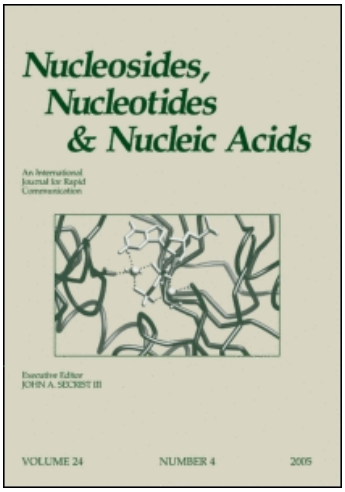

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CATABOLISM OF PYRIMIDINES IN YEAST: A TOOL TO UNDERSTAND DEGRADATION OF ANTICANCER DRUGS

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□ *The pyrimidine catabolic pathway is of crucial importance in cancer patients because it is involved in degradation of several chemotherapeutic drugs, such as 5-fluorouracil; it also is important in plants, unicellular eukaryotes, and bacteria for the degradation of pyrimidine-based biocides/antibiotics. During the last decade we have developed a yeast species,* Saccharomyces kluyveri*, as a model and tool to study the genes and enzymes of the pyrimidine catabolic pathway. In this report, we studied degradation of uracil and its putative degradation products in 38 yeasts and showed that this pathway was present in the ancient yeasts but was lost approximately 100 million years ago in the* S. cerevisiae *lineage.*

Keywords Uracil degradation; Pyrimidines; Yeast; Evolution; Cancer

INTRODUCTION

Pyrimidine bases, among which include several anticancer drugs, are degraded via a reductive or an oxidative pathway.[1] The reductive pathway is better studied primarily because it is important in humans. The enzymes involved in the reductive degradation have been purified from a

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variety of organisms, like mammals, insects, molds, yeast, and bacteria.[2–4] The crystal structures of pig dihydropyrimidine dehydrogenase (DPD, EC 1.3.1.2), catalyzing the conversion of uracil to dihydrouracil (DHU),[5] and *Saccharomyces kluyveri* β-alanine synthase (BS, EC 3.5.1.6), catalyzing the conversion of β-ureidopropionate (βUP) to β-alanine (βALA),[6] have been solved. *S. kluyveri* dihydropyrimidinase (DHP, EC 3.5.2.2), which opens the DHU ring resulting in βUP recently has been crystallized.[7] In addition, 2 *S. kluyveri* catabolic genes, *PYD2* and *PYD3*, encoding DHP, BS, and their expression have been characterized in detail.[8,9] Thereby, *S. kluyveri*, is becoming a useful model to understand the degradation of pyrimidines. However, the fate of uracil in yeast and the involved genes and enzymes still are largely unknown. In this article, we address the origin of the present situation regarding the pyrimidine catabolism in yeast. Thirty-eight yeast species belonging to the genera *Saccharomyces*, *Arxiozyma*, *Kluyveromyces*, *Candida*, *Zygosaccharomyces*, *Torulaspora*, and *Hanseniaspora* were analyzed for their growth on uracil, DHU, βUP, and βALA as the sole nitrogen source. One should keep in mind that the ability to utilize uracil as the sole nitrogen source is a complex of several biochemical pathways and the corresponding regulatory mechanisms.

MATERIALS AND METHODS

Strains

The strains used in growth experiments and their accession numbers are listed in Table 1. All strains are maintained in the ARS Culture Collection (NRRL), National Center for Agricultural Utilization Research (Peoria, IL), except *Zygosaccharomyces bailii* (ISA 1307) from Culture Collection of the Instituto Superior de Agronomia (Lisbon, Portugal), *Zygosaccharomyces bailii* (ATCC 36947), and *Zygosaccharomyces bailii* (ATCC 60483) from American Type Culture Collection (Manassas, VA) and *Kluyveromyces lactis* (CBS 2359) from Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands).

Growth Test

Uracil, DHU, βUP, and βALA were purchased from Sigma-Aldrich (St. Louis, MO, USA). Yeast Nitrogen Base w/o amino acids and ammonium sulphate was purchased from Difco (Beckton Dickinson and Co., Franklin Lakes, NJ, USA). The growth test was done by spotting 5 μL of culture on synthetic defined (SD) plates (1% succinic acid, 0.6% sodium hydroxide, 2% glucose, 0.17% yeast nitrogen base without amino acids and ammonium sulfate, 2% agar) supplemented with 0.5% ammonium sulfate (control) or 0.1% uracil, DHU, βUP, and βALA, respectively. Growth was determined after 7 days at 30°C. All given percentages are in w/v.

TABLE 1 Utilization of Different Nitrogen Sources: Growth on Uracil, DHU, βUP, and βALA was Tested on Minimum Medium Plates. The Yeast Strains are Listed According to the Phylogenetic Relationship Presented by Kurtzman and Robnett, 2003.[10]

Strain (Accesion number)	Uracil	DHU	βUP	βALA
Saccharomyces cerevisiae (CEN.PK 113-7D)	−	−	−	−
Saccharomyces paradoxus (Y-17217)	−	−	−	−
Saccharomyces pastorianus (Y-27171)	−	−	−	−
Saccharomyces bayanus (Y-12624)	−	−	−	−
Saccharomyces servazzii (Y-12661)	−	−	−	−
Saccharomyces unisporus (Y-1556)	−	−	−	++
Arxiozyma telluris (YB-4302)	−	−	−	−
Saccharomyces spencerorum (Y-17920)	−	−	−	−
Saccharomyces rosinii (Y-17919)	−	−	−	−
Kluyveromyces lodderae (Y-8280)	−	−	+	++
Saccharomyces exiguus (Y-12640)	−	−	−	++
Saccharomyces barnettii (Y-27223)	−	−	−	++
Candida humilis (Y-17074)	−	−	−	+
Saccharomyces castellii (Y-12630)	−	−	−	++
Candida glabrata (Y-65)	−	−	−	++
Kluyveromyces delphensis (Y-2379)	−	−	−	−
Kluyveromyces bacillisporus (Y-17846)	−	−	−	++
Candida castellii (Y-17070)	−	−	−	−
Kluyveromyces blattae (Y-10934)	−	−	−	++
Kluyveromyces phaffii (Y-8282)	−	−	−	+
Zygosaccharomyces rouxii (Y-229)	++	++	++	++
Zygosaccharomyces bailii (ISA 1307)	++	++	++	++
Zygosaccharomyces bailii (ATCC 36947)	++	++	++	++
Zygosaccharomyces bailii (ATCC 60483)	++	++	++	++
Zygosaccharomyces bisporus (Y-12626)	++	++	++	++
Zygosaccharomyces florentinus (Y-1560)	++	++	++	++
Torulaspora globosa (Y-12650)	++	+	+	++
Torulaspora pretoriensis (Y-17251)	+	+	+	++
Torulaspora delbrueckii (Y-866)	+	+	++	++
Zygosaccharomyces microellipsoides (Y-1549)	++	++	++	++
Zygosaccharomyces fermentati (Y-7434)	++	++	++	++
Kluyveromyces thermotolerans (Y-8284)	++	+	++	++
Saccharomyces kluyveri (Y-12651)	++	++	++	++
Kluyveromyces wickerhamii (Y-8286)	++	++	++	++
Kluyveromyces lactis (CBS 2359)	++	++	++	++
Hanseniaspora valbyensis (Y-1626)	++	−	−	++
Hanseniaspora vineae (Y-17529)	++	+	+	++
Hanseniaspora occidentalis (Y-7946)	−	−	−	++

RESULTS

Utilization of Uracil, DHU, βUP, and βALA

The ability of different strains to grow on uracil, DHU, βUP, or βALA, as the sole nitrogen source, is shown in Table 1. The different species analyzed are listed according to their phylogenetic relationship, as reported by Kurtzman and Robnett.[10] Note that the present yeast nomenclature

does not reflect their phylogenetic relationship. For example, *S. kluyveri* is not very closely related to other *Saccharomyces* yeasts. In other words, the higher a species is listed in the table, the more closely it is related to *S. cerevisiae*. The growth was classified as no growth (−), some growth (+), and full growth (++), compared to the control plates (with ammonium sulfate as the sole nitrogen source). It is interesting to point out that the growth on uracil, DHU, or βUP, in general, is linked in all species, except for *K. lodderae* and *H. valbyensis* (Table 1).

Loss of Pyrimidine Catabolic Pathway

Figure 1 shows a simplified tree of the *Saccharomyces* complex based on data from Kurtzmann and Robnett (2003)[10] and summarizes the loss of the ability to grow on uracil, DHU, βUP, and βALA. In general, the presence or absence of the tested abilities can be well explained as a function of the gene-loss events at various time-points in the evolutionary history. Uracil, DHU, and βUP phenotypes are linked, and the ability to grow on these three compounds was "lost" independently and before the loss of the βALA phenotype (Figure 1). A few minor discrepancies are found within the ability to degrade βALA. The ability to utilize βALA was lost in the *S. cerevisiae* –*S. rosinii* lineage (Table 1). Surprisingly, it is still found in *S. unisporus*, which is a very close relative of *S. servazzii*. *S. unisporus* has kept the ability even though it was lost in *A. telluris*, *S. spencerorum*, and *S. rosinii*. Apparently, this ability, independently also has been lost in the *K. delphensis* lineage (a close relative of *C. glabrata*) and *C. castellii*. *K. delphensis* and *C. castellii*, in fact, has lost this ability, even though all closely related species still posses it.

DISCUSSION

The yeast *S. kluyveri* can grow on uracil, DHU, βUP, and βALA, which all are components of the reductive pyrimidine pathway known from humans,

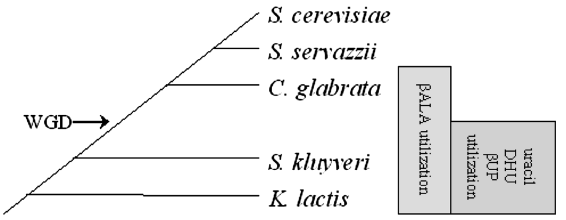

FIGURE 1 The presence of pyrimidine degradation pathway. A simplified phylogenetic tree of 5 prominent yeast species is shown and the occurrence of the whole-genome duplication (WGD), which took place approximately 100 million years ago, is indicated. The ability to utilize uracil, DHU, βUP, and βALA is shown next to the species.

while *S. cerevisiae* cannot. The growth tests of 38 strains from the *Saccharomyces* complex on uracil and the intermediates of the reductive pathway was done in order to understand the diversity and evolution of the ability to degrade pyrimidines. It seems that the ability to utilize uracil, DHU, and βUP as sole nitrogen source was lost at approximately the same time, when the yeast genome was duplicated,[11] while the ability to use βALA was lost much later and perhaps independently in a few lineages. Apparently, the major metabolic changes that followed the yeast genome duplication made the possibility to regulate pyrimidine pools via degradation and to produce βALA from βUP (for pantothenate synthesis) obsolete. The extensive sequencing of the yeast genomes[11] now provides a tool to find the genetic background for many phenotypes and to deduce their evolutionary history. However, one should keep in mind that we still do not understand the genetic or biochemical background of uracil degradation, which likely represents a novel pathway, in any yeast. In addition, the ability to utilize uracil, DHU, βUP, and βALA is a complex process which requires the presence of the genes coding for the degradation enzymes, presence of the recipients of the nitrogen originating from "poor" N-sources, an efficient uptake system, and a complex regulatory net. When our knowledge on all these elements is improved in at least one yeast species, a comparative genome analysis will add an even additional insight on the evolution of the uracil, DHU, βUP, and βALA degradation.

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