Biotechnology in Medicine, Foodstuffs, Biocatalysis, Environment and Biogeotechnology

Sergey D. Varfolomeev
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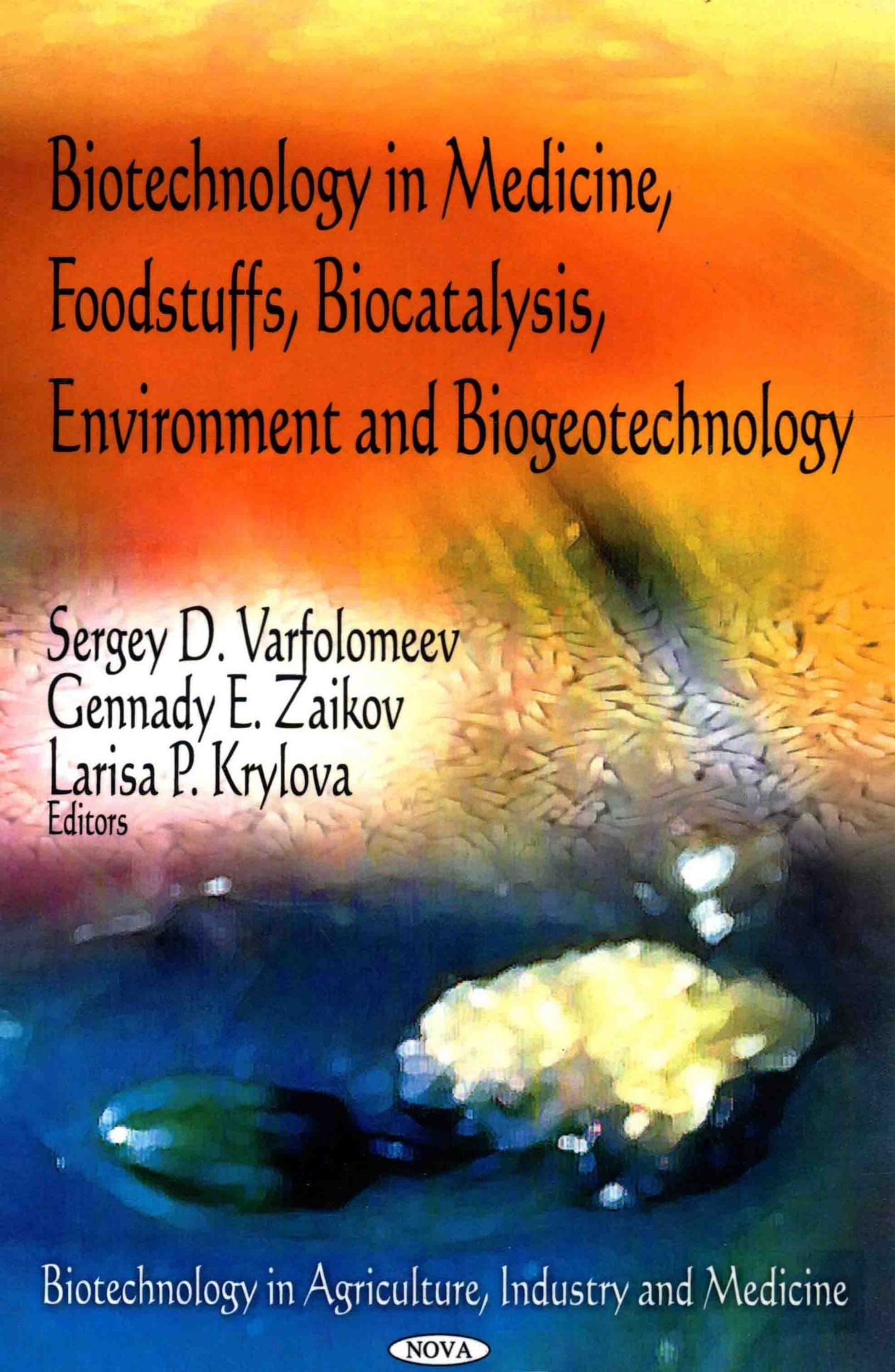

Biotechnology in Agriculture, Industry and Medicine

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BIOTECHNOLOGY IN AGRICULTURE, INDUSTRY AND MEDICINE

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PREFACE

Chapter 1 - In this study, we have engineered *Escherichia coli* strains overproducing recombinant enzymes: uridine phosphorylase, thymidine phosphorylase, purine nucleoside phosphorylase, adenosine deaminase, lysyl-tRNA synthetase and nucleoside phosphotransferase. The whole cells or isolated enzymes were applied as biocatalysts for the synthesis of compounds of pharmaceutical interest, particularly arabinosides of adenine and guanine, ribosides of thymine, 6-furfurylaminopurine (kinetin) and 6-benzylaminopurine, 5-bromovinyl-2′-deoxyuridine, 2′,3′-dideoxy-adenosine and -guanosine, arabinofuranosyladenine-5′-monophosphate, diadenosine-5′,5‴-P^1,P^4-tetraphosphate (Ap_4A) *etc*. Under optimal conditions the indicated compounds were formed in the reaction mixtures in 55–96% yields after 1.5–7 h.

Chapter 2 - The purpose of the present study was to receive the sp-2 protein possessing antitumor activity from soybean cake. To determine the antitumor activity of the sp-2 protein we used a model test system for screening of antitumor substances in vitro on cell lines of KML mice melanoma (mice melanoma B-16 culture). The dose of 100.0 mcg/ml caused suppression of marker incorporation by 58,5%.The research of influence of sp-2 protein on cancer cells used tumor cells samples obtained from biopsy and operational material of patients with verified breast cancer diagnosis, undergoing treatment at the National Cancer Research Center of Ministry of Health of the Republic of Uzbekistan, Tashkent.

Sp-2 soy protein has a significant antitumor effect on breast cancer cells with absent or reduced expression of receptors to estrogen and progesterone.

Chapter 3 - The analyses of lipid peroxidation demonstrate the absence of authentic pathological changes and their manifestations upon introduction of phytohormone epibrassinolide and preparations on its basis into the organism of mammals in the period of early ontogenesis. The biologically active substance epibrassinolide due to its revealed antioxidant properties contributes to stabilization and approximation of the whole complex of physiological parameters of vertebrates to the testing level in early ontogenesis in case vertebrates are kept in unfavorable conditions of the environment or under experimental influence of different toxic materials.

Chapter 4 - This article presents the practice of lavage and perfusion of wounds and cavities by oxygenated solutions in surgical patients during the early post-operation period for the prevention and treatment of complications. Balanced oxygenated electrolytic solutions containing antibiotics and heparin improve tissue respiration in wounds and cavities, wash out necrotoxins and fibrin, and step up tissue regeneration.

Hyperbaric oxygenation is a key factor in the application of the method based on diffusion and convection. A solution is activated by UV irradiation with its temperature stabilized.

Recurrent perfusions by oxygenated solutions speed up microcirculation in cavity tissue, leading to an early cavity closing and a smaller wound size. By activating coagulation, oxygenated solutions stop capillary bleeding without hindering granulation.

Being an essential supplement to standard therapy, the method neither replaces nor excludes other types of treatment and surgery. However, it speeds up regeneration considerably.

Chapter 5 - A peculiar method for immobilization of the whole non-growing microorganisms by entrapment of microbial biomass into silica xerogel was developed. The silica hydrogel was prepared by a sol-gel procedure and then was used as a precursor for preparation of the heterogeneous biocatalysts. The optimal compositions (ratio of microbial biomass to SiO_2) of the biocatalysts with the high enzymatic activity and stability were found to depend strongly on the species of microorganisms used. As an example, for bacteria Arthrobacter nicotianae the biomass content did not exceed 15 wt. % of dry-weight substances. In this case, the inactivation half-time of the biocatalyst with glucose isomerase activity was more than 500 h of continuous operation in monosaccharide isomerization at 65±5°C. As another example, the biocatalysts based on the bakers' yeast contained up to 80 wt. % of dry-weight microbial biomass, and the invertase activity of these biocatalysts reached 600 U/g at 50°C.

Chapter 6 - The present work is devoted to development of β-galactosidase immobilization on the basis of organosilicone templates with a method of a porous structure formation to product stable preparations with high percent of activity preservation.

The obtained preparations can be used in medical diagnosis for the definition of some tumours of a digestive tube and also in the food-processing industry for making of delactosed and lowlactosed food stuffs on the base of milk.

Chapter 7 - Glycolysis was investigated as a source of energy for microbiological synthesis of nucleosidephosphates (ATP,GTP). Model experiments on cell-free extracts of producer-strains of nucleosidephosphates and NAD belonging of Corynebacterium were used.

Chapter 8 - Employment of two independent approaches revealed that O-palmitoyl-L-malate does not penetrate through plasma membrane of *Saccharomyces cerevisiae* cells. This substrate derivative completely inhibited yeast succinate respiration. Linear behavior of the dependence of the respiration rate on inhibitor concentrations in Dixon plots suggests that plasmalemma transport is the rate limiting step for succinate oxidation and transport of this substrate involves a single type of transporter. This rapid test is applicable at any changes of assay conditions. Affinity of this transporter for succinate was evaluated using endogenous coupled mitochondrial succinate oxidase system of cells, which were grown under conditions favoring mitochondria proliferation. At pH 6.5 the K_m value for the plasmalemmal transporter was one order of magnitude higher then the K_m value for mitochondrial dicarboxylate transporter determined using mitochondria preparations, whereas the activity of the plasmalemmal transporter was rather low. Consequently, during succinate oxidation in yeasts the endogenous coupled system of cells maintains low concentration of this substrate in cytoplasm. This is independent evidence that oxidation is limited by plasmalemmal transport.

Chapter 9 - In order to receive stable and highly active heterogeneous biocatalysts for the industrial manufacture of functional nutrition products and pharmaceutical preparations,

immobilization of inulinase, lipase and glucoamylase has been carried out by various methods on synthetic and natural carriers. It has been shown that inulinase, adsorbed on macroporous anion-exchange rezin AV-17-2P, and lipase, adsorbed on high-molecular hytozan, have the highest catalytical activity.

It is established that at the creation of heterogeneous preparations of glucoamylase, not only should a percent of activity preservation act as leading criterion, but a durability degree of a complex enzyme-carrier too, because apart from inulinase and lipase, this parameter for glucoamylase varies strongly. Thus at industrial scaling of starch hydrolysis by enzymes, we would recommend the use of ion-exchangers KB-2e-0,5 and KB-4-10P as carriers for adsorption and immobilization of glucoamylase and ion-exchangers KB-2 and KB-4 as carriers for covalent immobilization of glucoamylase by the aside method.

Chapter 10 - Taking into account the ability of chemical analogues of microbial auto-regulatory factors to alter the catalytic activity of hydrolases, we have studied thermodynamic aspects of influence of one of the homologues (methylresorcinol – MR) on the conformational stability of lysozyme while in storage of their combined solutions. The range of methylresorcinol concentrations (0.2-20 mg/ml at the molar ratio MR-lysozyme r = 5-422), as well as the conditions for carrying out modifications (I hr incubation at 5°C), which raise 200% - 470% the activity of lysozyme regarding a non-specific substrate - colloid chitin, have been selected. Increase in the time of enzyme modification up to 120 hours has been shown to result in a significant drop of an activating effect of MR up to the maximum value 135% of the control. It has been revealed that dependence of the molar enthalpy of lysozyme denaturation acquires a weakly defined extreme nature during storage; a more significant augmentation of difference between the heat capacity of a native and a denatured state of the protein (ΔC_p) taking place. Introduction of methylresorcinol causes the same drop in temperature of lysozyme denaturation irrespective of the incubation time. The obtained data provide evidence that under a long-term exposure of lysozyme in a combined solution with MR, hydrophobic hydration of the protein grows and the cooperative unit of a conformational transition alters.

Chapter 11 - The paper presented observed the ultrasonic low-frequency (30kHz) extraction and investigates the basic physicochemical properties of flax seed polysaccharides.

Chapter 12 - There has been evaluated the AOA of a number of medicinal, synthesized preparations, BAAs and medicinal herbs intended for prophylactic and treatment of illnesses of the cardiac-vascular system, motor organs and digestion tract. For the first time, there has been obtained quantitative data on fat- and water-soluble antioxidants (AO) in the investigated preparations. These data fluctuate for fat-soluble AO in the range from $9.4 \cdot 10^{-4}$ to $4.3 \cdot 10^{-2}$ mole/kg and for water-soluble AO in the range from $5.1 \cdot 10^{-4}$ to $1.6 \cdot 10^{-2}$ mole/kg. In the main, samples contain more than $1.0 \cdot 10^{-3}$ mole/kg of AO, i.e. are a potential source of AO for the organism.

Chapter 13 - In the presented work, we studied the influence of high frequency noise radiation of low intensity (EHF), with frequency range from 42 to 100 GHZ and flow density of impulse power of $5H10^{-10}$ megaWtHh/sm^2, on spores and mycelia of microscopic fungi and vegetative cells, spores of gram-negative and gram-positive bacteria; as well as on water solutions of different disinfecting agents.

It was shown that the influence of EHF-radiation on spores of microscopic fungi can cause their destruction, at the same time, vegetative fungi mycelium is not sensible to the influence of this kind of radiation. The influence of EHF-radiation on vegetative spores and

cells of gram-positive bacteria can also cause their death. Thus, this diapason of EHF-radiation can be recommended for creation of sterilizing devices for the needs of biotechnological manufacturing facilities. It can also be used for intensifying antimicrobial activity of water soluble technical biocides, used in production facilities and at home.

Chapter 14 - The purpose of this work is callus induction in the medicinal plants. The callus-formating ability of cells and tissues of rare and endangered medical plants *as Convallaria transcaucasica* Utkin ex Grossh., *Adonis vernalis*L., *Paeonia tenuifolia* L. are studied. We obtained the callus from fragments of the leaves, petioles, inflorescences, buds of all studied plants.

Chapter 15 - During the research work we developed biotechnologies of gonads and germ cell cryoconservation of commercially valuable fish species of the Volga-Caspian basin for the purpose of their genetic preservation. Cytomorphological and histological analyses of defrosted male gonads of various fish species have shown vitality of the cell material, and so the possibility of their cultivation in vitro. During the research we developed some isolation methods for the cells, composed culture media and selected optimal cultural conditions sustaining high biomass production.

Chapter 16 - A combined EOR method integrates total effect resulting from oil-washing capacity of surfactant- based systems and activated hydrocarbon-oxidizing reservoir microflora. Surfactants and nitrogen-containing compounds, composing ammonium buffer solution, are the components of the systems. Nitrogen-containing compounds serve as a nutrient substrate stimulating reservoir biocenosis. Variants were developed taking into account geochemical and microbiological conditions of viscous oil deposit in White Tiger oil field, Vietnam. The combined EOR technology provides for active and purposeful development of microbiological processes generating microbial metabolites *in situ.*

The studies of filtration characteristics and oil displacement capacity of the proposed method as applied to "White Tiger" oil field were conducted at the Laboratory for Simulating Processes of Subsurface Hydrodynamics, Research Institute for Offshore Oil & Gas Development, Joint Venture "VIETSOVPETRO" using a core unit. The efficiency oil displacement with sea-water amounted to 44 %. Due to employment of the combined EOR method the efficiency increased up to 57 %.

Chapter 17 - Stimulating effect of polyethylene photoluminescence films on the growth of autochthonous microflora population in oil-polluted soils has been determined under the laboratory conditions, where oil concentration in the soil amounted to 50 g/kg. The increase in number of basic groups of microflora was accompanied with the increased activity of the ferments, which catalyzed oxidizing processes. At the end of the experiment, on the 30^{th} day, oil content decreased to 15 g/kg. The analysis of the residual hydrocarbons by IR-spectroscopy has revealed absorption bands in the regions of 1710 cm^{-1} and 1600 cm^{-1}. Chromatographic analysis proved the intensity of hydrocarbon biodegradation. Hydrocarbons C_{11}-C_{14} completely eliminated in the test samples and concentration of hydrocarbons with a great molecular weight decreased by 70-80 %. At the same time hydrocarbon biodegradation index, determined as the ratio of the sum of isoprenoids (pristine + phytane) to the sum of n-alkanes (C_{17}+C_{18}), increased 5-6 times due to the use of photoluminescence films.

CONTENTS

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Chapter 1

APPLICATION OF RECOMBINANT ENZYMES FOR THE SYNTHESIS OF PHARMACEUTICALLY VALUABLE NUCLEOSIDES AND NUCLEOTIDES

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ABSTRACT

In this study, we have engineered *Escherichia coli* strains overproducing recombinant enzymes: uridine phosphorylase, thymidine phosphorylase, purine nucleoside phosphorylase, adenosine deaminase, lysyl-tRNA synthetase and nucleoside phosphotransferase. The whole cells or isolated enzymes were applied as biocatalysts for the synthesis of compounds of pharmaceutical interest, particularly arabinosides of adenine and guanine, ribosides of thymine, 6-furfurylaminopurine (kinetin) and 6-benzylaminopurine, 5-bromovinyl-2′-deoxyuridine, 2′,3′-dideoxy-adenosine and -guanosine, arabinofuranosyladenine-5′-monophosphate, diadenosine-5′,5‴-P^1,P^4-tetraphosphate (Ap_4A) *etc.* Under optimal conditions the indicated compounds were formed in the reaction mixtures in 55–96% yields after 1.5–7 h.

Keywords: nucleoside; nucleoside-5′-monophosphate; diadenosine-5′,5‴-P^1,P^4-tetraphosphate; recombinant enzyme; enzymatic transformation; *Escherichia coli*

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INTRODUCTION

Natural nucleosides and nucleotides and their modified analogues either directly or as intermediates are of great pharmaceutical interest as antiviral and antitumor agents and as precursors in preparation of antisense oligonucleotides for diagnostic or therapeutic use [1, 2].

Nucleosides and nucleotides bearing different modifications either on the ribofuranosyl moiety or on the sugar-linked heterocyclic nitrogen bases can be prepared by chemical synthesis through multistage processes requiring protection and deprotection of labile groups [3, 4]. These synthetic approaches are often plagued by formation of regio- and stereochemical isomers and by low overall yields (for a review, see [5]).

An alternative process that involves the enzymatic or microbiological synthesis has been applied during recent years in order to obtain simply and efficiently modified nucleosides and nucleotides in a stereospecific way. The results were exhaustively reviewed in [6, 7].

We previously reported a chemo-enzymatic method for synthesis of natural and modified nucleosides and nucleotides by enzymes produced by wild-type or mutant bacteria of different genera [8–10]. In this study, we have engineered *Escherichia coli* strains which overproduce a set of recombinant bacterial enzymes. These enzymes were applied as biocatalysts for the synthesis of some pharmaceutically useful nucleosides and nucleotides.

MATERIALS AND METHODS

General

Kanamycin, ampicillin, isopropyl-β-thiogalactopyranoside (IPTG), ATP, *p*-nitrophenylphosphate (*p*-NPP), nucleosides and nucleobases were purchased from Sigma-Aldrich (USA). Inorganic pyrophosphatase, T4 DNA ligase, restriction endonucleases (*Eco*RI, *Nde*I, *Sal*I and *Bam*HI), *Taq* and *Pfu* DNA polymerases were purchased from Fermentas (Lithuania). Cultural media chemicals were from Merck (Germany).

The course of reactions was routinely monitored by high-performance liquid chromatography (LKB, Sweden; column Ultropac TSK ODS-120T) and thin-layer chromatography (TLC) on Merck F_{254} silica gel 60 aluminium sheets as described in [8, 11, 12]. Centrifugation steps were performed on a Beckman Coulter Allegra 25R apparatus. UV spectra were recorded with UV-1202 spectrophotometer (Shimadzu Corporation). SDS polyacrylamide gel electrophoresis was carried out by the method of Laemmli [13].

Analytical Methods

Activities of uridine phosphorylase (UPase), thymidine phosphorylase (TPase) and purine nucleoside phosphorylase (PNPase) were determined spectrophotometrically according to [14] using uridine, thymidine and inosine as substrates. Adenosine deaminase (ADase) activity was assayed by the rate of adenosine transformation into inosine [15]. Nucleoside phosphotransferase (NPTase) activity was measured *via* adenosine phosphorylation rate, using *p*-NPP as phosphate donor as described earlier [12].

Lysyl-tRNA synthetase (LysU) was assayed in a standard reaction mixture (1 ml) containing 20 mM MOPS-KOH (pH 8.0), 10 mM $MgCl_2$, 160 μM $ZnSO_4$, 4.3 mM ATP, 2.4 mM L-lysine and 0.5 units of inorganic pyrophosphatase. The reaction mixture was incubated for 5 min at 42°C. Aliquots of the reaction medium were analyzed by TLC, using solvent system dioxane/25% aq. ammonia/water, 6:1:4 (v/v). The amount of each enzyme producing 1 nmol of product per min was taken to be one unit.

The protein concentration was determined by modified Lowry method [16] with bovine serum albumin as the standard.

Construction of Recombinant Stains

Construction of *E. coli* strain overproducing *Erw. herbicola* NPTase, cultivation and preparation of the enzyme solution were performed as described previously [17].

Microorganisms, Plasmids, and Media

E. coli strain DH5α (Stratagene, USA) was used for gene cloning. *E. coli* strain BL21(DE3) (Stratagene, USA) was chosen for gene expression. The vector pET24b+ (Novagen, USA) was applied for *udp*, *tpp*, *pup* and *add* genes expression. The vector pRsetB (Invitrogen, USA) was used for *lysU* gene expression. *E. coli* strains harboring the expression plasmids were grown at 37°C with orbital shaking at 200 rpm in 250 ml Erlenmeyer flasks containing 50 ml of Luria-Bertani (LB) culture medium: 1% (w/v) tryptone, 0.5% (w/v) yeast extract and 0.5% (w/v) NaCl in deionized water adjusted to pH 7 with KOH. Also in the medium when necessary, 50 μg/ml kanamycin for *E. coli* containing plasmid pET24b+ and 150 μg/ml ampicillin for *E. coli* containing plasmid pRsetB were added, respectively, to inhibit the growth of plasmid-free cells. Cultivation was continued until the optical density at 600 nm reached 0.6. Then IPTG up to concentration of 0.2–1 mM was added and the fermentation was carried out for another 4–5 h. Cells were harvested by centrifugation for 10 min at 12,000 × g, washed once with 30 mM potassium phosphate buffer (pH 7) and recentrifuged. The cells of all recombinant stains except the strain producing LysU were directly used as biocatalysts.

The biomass production was determined from the absorbance values at 600 nm obtained in the cell culture medium. Previously, a straight line was obtained by plotting the absorbance at 600 nm versus concentration (mg/ml).

Recombinant DNA Techniques

Expression plasmids encoding UPase, TPase, PNPase, ADase and LysU were constructed accordingly to standard molecular biology protocols [18]. Genes encoding UPase (*udp*), TPase (*tpp*), PNPase (*pup*), ADase (*add*) and LysU (*lysU*) were amplified by the polymerase chain reactions (PCR) from the chromosomal DNA of *E. coli* strain BMT-4D/1A [8] using the following synthetic primers:

5′-GGAATT***CATATG***TCCAAGTCTGATGTTTTTC-3′ and 5′-ACGC***GTCGAC***GAATTACA-GCAGACGACGCGCCGC-3′ for *udp*,

5′-GGAATT***CATATG***TTGTTTCTCGCACAA-3′ and 5′-TTTT***GTCGAC***T-TATTCGCTGATACGG-3′ for *tpp*,
5′-GGAATT***CATATG***GCTACCCCACACATTAA-3′ and 5′-CG***GAATTC***TATTA-CTCTTTATCGCCCAGCA-3′ for *pup*,
5′-GCCCGA***CATATG***ATTGATACCACCCTGCCA-3′ and 5′-CG***GGATCC***CG-TTACTTCGCGGCGACTTTTTC-3′ for *add*,
5′-CG***GGATCC***GTCTGAACAAGAAACACGGGGAGCCAATG-3’ and 5′-GACTAG***AAGCTT***TTATTTCTGTGGGCGCATCGCCG-3’ for *lysU*.

*Nde*I-*Sal*I (for *udp* and *tpp*), *Nde*I-*Eco*RI (for *pup*), *Nde*I-*Bam*HI (for *add*) and *Bam*HI-*Hind*III (for *lysU*) restriction sites were inserted at 5′ and 3′ end of the genes (the bases are in italic).

Amplification was performed using *Taq* or *Pfu* polymerase. The resulting PCR products were isolated using Wizard SV gel and PCR clean-up system (Promega, USA) digested with restriction endonucleases and ligated into the pET24b(+) or pRsetB expression vectors, which had been restricted with the same enzymes.

The resultant recombinant plasmids were introduced into *E. coli* strain BL21(DE3) and antibiotic resistant clones were selected on LB medium containing kanamycin or ampicillin. The isolated *E. coli* recombinant strains encoded pUDP4, TDP, PD10, pADD3 and LysU-12 overexpress UPase, TPase, PNPase, ADase and LysU, respectively.

LysU Purification

The cells of *E. coli* LysU-12 were disrupted by sonication at 4°C for 5 min. After centrifugation of the sample for 30 min at 20,000 × g, the resulting supernatant was purified, using metal affinity chromatography with Ni-NTA agarose (Qiagen, USA).

Synthesis of Modified Nucleosides

The reaction mixture comprising: wet cell paste (0.1–0.4% dry wt.), potassium phosphate buffer (10–30 mM; pH 7), nucleoside and nucleobase, was gently stirred at 50–60°C. Samples were centrifuged at 10,000 × g for 30 s and the supernatants were analyzed by HPLC or TLC.

Synthesis of Nucleoside 5′-Monophosphates (NMP)

Reaction mixture (2 ml) consisting of 10 mM ara-A, 30 mM *p*-NPP, 0.2 M sodium acetate buffer (pH 4.75) and NPTase (130–200 units/ml) was incubated at 45°C.

Synthesis of Diadenosine-5′,5‴-P^1,P^4-tetraphosphate (Ap_4A)

Ap_4A synthesis was performed in the a standard reaction mixture (100 ml), containing 237 mg ATP, 53 mg L-lysine, 20 mM MOPS-KOH (pH 8.0), 10 mM $MgCl_2$, 160 μM $ZnSO_4$ and 0.5 units of inorganic pyrophosphatase. The reaction mixture was incubated for 60 min at 42°C. After the reaction was completed, mixture was heated at 100°C for 10 min, centrifuged, and the supernatant was applied onto DEAE-cellulose column previously equilibrated with a 20 mM Tris-buffer (pH 7.5). Products were eluted with a linear gradient of ammonium bicarbonate, from 20 mM to 400 mM, in the same buffer. The Ap_4A fractions were combined and rechromatographed in the same conditions, then evaporated to dryness in a rotary evaporator.

RESULTS AND DISCUSSION

Synthesis of Modified Nucleosides

Modified nucleosides can be obtained using enzymes such as lipases, proteases, glycosyltransferases or nucleoside phosphorylases [7, 19, 20]. The latter enzymes are especially valuable because purine and pyrimidine nucleosides may be produced in-one-pot reactions from other lower price pyrimidine or purine nucleobases, respectively. Usually, for this synthesis two enzymes are necessary (figure 1). Nucleoside phosphorylases are intracellular enzymes. Three kinds of purine nucleoside phosphorylases displaying different substrate specificity have been described. Also, two different pyrimidine nucleoside phosphorylases have been reported. One of them recognizes uridine and the other thymidine [21, 22].

The mechanism is well established in literature. The phosphorolysis takes place to yield α-pentose-1-phosphate. At the second step phosphate is substituted by a base to form β-nucleoside. The synthesis of modified nucleosides using nucleoside phosphorylases is well documented including our paper [6, 7, 23] but in most cases it was conducted with non-recombinant enzymes.

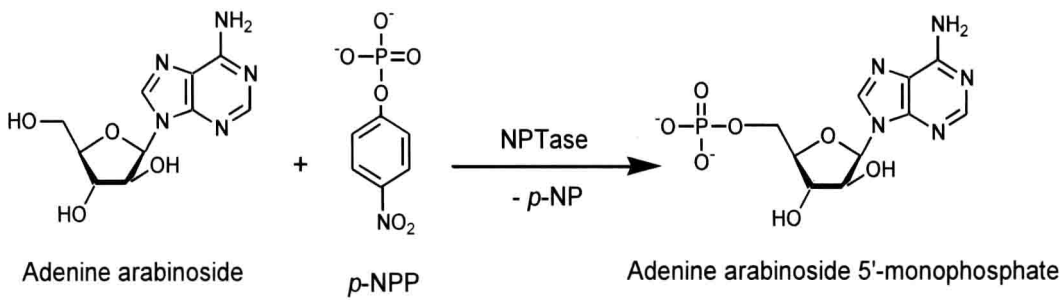

Figure 1. Scheme of the transfer of sugar moiety from uracil arabinoside to adenine by the action of UPase and PNPase.

Table 1. Adenine arabinoside synthesis by enzymatic transglycosylation

Strains	Uracil arabinoside: adenine ratio (mM:mM)	Cell paste concentration (%)	Reaction time (h)	Reaction yield (mol%)	Volumetric yield (g/l)	Ref.
Enterobacter aerogenes	40:40	5	20	55	5.9	[20]
E. coli DH5α/pGM716	75:75	0.5	4	70	14	[24]
E. coli BL21	30:10	5	24	85	2.3	[25]
E. coli BL21(DE3)/-pERPUPHO1 + *E. coli* BL21(DE3)/-pERUPHO1	5:6	Immobilized recombinant enzymes	24	46	0.6	[26]
E. coli pUDP4 + *E. coli* pD10	50:50	0.1	3	77	10.3	[This study]
E. coli pUDP4 + *E. coli* pD10	75:50	0.1	3	93	12.4	[This study]
E. coli pUDP4 + *E. coli* pD10	100:50	0.1	3	96	12.8	[This study]
E. coli pUDP4 + *E. coli* pD10	400:200	0.1	72	98	57.4	[This study]

We have engineered recombinant *E. coli* strains overproducing UPase, TPase and PNPase. The genes encoding these enzymes were amplified by PCR from the chromosomal DNA of *E. coli* using conformable synthetic primers. Expression vectors encoding UPase, PNPas and TPase were constructed according to standard molecular biology protocols [18]. *E. coli* strain DH5α was used as the host for DNA manipulation and as recipient of the expression vectors.

All enzymes were found in a soluble and functional form by assaying their enzymatic activities in soluble cell extracts. UPase activity in recombinant strain showed 400 fold higher activity than the corresponding wild type strain, while PNPase and TPase showed 800 and 900 fold higher activity, respectively. The use of these new recombinant cells as biocatalysts in the transglycosylation reaction was studied in comparison with cells of *Enterobacter aerogenes* [20], *E. coli* DH5α/pGM716 [24], and *E. coli* BL21 [25] which, according to the literature, are the best biocatalysts for enzymatic preparation of adenine arabinoside. We chose synthesis of adenine arabinoside as a model reaction since transglycosylation involving arabinose moiety is less efficient in comparison to other sugars. As reported in Table 1, the comparative results show that the use of new recombinant strains significantly improved the bioconversion process and provided better yields in a shorter reaction time at a lower concentration of cells with a higher volumetric yields of end product.