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Genes Encoding Ribonucleoside Hydrolase 1 and 2 from *Corynebacterium ammoniagenes*

Jin-Ho Lee[2*], Hyun-Soo Kim[2†], Won-Sik Lee[2], Young-Hoon Park[2], and Won-Gi Bang[1†]

[1]*Department of Biotechnology and Genetic Engineering, College of Life and Environmental Sciences, Korea University,* [2]*R&D Center for Bioproducts, CJ Corp.*

[†] *These authors equally contributed.*

Abstract

Two kinds of nucleoside hydrolases (NHs) encoded by rih1 and rih2 were cloned from *Corynebacterium ammoniagenes* using *deoD*- and *gsk*-defective *Escherichia coli*. Sequence analysis revealed that NH 1 was a protein of 337 aa with a deduced molecular mass of 35,892 Da, whereas NH 2 consisted of 308 aa with a calculated molecular mass of 32,310 Da. Experiments with crude extracts of IPTG-induced E. coli CGSC 6885(pTNU23) and 6885(pTNI12) indicated that the Rih1 enzyme could catalyse the hydrolysis of uridine and cytidine and showed pyrimidine-specific ribonucleoside hydrolase activity. Rih2 was able to hydrolyse both purine and pyrimidine ribonucleosides with the following order of activity – inosine>adenosine> uridine>guanosine>xanthosine>cytidine – and was classified in the non-specific NHs family. rih1 and rih2 deletion mutants displayed a decrease in cell growth on minimal medium supplemented with pyrimidine and purine/pyrimidine nucleosides, respectively, compared with the wild-type strain. Growth of each mutant was substantially complemented by introducing *rih1* and *rih2*, respectively. Furthermore, disruption of both *rih1* and *rih2* led to the inability of the mutant to utilize purine and pyrimidine nucleosides as sole carbon source on minimal medium. These results indicated that *rih1* and *rih2* play major roles in the salvage pathways of nucleosides in this micro-organism.

Introduction

The N-ribosidic cleaving reactions of nucleosides occur through actions of nucleoside phosphorylases (EC 2.4.2.–) and nucleoside hydrolases (NHs; EC 3.2.2.–) (West, 1988; Camici et al., 1990). Nucleoside phosphorylases catalyse the phosphorolytic cleavage of nucleosides and have a reversible ribosyl transferase activity. These enzymes have been well studied and used as catalysts for the synthesis of nucleoside analogues through base-exchange reactions (Krenitsky et al., 1981; Ling et al., 1990;

Hamamoto et al., 1996). In contrast, the NHs mediating the irreversible hydrolysis of nucleosides to ribose and free nucleic bases are widely distributed in bacteria, fungi, protozoa, insects, fish, amphibians and plants, but so far not in mammals. They have been studied extensively in pathogenic protozoa as potential targets for chemotherapy, since pathogenic protozoa lack de novo purine biosynthetic pathways and rely on exogenous purines salvaged from the hosts (Degano et al., 1996; Miles et al., 1999; Shi et al., 1999).

Enzymes responsible for nucleoside salvage are quite different depending on the organism. In *Escherichia coli,* exogenous ribonucleosides are predominantly metabolized by nucleoside phosphorylases encoded by *deoD, udp* and *xapA*, while NHs encoded by *rihA*, *rihB* and *rihC* have been reported and play a minor role (Koszalka et al., 1988; Petersen & Moller, 2001). In the case of the genus *Bacillus*, the metabolic pathways mediated by only nucleoside phosphorylases have been reported and characterized (Hamamoto et al., 1996; Rocchietti et al., 2004), whereas in the yeast *Saccharomyces cerevisiae*, purine ribonucleosides, inosine and guanosine, and pyrimidine ribonucleosides, cytidine and uridine, were salvaged by purine nucleoside phosphorylase (encoded by *pnp1*) and uridine ribohydrolase (encoded by *urh1*), respectively (Desgranges et al., 2001; Kurtz et al., 2002; Mitterbauer et al., 2002). In addition, NHs, but not nucleoside phosphorylases, are crucial enzymes in purinepyrimidine salvage in protozoan parasites (Parkin et al., 1991): an inosine-uridine NH and a guanosine-inosine NH from *Crithidia fasciculata* (Estupinan & Schramm, 1994; Degano et al., 1996), a purine-specific inosine-adenosineguanosine NH from *Trypanosoma brucei* subsp. brucei (Parkin, 1996) and a non-specific NH from *Leishmania major* (Shi et al., 1999).

Corynebacterium ammoniagenes is a Gram-positive microorganism that has been used for the industrial production of flavour-enhancing purine nucleotides and other compounds (Chung et al., 1996; Koizumi et al., 2000; Noguchi et al., 2003). The biosynthetic pathways leading to nucleotides/nucleosides have been reported by Chung et al. (1996) and Noguchi et al. (2003); however, very little is known about the N-ribosidic cleavage reactions of the salvage pathways in this organism. Although several enzymes of other coryneform bacteria were predicted to be putative NHs during genome sequencing projects, to date their functions have not been verified experimentally.

In this work, we cloned and identified two major genes involved in the salvage of ribonucleosides from *C. ammoniagenes* ATCC 6872, and designated *rih1* and *rih2*. To investigate the characteristics and physiological function of the NHs, we have determined their substrate specificity and characterized their functions using deletion mutants and gene-bearing plasmids. This is the first report of the identification and characterization of putative NHs in *Corynebacterium* species.

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