An analysis of initiation codon utilization in the Domain *Bacteria* – concerns about the quality of bacterial genome annotation

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Using custom software (Inidon) we have examined the initiation codon utilization in 620 complete bacterial genomes downloaded from the National Center for Biotechnology Information (NCBI). The mean utilization of ATG, GTG and TTG codons is 80.1, 11.6 and 7.8 %, respectively. In most cases in which similar species or strains have been analysed the utilization percentages of the three initiation codons are remarkably similar, but in certain cases the results exhibit significant differences.

While numerous programs are available [e.g. GCUA (McInerney, 1998), OPTIMIZER (Puigbo *et al.*, 2007), ACUA (Vetrivel *et al.*, 2007), E-CAI (Puigbo *et al.*, 2008), CAI Analyser (Ramazzotti *et al.*, 2007), CodonW (<http://codonw.sourceforge.net/culong.html>) and Countcodon (Nakamura *et al.*, 2000; <http://www.kazusa.or.jp/codon/countcodon.html>)] for the analysis and optimization of codon usage, little has been published on initiation codon usage in bacteria. Surprisingly, the only paper specifically to address this topic was published well before the genomic era (Rudd & Schneider, 1992). From that paper, one is left with the impression that, at least in the case of *Escherichia coli*, ATG(AUG) is the predominant initiation codon (92.0 % of characterized genes), while GTG(GUG) functions in 6.7 % of translational starts, and TTG(UUG) accounts for only 1.2 %.

We have reexamined the status of initiation codon usage using data from 620 bacterial chromosomes (GenBank data to February 14, 2008). Data on bacterial genomes were downloaded from the GenBank ftp site at the National Center for Biotechnology Information (NCBI; <ftp://ftp.ncbi.nih.gov/genbank/genomes/Bacteria/>). Information on the microbial phylum and genome mass was extracted from the genomic flat file (*.gbk), and on mol%G + C from the FASTA nucleotide (*.fna) files. Initiation codon usage was determined using Inidon (Initiation Codon; <http://molbiol-tools.ca/Inidon/>) software. This program was written as a Java applet and is easily accessible via a Java-enabled web browser. This tool accepts FASTA-formatted gene files (*.ffn – nucleotide coding regions). Inidon goes through each gene in these files and records the number of

occurrences for every initiation codon encountered. The program reports the total number of genes in the input file, the number of occurrences and the frequency of each encountered initiation codon in decreasing order. The results were then exported into MS Excel (the complete database can be viewed as Supplementary Table S1).

Although the total data show considerable scatter (Fig. 1), analysis using Lowess Spine analysis (GraphPad Software) suggests that the overall base composition of the host genome has little impact on initiation codon utilization between 30 and 65 mol%G + C (Fig. 1).

Fig. 1. Utilization of ATG, GTG and TTG initiation codons in 620 bacterial chromosomes as a function of their mol%G + C content.

A table of results exported from Inidon for 620 bacterial chromosomes is available with the online version of this paper. Access to Inidon software can be obtained at http://molbiol-tools.ca/InitCodon/Supplementary_TableS1.xls.

Table 1. Closely related genomes with significantly differing percentages of ATG initiation codon utilization

CDS, coding sequence.

Genomic pairs	Percentage utilization of ATG	Potential CDSs with different initiation codons*/mean number CDSs
<i>Bacillus thuringiensis</i> konkukian	80.3	935/4926
<i>Bacillus thuringiensis</i> Al Hakam	67.0	
<i>Brucella melitensis</i> 16M chromosome I	79.4	433/2122
<i>Brucella melitensis</i> biovar Abortus 2308 chromosome I	63.3	
<i>Burkholderia pseudomallei</i> K96243 chromosome I	80.3	123/3587
<i>Burkholderia pseudomallei</i> 1710b chromosome I	73.3	
<i>Chlamydomophila pneumoniae</i> CWL029	82.2	57/1082
<i>Chlamydomophila pneumoniae</i> TW 183	72.7	
<i>Ehrlichia ruminantium</i> str. Welgevonden	86.4	104/938
<i>Ehrlichia ruminantium</i> str. Welgevonden	71.9	
<i>Legionella pneumophila</i> Corby	89.4	675/3073
<i>Legionella pneumophila</i> Philadelphia 1	74.5	
<i>Leptospira interrogans</i> serovar Lai chromosome I	58.6	250/3825
<i>Leptospira interrogans</i> serovar Copenhageni chromosome I	67.9	
<i>Prochlorococcus marinus</i> MIT 9215	80.8	197/2490
<i>Prochlorococcus marinus</i> MIT 9303	67.7	
<i>Streptococcus pyogenes</i> SSI-1	88.2	295/1880
<i>Streptococcus pyogenes</i> MGAS2096	70.9	
<i>Streptococcus thermophilus</i> LMD-9	84.8	111/1812
<i>Streptococcus thermophilus</i> CNRZ1066	69.9	
<i>Vibrio vulnificus</i> CMCP6 chromosome I	89.2	385/3117
<i>Vibrio vulnificus</i> YJ016 chromosome I	69.5	
<i>Yersinia pestis</i> Pestoides F	87.7	615/3920
<i>Yersinia pestis</i> KIM	67.5	

* (Percentage ATG × number CDSs)_{organism 1} − (percentage ATG × numberCDSs)_{organism 2}

Bacterial genomes with extremely low GC contents tended to exclusively utilize ATG, while at high host mol%GC the utilization of ATG and TTG decreased and that of GTG increased. The mean utilization of these three codons was ATG, 80.1 %, GTG, 11.6 %, and TTG, 7.8 %, values considerably different from those reported by Rudd & Schneider (1992).

In most cases where similar species or strains were analysed, the utilization percentages of the three initiation codons were remarkably similar. In certain cases (see Table 1) the results exhibited significant differences.

In part this may be due to differences in recognizing coding regions, since many of these pairs showed significant differences in the total number of genes. This was not due to the massive presence of pseudogenes, which only had an impact on 'initiation codon' percentages in *Mycobacterium leprae* and *Rickettsia massiliae* (see Supplementary Table S1, column M).

In addition, in the case of *Mycoplasma gallisepticum*, ATT and ATC contributed significantly to initiation codon utilization, yet apparently are not utilized in related species. Examination of the data further suggests that the major

discrepancies are due to over or under estimation of the role of TTG codons, and to a lesser extent the utilization of GTG. These results clearly indicate that the identification of initiation codons in bacterial genes is still far from precise and therefore warrants new software developments plus the reexamination, and possible third-party correction, of existing GenBank genomic data.

REFERENCES

- McInerney, J. O. (1998). GCUA: general codon usage analysis. *Bioinformatics* **14**, 372–373.
- Nakamura, Y., Gojobori, T. & Ikemura, T. (2000). Codon usage tabulated from the international DNA sequence databases: status for the year 2000. *Nucleic Acids Res* **28**, 292.
- Puigbo, P., Guzman, E., Romeu, A. & Garcia-Vallve, S. (2007). OPTIMIZER: a web server for optimizing the codon usage of DNA sequences. *Nucleic Acids Res* **35**, W126–W131.
- Puigbo, P., Bravo, I. G. & Garcia-Vallve, S. (2008). E-CAI: a novel server to estimate an expected value of Codon Adaptation Index (eCAI). *BMC Bioinformatics* **9**, 65.
- Ramazzotti, M., Brilli, M., Fani, R., Manao, G. & Degl'innocenti, D. (2007). The CAI Analyser Package: inferring gene expressivity from raw genomic data. *In Silico Biol* **7**, 507–526.

Rudd, K. E. & Schneider, T. D. (1992). Compilation of *E. coli* ribosome binding sites. In *A Short Course in Bacterial Genetics*, pp. 17.19–17.45. Edited by J. H. Miller. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Vetrivel, U., Arunkumar, V. & Dorairaj, S. (2007). ACUA: a software tool for automated codon usage analysis. *Bioinformation* 2, 62–63.

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