyridine nucleotide derivatives with nucleotidase Japanese Patent Application, Japan kokai 7826396 (29840A/16; J5 3026–3096) (Chem. Abstr. 89 (1978) P44137s). 1976.

[7] B.H. Lipshuts, H. Hayakawa, K. Kato, R.F. Lowe, K.L. Stevens, A novel route to nucleosides via intramolecular coupling of bases with 2′-deoxyribosides: quick and stereospecific...but with a 'twist', Synthesis 12 (1994) 1476–1484.

[8] A. Takami, M. Imazawa, M. Irie, Ch. Ukida, A preparation of nucleosides by the hydrolysis of 5′-nucleotides, Yakugaku Zasshi 85 (1965) 658–661 (Chem. Abstr. 67 (1967) 44047h).

[9] O.M. Friedman, Controlled degradation of phosphoric acid links in deoxyribose nucleic acid USA Patent Application 3010954. 1966.

[10] A.M. Maxam, W. Gilbert, A new method for sequencing DNA, Proc. Nat. Acad. Sci. 74 (1977) 560–564.

[11] F.R. Bryant, S.J. Benkovic, Stereochemical course of the reaction catalyzed by 5′-nucleotide phosphodiesterase from snake venom, Biochemistry 18 (1979) 2825–2828.

[12] E. Kuligowska, D. Klarkowska, J.W. Szarkowski, Purification and properties of endonuclease from wheat chloroplasts, specific for single-stranded DNA, Phytochemistry 27 (1988) 1275–1280.

[13] H. Imagawa, H. Toryu, T. Ozawa, Purification and characterization of nucleases from tea leaves, Agricul. Biol. Chem 46 (1982) 1261–1270.

[14] H. Joenje, G. Venema, Different nuclease activities in competent and noncompetent *Bacillus subtilis*, J. Bacteriol 122 (1975) 25–33.

[15] S. Stubbs, M. Lewis, R. Waddington, G. Embery, Hydrolytic and depolymerising enzyme activity of Prevotella intermedia and Prevotella nigrescens, Oral Dis 2 (1996) 272.

[16] U. Chmielowiec, H. Kruszewska, J. Cybulski, Selective hydrolysis of nucleotides to nucleosides and free bases, Il Farmaco 54 (1999) 611–614.

[17] U. Chmielowiec, H. Kruszewska, J. Cybulski, A. Głowacka, Method of hydrolysis of nucleotides derived from DNA and production of nucleosides and free amines Polish Patent Application PL 183827 B1. 1996.

[18] J.W. Blackburn, R.K. Jain, G.S. Sayler, Molecular microbial ecology of a naphthalene-degrading genotype in activated sludge, Environ. Sci. Technol 21 (1987) 884–890.

[19] M.G. Lorenz, W. Wackernagel, Bacterial gene transfer by natural genetic transformation in the environment, Microbiol. Rev 58 (1994) 563–602.

[20] S. Nakamura, H. Shirota, Behaviour of drug resistant faecal coliforms and R plasmids in a wastewater treatment plant, Nippon Koshu Eisei Zasshi 37 (1990) 83–90.

[21] J. Trevors, Molecular evolution in bacteria, Antonie van Leeuwenhoek 67 (1995) 315–324.

[22] N.J. Palleroni, J.F. Bradbury, *Stenotrophomonas*, a new bacterial genus for *Xanthomonas maltophilia* (Hugh 1980) Swings et al. 1983, Int. J. Syst. Bacteriol 43 (1993) 606–609.

[23] A.M. Elshafei, M.R. Abbu-Shady, F.M. El-Beih, L.A. Mohamed, Mode and extent of degradation of adenosine and guanosine by extracts of *Aspergillus terricola*, Microbiol. Res. 150 (1995) 291–295.