Iranian Journal of Pharmaceutical Sciences
Spring 2012: 8(2): 99-102
ijps.sums.ac.ir

Original Article

Phylogeny of urate oxidase producing bacteria: on the basis of gene sequences of 16S rRNA and uricase protein

Fatemeh Dabbagh[a,b], Zahra Moradpour[a,b], Abdollah Ghasemian[a,b,*], Younes Ghasemi[a,b]

[a]*Department of Pharmaceutical Biotechnology,* [b]*Pharmaceutical Sciences Research Center, School of Pharmacy, Shiraz University of Medical Sciences, P.O. Box 71345-1583, Shiraz, Iran*

Abstract

Uricase or Urate oxidase (urate:oxygen oxidoreductase, EC 1.7.3.3), a peroxisomal enzyme which is found in many bacteria, catalyzes the oxidative opening of the purine ring of urate to yield allantoin, carbon dioxide, and hydrogen peroxide. In this study, the phylogeny of urate oxidase (uricase) producing bacteria was studied based on gene sequences of 16S rRNA and uricase protein. Representative and type strains (52 strains total) of most of the known species were analyzed. The acquired sequences (rDNA sequences of the 16S rRNA genes and the amino acid sequences of uricase) were aligned with the Clustal W program using MEGA software version 4.0. Phylogenetic trees were constructed with the neighbor-joining method, and were bootstrapped with 500 replications of each sequence. The large congruence of phylogenetic relationship between the uricase gene and of 16S rRNA gives considerable support to the phylogeny of urate oxidase producing bacteria which was previously suggested on the basis of 16S rRNA sequences. The observed consistency promotes the idea that each of these genes shared a common evolutionary history in uricase producing bacteria we have analyzed.

Keywords: 16S rRNA; Phylogeny; Urate oxidae
Received: November 5, 2011; *Accepted:* January 12, 2012.

Introduction

Uricase or Urate oxidase (urate:oxygen oxidoreductase, EC 1.7.3.3), a peroxisomal enzyme, catalyzes the oxidative opening of the purine ring of urate to yield allantoin, carbon dioxide, and hydrogen peroxide [1-2]. The enzyme is a tetramer, consisting of two types of different subunits with a final molecular weight ranging from 145 to 150 kDa [3]. A number of bacteria are able to produce uricase, including, but not limited to, *Pseudomonas aeruginosa, Arthrobacter globiformis, Bacillus subtilis, Bacillus fastidious, Nocardia farcinica* and *Microbacterium sp.* [4-12]. This enzyme has also been reported in fungi, plants and animals [13-15]. It has a unique evolutionary feature, in that the enzyme has been lost during primate evolution with no obvious explanation [16]. The biological reason for the loss of urate oxidase activity in humans and certain primates is unknown. According to one view, this loss has had a distinctly beneficial effect.

*Corresponding author: Abdollah Ghasemian,
Department of Pharmaceutical Biotechnology and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
E-mail: ghasemiyan@sums.ac.ir

It has been shown that uric acid is a powerful antioxidant and a scavenger of free radicals; therefore, a high serum uric acid level caused by the loss of urate oxidase activity may have contributed to a decreased cancer rate and a lengthened hominoid life span [17]. In order to investigate the genetic relationships among the uricase producing bacteria further, sequences of another gene not related to the 16S rRNA should be considered. Therefore, in this report, we compare the genetic relationships of the uricase producing bacteria based on 16S rDNA sequences and on uricase amino acid sequences.

2. Material and Methods

Data Collection

Here, representative and type strains (52 strains total) of most of the known bacterial species were analyzed. Almost all 16S rDNA sequences and uricase amino acid sequences were collected from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). List of all these 52 strains and the GenBank accession numbers for genomes used in this study are listed in Table 1.

Phylogenetic analysis

The acquired sequences (rDNA sequences of the 16S rRNA genes and the amino acid sequences of uricase) were aligned with the Clustal W program using MEGA software version 4.0 [18]. Phylogenetic trees were constructed with the neighbor-joining method, and were bootstrapped with 500 replications of each sequence.

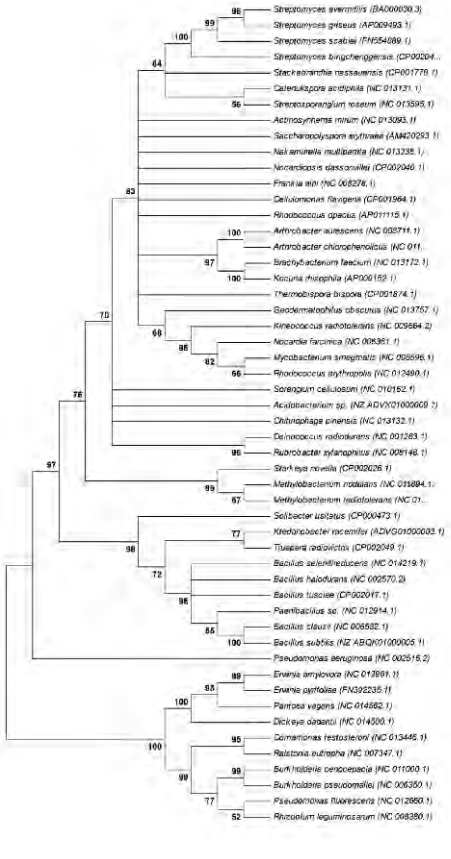

Figure 1. Neighbor-joining tree constructed from amino acid sequences of the uricase showing the phylogenetic relationships. Bootstrap values above 50 are indicated at the main nodes. Bootstrap values are given above branches.

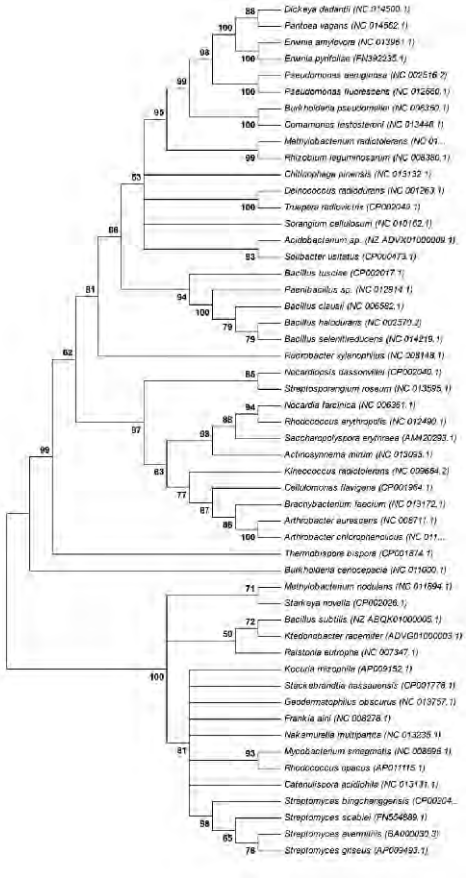

Figure 2. Neighbor-joining tree constructed from nucleotide sequences of the 16S rDNA gene showing the phylogenetic relationships. Bootstrap values above 50 are indicated at the main nodes. Bootstrap values are given above branches.

Table 1. Uricase producing bacteria included in this study.

Species	Strain	GenBank Accession number
Acidobacterium sp.	MP5ACTX8	NZ_ADVX01000009.1
Actinosynnema mirum	DSM 43827	NC_013093.1
Arthrobacter aurescens	TC1	NC_008711.1
Arthrobacter chlorophenolicus	A6	NC_011886.1
Bacillus clausii	KSM-K16	NC_006582.1
Bacillus halodurans	C-125	NC_002570.2
Bacillus selenitireducens	MLS10	NC_014219.1
Bacillus subtilis	str. 168	NZ_ABQK01000005.1
Bacillus tusciae	DSM 2912	CP002017.1
Brachybacterium faecium	DSM 4810	NC_013172.1
Burkholderia cenocepacia	J2315	NC_011000.1
Burkholderia pseudomallei	K96243	NC_006350.1
Catenulispora acidiphila	DSM 44928	NC_013131.1
Cellulomonas flavigena	DSM 20109	CP001964.1
Chitinophaga pinensis	DSM 2588	NC_013132.1
Comamonas testosteroni	CNB-2	NC_013446.1
Deinococcus radiodurans	R1	NC_001263.1
Dickeya dadantii	3937	NC_014500.1
Erwinia amylovora	CFBP1430	NC_013961.1
Erwinia pyrifoliae	DSM 12163	FN392235.1
Frankia alni	ACN14a	NC_008278.1
Geodermatophilus obscures	DSM 43160	NC_013757.1
Kineococcus radiotolerans	SRS30216	NC_009664.2
Kocuria rhizophila	DC2201	AP009152.1
Ktedonobacter racemifer	DSM 44963	ADVG01000003.1
Methylobacterium nodulans	ORS 2060	NC_011894.1
Methylobacterium radiotolerans	JCM 2831	NC_010505.1
Mycobacterium smegmatis	MC2 155	NC_008596.1
Nakamurella multipartite	DSM 44233	NC_013235.1
Nocardia farcinica	IFM 10152	NC_006361.1
Nocardiopsis dassonvillei	DSM 43111	CP002040.1
Paenibacillus sp.	JDR-2	NC_012914.1
Pantoea vagans	C9-1	NC_014562.1
Pseudomonas aeruginosa	PAO1	NC_002516.2
Pseudomonas fluorescens	SBW25	NC_012660.1
Ralstonia eutropha	JMP134	NC_007347.1
Rhizobium leguminosarum	bv. viciae 3841	NC_008380.1
Rhodococcus erythropolis	PR4	NC_012490.1
Rhodococcus opacus	B4	AP011115.1
Rubrobacter xylanophilus	DSM 9941	NC_008148.1
Saccharopolyspora erythraea	NRRL2338	AM420293.1
Solibacter usitatus	Ellin6076	CP000473.1
Sorangium cellulosum	So ce 56	NC_010162.1
Stackebrandtia nassauensis	DSM 44728	CP001778.1
Starkeya novella	DSM 44728	CP002026.1
Streptomyces avermitilis	DSM 506	BA000030.3
Streptomyces bingchenggensis	MA-4680	CP002047.1
Streptomyces griseus	NBRC 13350	AP009493.1
Streptomyces scabiei	87.22	FN554889.1
Streptosporangium roseum	DSM 43021	NC_013595.1
Thermobispora bispora	DSM 43833	CP001874.1
Truepera radiovictrix	DSM 17093	CP002049.1

3. Results

Phylogeny according to 16S rDNA sequences and *uricase amino acid sequences*

The phylogenetic tree based on uricase amino acid sequences (Figure 1) revealed a tree topology which was generally similar to the 16S rDNA tree (Figure 2). The large congruence of phylogenetic relationship

between the uricase gene and of 16S rDNA gives considerable support to the phylogeny of urate oxidase producing bacteria previously suggested on the basis of 16S rDNA sequences. The similar position of *Strepomyces, Bacillus, Arthrobacter* and *Erwinia* species indicate that the phylogenetic tree based on uricase amino acid sequences (Figure 1) was highly consistent with the 16S rDNA tree (Figure 2), suggesting that each of these genes shared a common evolutionary history in uricase producing bacteria we have analyzed in this study.

4. Discussion

The evolutionary history of tree constructed from nucleotide sequences of the 16S rDNA gene was inferred using the Neighbor-Joining method [19]. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed [20]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches [20]. The evolutionary distances were computed using the Maximum Composite Likelihood method [21] and are in the units of the number of base substitutions per site. The analysis involved 52 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 971 positions in the final dataset. On the other hand, The evolutionary distances for amino acid based tree were computed using the Poisson correction method [22] and are in the units of the number of amino acid substitutions per site. The analysis involved 52 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 142 positions in the final dataset.

The infrageneric groups retained in 16S rDNA nucleotide analysis only partly reflect the current taxonomical classification of these bacteria. It should be kept in mind that a more complete sample of urate oxidase species will considerably enhance the phylogenetic resolution and closely reflects the results of analysis.

The uricase-producing bacteria represent a phylogenetically coherent group of bacteria, which are also closely related according to the 16S rDNA sequence. By comparison of the sequences of both genes, an improved relationship for the uricase-producing bacteria could be established. This observation is quite remarkable and strongly supports the 16S-rDNA-based phylogeny of these bacteria. High bootstrap values at most of the branching points suggest that the analysis has a high degree of reliability. However, more detailed studies including a higher number of accessions are necessary to test this hypothesis.

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