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Molecular phylogeny of the genus *Pseudomonas* based on *rpoB* sequences and application for the identification of isolates

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Abstract

Phylogenetic relationships within the genus *Pseudomonas* were examined by comparing partial (about 1000 nucleotides) *rpoB* gene sequences. A total of 186 strains belonging to 75 species of *Pseudomonas* sensu stricto and related species were studied. The phylogenetic resolution of the *rpoB* tree was approximately three times higher than that of the *rrs* tree. Ribogroups published earlier correlated well with *rpoB* sequence clusters. The *rpoB* sequence database generated by this study was used for identification. A total of 89 isolates (79.5%) were identified to a named species, while 16 isolates (14.3%) corresponded to unnamed species, and 7 isolates (6.2%) had uncertain affiliation. *rpoB* sequencing is now being used for routine identification of *Pseudomonas* isolates in our laboratory.

Keywords: *Pseudomonas*; Phylogeny; *rpoB*; Sequencing

1. Introduction

For over a century, the genus *Pseudomonas* Migula 1894 served as a repository for straight, strictly aerobic Gram-negative rods that were motile by one or several polar flagella. The nutritional studies of Stanier et al. [24] showed that the ability of *Pseudomonas* strains to utilize different compounds as sole source of carbon and energy provided essential phenotypic characterization. Groups of species differed considerably in their nutritional versatility.

Later, DNA–DNA [20] and rRNA–DNA hybridization [7,21] split the genus *Pseudomonas* into five groups called rRNA groups I to V.

Finally, sequencing 16S rRNA [28] or its gene (*rrs)* [27] supported the transfer of many species to other (often new) genera in the alpha subclass (e.g., *Aminobacter, Brevundimonas*, *Devosia*, *Oligotropha*, *Sphingomonas*, *Zavarzinia*), beta subclass (e.g., *Acidovorax, Burkholderia, Comamonas, Hydrogenophaga*, *Ralstonia*, *Telluria*), gamma subclass (e.g., *Chryseomonas, Flavimonas, Pseudomonas sensu stricto*), or between the beta- and gamma-subclasses (e.g., *Stenotrophomonas*) of the Proteobacteria (reviewed by Kersters et al.) [13].

The genus *Pseudomonas* sensu stricto contains all species which corresponded to the RNA group I, i.e., fluorescent pseudomonads and related bacteria [19]. The list of species (145 when this paper was written) and subspecies of the genus *Pseudomonas* which have standing in nomenclature is available on the WorldWide Web (http://www.bacterio.cict.fr/p/pseudomonas.html).

Although the taxonomy of the genus *Pseudomonas* has progressed steadily thanks to rRNA sequencing (for gross phylogeny) and DNA–DNA hybridization (as species delimiter), identification of species is often a nightmare. The finest phenotypic systems (e.g., Biotype-100 strips) cannot resolve species within the *P. fluorescens*, *P. putida*, or *P. syringae* complexes [10].

Siderophore typing has been proposed for the identification of *Pseudomonas* species producing fluorescent pyoverdine [14].

Ribotyping, using endonucleases *Sma*I and *Hin*cII, showed high resolution in separating DNA hybridization groups

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[3]. However, DNA sequencing is becoming less expensive, less tedious, and far more portable than ribotyping.

The high degree of conservation of the *rrs* gene (an advantage for its universality) led to a small number of informative sites in its sequence. Its utility has been questioned because of its heterogenity [5], and it often fails to reveal precise and statistically supported phylogeny at the species level [9,11,25].

Therefore, other genes have been used to aim at a more precise phylogeny, such as *gyrB*, *rpoD* and *oprI* for the genus *Pseudomonas* [6,29].

The *rpoB* gene, which codes for the RNA polymerase beta subunit, has been used as a signature for bacterial identification as well as a locus for phylogenetic analysis [15]. Moreover, *rpoB* is a highly conserved housekeeping gene, and one copy is present in all bacteria because of its essential role in cellular metabolism [22].

Due to its discriminatory power, the *rpoB* gene has been used for developing probes for specific detection and phylogenetic analysis of several bacterial groups [4,8,16,22].

In this study, *rpoB* gene partial sequences of 900–1200 bp were determined for examining the phylogenetic structure of the genus *Pseudomonas* and the results were compared to those obtained with the *rrs* gene. Isolates submitted to *rpoB* sequencing were identified against the sequence database built in this study.

2. Materials and methods

2.1. Bacterial strains and DNA preparation

A total of 186 strains belonging to 75 species of the genus *Pseudomonas* were studied. These strains are listed in Table 1 and Table 2. They were obtained from the Laboratorium voor Microbiologie Gent Culture Collection (LMG), Ghent, Belgium; Collection de l'Institut Pasteur (CIP), Paris, France; Collection de la Faculté de Médecine de Lille (CFML), Lille, France; American Type Culture Collection (ATCC), Manassas, VA; or received for identification in our laboratory. Strains were grown in appropriate media and temperatures following the recommendations of the culture collections, and stored at −80 °C in brain–heart infusion supplemented with 50% (vol/vol) glycerol.

Table 1
GenBank accession numbers of *rrs* and *rpoB* gene sequences and ribogroup of type strains studied

Species	Strain	Ribogroup *rrs*	Accession no.	*rpoB* accession no.
Marinobacterium georgiense	CIP 106746T		AB021408	AJ717489
Marinobacterium stanieri	LMG 6847T		AB021367	AJ717490
Oceanimonas doudoroffii	CIP 74.9T		AB021371	AJ717491
P. abietaniphila	CIP 106708T		AJ011504	AJ717416
P. alcaliphila	CIP 108031T		AB030583	AJ717463
P. aeruginosa	LMG 1242T	R50	Z76651	AJ717442
P. agarici	LMG 2112T	R40	Z76652	AJ717477
P. alcaligenes	LMG 1224T	R52	Z76653	AJ717475
P. amygdali	LMG 2123T	R18	Z76654	AJ717462
P. anguilliseptica	CIP 106711T		AB21376	AJ717417
P. asplenii	LMG 2137T	R33	Z766655	AJ717432
P. aurantiaca	CIP 106718T		AB021412	AJ717421
P. aureofaciens	LMG 1245T	R30	Z76656	AJ717426
P. avellanae	CIP 105176T		U49384	AJ717469
P. azotoformans	CIP 106744T		D84009	AJ717458
P. balearica	CIP 105297T		U26418	AJ717480
P. brassicacearum	CIP 107059T		AF100321	AJ717436
P. brenneri	CIP 106646T		AF268968	AJ717482
P. cannabina	CIP 106140T		AJ492827	AJ717453
P. caricapapaye	LMG 2152T	R14	D84010	AJ717437
P. cedrina	CFML 96-198T		AF064461	AJ717424
P. chlororaphis	LMG 5004T	R30	D84011	AJ717478
P. cichorii	LMG 2162T	R25	Z76658	AJ717418
P. citronellolis	CIP 104381T		Z76659	AJ717460
P. coronafaciens	LMG 13190T	R24	Z76660	AJ717443
P. corrugata	LMG 2172T	R10	D84012	AJ717487
P. cremoricolorata	CIP 107616T		AB060137	AJ717476
P. ficuserectae	LMG 5694T	R17	AB021378	AJ717457
P. flavescens	CIP 104204T		U01916	AJ717468
P. fluorescens bv. 1	LMG 1794T	R42	D11188	AJ717451
P. fragi	LMG 2191T	R34	AB021413	AJ717444
P. frederiksbergensis	CIP 106887T		AJ249382	AJ717465
P. fulva	CIP 106765T		D84015	AJ717419
P. fuscovaginae	LMG 2158T	R32	AB021381	AJ717433

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