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# Phylogenetic analysis of enteric species of the family Enterobacteriaceae using the *oriC*-locus

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## Abstract

Phylogenetic analysis of 38 enteric species belonging to the Enterobacteraceae family was carried out using the non-coding locus *oriC*, the chromosomal replication origin. The *oriC* loci were amplified with conserved oligonucleotides and the PCR fragments were sequenced directly. The results establish a phylogenetic tree for the classification of different species of the genera *Escherichia*, *Shigella*, *Salmonella*, *Enterobacter*, *Citrobacter*, *Klebsiella*, *Raoultella*, *Kluyvera*, *Cedecea* and *Buttiauxella*. Functional important protein-binding sites located in *oriC* are well conserved throughout the enteric group. More over, due to a high overall divergence value phylogenetic trees were robust and well supported by bootstrap analysis. In comparison with 16S rDNA analysis, the *oriC* sequences indicated a greater evolutionary divergence for bacteria. We propose that the *oriC* locus might be a suitable phylogenetic marker for the identification and classification of bacteria, in particular for closely related species.


***Keywords:*** Oric; Enteric species; Enterobacteriaceae; Coliform group; Phylogenetic study

## Introduction

Replication is the central event in the life of a bacterium. DNA replication of prokaryotes is started at the chromosomal replication origin, *oriC* and involved the initiator protein DnaA and the accessory proteins FIS and IHF [19,21]. Different recognition sites for DnaA, FIS and IHF have been identified in *oriC*. The process of bacterial replication has been studied in Gram-positive and Gram-negative eubacteria and sequence analysis demonstrated that the *oriC* locus is not highly conserved. Distinct classes of replication origins have been identified in γ-proteobacteria [4,27]. The minimal enteric-type origin is about 245 bp in size and contained eight different recognition sites for DnaA, three high to medium affinity sites (R1, R4 and R2), and five lower affinity sites (R5 M, I2, I3, I1 and R3) [11,16,24]. The binding sites for FIS and IHF are unique. Three AT-rich, 13 mer repeats required for DNA unwinding are located in the vicinity of R1. In opposite to the *oriC* of *Pseudomona* the enteric *oriC* contain 9–14 GATC sites, the recognition site for the DAM methylase. The high degree of conservation of the GATC sites, eight are positionally conserved, implies an important functional role for methylation in initiation of replication in enteric bacteria.

16S rDNA is most frequently used for phylogenetic analyses; however, phylogenetic relationships between closely related species are weakly defined by this

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approach, owing to the high similarities in this gene region. The enteric species of the family Enterobacteriaceae containing *Citrobacter*, *Enterobacter*, *Escherichia*, *Salmonella, Shigella* and some other genera are closely related and phylogenetic tree analyses are difficult [3,26]. Resulting trees are not supported by statistical analysis and same taxa did not form a coherent cluster. Protein-coding genes, such as *gyrB*, *hsp60*, *infB*, *rpoB* and others, allowed the construction of reliable trees and established a well-accepted classification for the members of the family Enterobacteriaceae [5,9,12,13,20]. However, analyses of multiple genes are necessary to identify horizontal transfer of single genes. Moreover, different levels of selection in certain gene-domains might cause constraints or distortions between the organisms being considered.

We amplified and sequenced the *oriC* locus of 39 reference strains from 10 genera of the family Enterobacteriaceae to investigate the impact of this locus on the phylogenetic relationship of these closely related group of γ-proteobacteria.

## Material and methods

### Bacterial strains

Bacterial strains used in this study are listed in Table 1. Type and reference strains were obtained from the German Collection of Microorganisms and Cell Culture (DSMZ), the American Type Culture

**Table 1.** Strains used in this study and results of different PCR assays

| Species | Strain | PCR 1[a] | PCR 2[b] | PCR 3[c] | PCR 4[d] | PCR 5[e] |
|---|---|---|---|---|---|---|
| *Escherichia coli* | K12, DH5α | + | – | + | + | + |
| *E. coli* | ATCC 25922 | + | – | + | + | + |
| *Escherichia fergusonii* | DSMZ 13698 | + | – | + | + | + |
| *Escherichia hermanii* | DSMZ 4560 | – | – | + | + | – |
| *Escherichia vulneris* | DSMZ 4564 | – | + | + | + | – |
| *Shigella flexneri* | MvP[f] | + | – | + | + | + |
| *Salmonella typhimurium* | MvP | + | n.d.[g] | + | + | – |
| *Kluyvera ascorbata* | DSMZ 4611 | + | – | + | + | – |
| *Klebsiella pneumoniae* | DSMZ 30104 | + | + | + | + | – |
| *Klebsiella oxytoca* | DSMZ 5175 | – | + | + | + | – |
| *Enterobacter aerogenes* | DSMZ 30053 | – | + | + | + | – |
| *Raoultella ornithinolytica* | DSMZ 7464 | n.d. | – | + | + | – |
| *Raoultella planticola* | DSMZ 3069 | n.d. | – | + | + | – |
| *Raoultella terrigena* | DSMZ 2687 | – | + | + | + | – |
| *Enterobacter cloacae* subsp. *cloacae* | ATCC 13047 | + | + | + | + | – |
| *Enterobacter cloacae* subsp. *dissolvens* | ATCC 23373 | n.d. | + | + | + | – |
| *Enterobacter kobei*-II | CDC 1347-71 | + | + | + | + | – |
| *Enterobacter kobei* | DSMZ 13645 | n.d. | + | + | + | – |
| *Enterobacter hormaechei* subsp. *hormaechei* | ATCC 49162 | + | + | + | + | – |
| *Enterobacter nimipressuralis* | ATCC 9912 | n.d. | n.d. | + | + | – |
| *Enterobacter asburiae* | ATCC 35953 | n.d. | n.d. | + | + | – |
| *Enterobacter cancerogenes* | ATCC 33241 | n.d. | n.d. | + | + | – |
| *Enterobacter intermedius* | ATCC 33110 | – | – | + | + | – |
| *Enterobacter amnigenus* | ATCC 3072 | + | – | + | + | – |
| *Enterobacter sakazakii* | ATCC 29544 | – | – | + | + | – |
| *Enterobacter gergoviae* | ATCC 33028 | – | + | + | + | – |
| *Enterobacter pyrinus* | ATCC 49851 | + | + | + | + | – |
| *Enterobacter cowenii* | CIP 107300 | n.d. | n.d. | + | + | – |
| *Enterobacter hormaechei* subsp. *oharae* | DSMZ 16687 | n.d. | n.d. | + | + | – |
| *Enterobacter cloacae* genogroup IX | Strain 25 | n.d. | n.d. | + | + | – |
| *Enterobacter hormaechii*, subsp. *steigerwaltii* | DSMZ 16691 | n.d. | n.d. | + | + | – |
| *Enterobacter ludwigii* | DSMZ 16688 | n.d. | n.d. | + | + | – |
| *Citrobacter freundii* | DSMZ 30039 | + | + | + | + | – |
| *Citrobacter koseri* | DSMZ 4595 | + | – | + | + | – |
| *Citrobacter amalonaticus* | DSMZ 4593 | + | + | + | + | – |
| *Citrobacter gillenii* | DSMZ 13694 | + | + | + | + | – |
| *Citrobacter murliniae* | DSMZ 13695 | – | + | + | + | – |