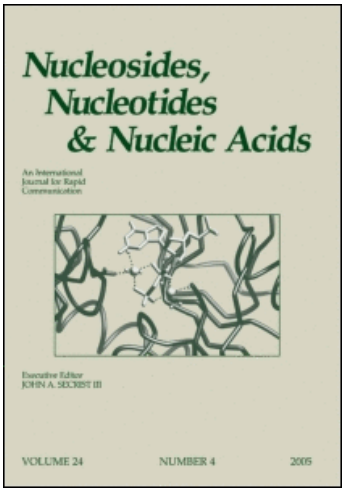

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RECOMBINANT BACTERIAL CELLS AS EFFICIENT BIOCATALYSTS FOR THE PRODUCTION OF NUCLEOSIDES

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ABSTRACT

The preparation of nucleosides as well as their base-modified analogues using purified nucleoside phosphorylase enzymes or, more conveniently, using whole bacterial cells is described. The development of genetically modified strains of *Escherichia coli*, able to over-produce Uridine-phosphorylase and Purine-nucleoside-phosphorylase in the same cells, improves the specific biocatalytic activity and the consequent industrial scale approach.

Bioconversion reactions catalyzed by the combined actions of UdPase (E.C. 2. 4.2.2.) and PNPase (E.C.2.4.2.1.), as reported in literature (1,2,3), are an alternative approach for preparing nucleosides and their modified analogues instead of conventional chemical syntheses, plagued by formation of regio- and stereochemical isomers and by low overall yields. The phosphorylase enzymes, in the presence of phosphate ions, promote the phosphorolysis of purine and pyrimidine nucleosides into pentose-1-phosphate and their corresponding free bases as well as the reverse reaction, that is attachment of the sugar moiety to a suitable heterocyclic base. Due to different equilibrium rates the coupling of UdPase and PNPase,

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as shown in the following general scheme,

$$\begin{array}{c} \text{Pyrimidine nucleoside (sugar donor)} + \text{Purine base (base acceptor)} \\ (UdPase + PNPase)\downarrow \quad \uparrow \\ \text{Purine nucleoside (product)} \quad + \quad \text{Pyrimidine base} \end{array}$$

efficiently transfer a sugar moiety from a donor nucleoside to an acceptor base enabling the "one-pot" preparation of a new nucleoside analogue. Important features of the transglycosylation reactions are stereoselectivity and regioselectivity. Furthermore transglycosylation reactions do not require cofactors and are carried out in an aqueous medium with reduction of environmental risks. Despite these potential advantages the industrial scale application of transglycosylation reactions has been hampered by the low specificic activity associated with the wild-type microorganisms. To overcome this drawback we developed genetically modified strains of *Escherichia coli* which co-expressed UdPase and PNPase (products of *udp* and *deoD* genes respectively) inside the same cells at very high level with respect to wild-type strains. The availability of biocatalysts with high specific activity enabled the optimization of nucleoside preparation in terms of bioconversion yields, productivity and costs as well as the direct implementation of the process at preparative scale. The better recombinant strains were obtained transforming two different hosts, namely *Escherichia coli* MG1655 and DH5α, with a single expression vector containing *Escherichia coli udp* and *deoD* genes cloned *in tandem*. The recombinant strains, namely MG1655/pGM716 and DH5α/pGM716, expressed simultaneously in the same cell both UdPase and PNPase as major cellular proteins when assayed by SDS-PAGE. The high expression levels were confirmed by RP-HPLC analysis which determined 55 mg UdPase and 15 mg PNPase per gram of wet cell paste. Both enzymes were found in a soluble and functional form by assaying their enzymatic activities in soluble cell extracts. UdPase activity in recombinant strains showed 600 fold higher activity than the corresponding wild type strain, while PNPase showed 200 fold higher activity. The use of these new recombinant cells as biocatalysts in large scale transglycosylation reactions was studied in comparison with cells of *Enterobacter aerogenes* which, according to the literature (4), is the best wild type microorganism for the enzyme catalysed preparation of nucleosides.

We selected the preparation of Ara-A as a model reaction since it is known that transglycosylations involving arabinose moiety is less efficient in comparison to other sugars; besides, Ara-A is also an important antiviral agent. 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 products.

In conclusion, we describe the construction of recombinant *Escherichia coli* cells co-expressing high levels of both UdPase and PNPase enzymes and their application as biocatalysts of high specific activity for industrially relevant

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Table 1.

Operating Conditions	DH5α/pGM716	E. aerogenes
Ara-U : adenine ratio	75:75	40:40
Cell paste concentration	0.5%	5%
Reaction time	4 hours	20 hours
Bioconversion yield	70%	55%
Volumetric yield	14 g. Ara-A/litre	5.9 g. Ara-A/litre

preparation of nucleosides with advantages in terms of bioconversion yields, productivity, cost saving and ease of scale-up. The availability of these novel recombinant strains enabled natural and modified nucleosides to be prepared with significantly better results than those obtained by enzymatic techniques known hitherto which are based on the use of isolated enzymes or on the use of whole cells of wild-type microorganisms.

REFERENCES

1. Hutchinson, DW (1990) *Trends Biotechnol*. **8**, 348–353.
2. Hanrahan JR, Hutchinson DW (1992) *J. Biotechnol*. **23**, 193–210.
3. Votruba I *et al*. (1994) *Collect. Czech. Chem. Commun*. **59**, 2303–2330.
4. Utagawa T (1999) *J. Molec. Catalysis B.: Enzymatic* **6**, 215–222.

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