

**Analysis of Codon Usage Bias of Delta 6 Fatty Acid Elongase Gene in *Pyramimonas cordata* isolate CS-140**Xue Wei Dong<sup>1</sup>, You Zhi Li<sup>1</sup>, Yu Ping Bi<sup>2</sup>, Zhen Ying Peng<sup>2</sup>, Qing Fang He<sup>2,3\*</sup><sup>1</sup> College of Life Science and Technology, State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, Guangxi University, Nanning, Guangxi, 530004, PR China.<sup>2</sup> High-Tech Research Center, Shandong Academy of Agricultural Science, Key Laboratory for Genetic Improvement of Crop, Animal and Poultry of Shandong Province, Key Laboratory of Crop Genetic Improvement and Biotechnology, Huanghuaihai, Ministry of Agriculture, Jinan, Shandong, 250100, PR China.<sup>3</sup> Department of Applied Science, University of Arkansas, Little Rock, Arkansas, 72204, US.[qfhe@ualr.edu](mailto:qfhe@ualr.edu)

**Abstract: Objectives:** The study was designed to provide a basis for understanding the codon usage bias of the *Pyramimonas cordata* isolate CS-140 delta 6 fatty acid elongase and selecting suitable expression systems for heterologous protein production. **Methods:** CodonW and CUSP programs were applied to calculate the effective number of codon (ENC) value, nucleotide contents, the relative synonymous codon usage (RSCU) of *Pyramimonas cordata* isolate CS-140 delta 6 fatty acid elongase genes. **Results:** *Pyramimonas cordata* isolate CS-140 delta 6 fatty acid elongase gene was biased toward the G-ended and C-ended synonymous codons. In addition, there were 17 codons showing distinct usage differences between PcD6FAE and *E.coli*, 23 codons between PcD6FAE and *Synechocystis sp.* PCC 6803, 26 codons between PcD6FAE and yeast, 25 codons between PcD6FAE and *Arabidopsis thaliana*. Therefore the *E.coli* and *Synechocystis sp.* PCC 6803 as prokaryotic expression systems might be suitable for the expression of PcD6FAE gene.

[Dong XW, Li YZ, Bi YP, Peng ZY, He QF. **Analysis of Codon Usage Bias of Delta 6 Fatty Acid Elongase Gene in *Pyramimonas cordata* isolate CS-140.** *Life Sci J* 2013;10(2):2987-2991] (ISSN:1097-8135). <http://www.lifesciencesite.com>. 413

**Keywords:** *Pyramimonas cordata* isolate CS-140; delta 6 fatty acid elongase (D6FAE); codon usage bias; gene expression

**1. Introduction**

Delta 6 fatty acid elongase is a rate-limiting enzyme that extends saturated and monounsaturated fatty acids (Saito, 2011). *Pyramimonas cordata* isolate CS-140 delta 6 fatty acid elongase (PcD6FAE) which was isolated from the microalgae *Pyramimonas cordata* isolate CS-140 converts its substrate efficiently when transformed in yeast (65.6% conversion for n3 fatty acid). And it is likely that, when assembled in a transgenic plant, these genes will produce an efficient pathway leading to the synthesis of DHA (Petrie, 2010).

Synonymous codons are not used equally both within and between genomes (Zhou, 2009). The genes will form a specific synonymous codon usage preference after long-term evolution, called codon bias (Biro, 2008). Researches on codon usage bias carried out in different species find that synonymous codon encoding amino acids in the synthesis of protein don't use randomly (Dittmar, 2006). Codon usage bias affected by many causes, such as mutations preferences, translator selection, protein secondary structure, replication and transcription alternatives, hydrophilic and hydrophobic of proteins (Levin, 2000; Gupta, 2001). These systematic biases can be exploited to perform many analyses of great theoretical and practical interest.

We can choose a suitable expression system

or optimize codon to improve expression of target gene through analysis of the PcD6FAE gene's codon usage bias based on EMBOSS and CondonW online program. Comparison the PcD6FAE gene with genome of *E.coli*, yeast, *Synechocystis sp.* PCC 6803 and *Arabidopsis thaliana* provides a basis for understanding the corresponding mechanism for biased usage of synonymous codons and selecting an proper prokaryotic or eukaryotic expression system to improve expression of a target gene. It lays the foundation for further study of structure and function of PcD6FAE.

**2. Materials and methods****2.1 Sequence data and Analysis Software**

The sequence used in the research is extracted from the GenBank database. Codon usage was estimated by using codonw 1.4.4 through online website <http://mobyle.pasteur.fr/cgi-bin/portal.py?#forms::codonw> and CUSP program of EMBOSS (The European Molecular Biology Open Software Suite, <http://bioinfo.pbi.nrc.ca:8090/EMBOSS/>) (Li et al., 2012).

**2.2 Codon usage indices analysis**

Relative synonymous codon usage values are estimated as the ratio of the observed codon usage to

that value expected if there is uniform usage within synonymous groups (Sharp, 1986). RSCU value which is independent of the amino acid and abundance of codon usage can reflect the codon usage preference directly. The codon RSCU value is equal to 1 if no preference codon usage. When a codon RSCU value is greater than 1, it indicates that the frequency of codon usage is relatively high, and vice versa. Effective number of codons (ENc) which ranges from 20 to 61 is used to describe the degree of codon usage preferences. The GC index is used to calculate the overall GC content in the gene. The GC3s which represents the frequency of the nucleotide G + C at the third synonymous codon position (excluding Met, Trp and termination codons) is a good indicator in the extent of base composition bias (Elhaik, 2009).

### 2.3 Comparison of codon preferences of PcD6FAE gene with those of *E.coli*, yeast, *Synechocystis sp.* PCC 6803 and *Arabidopsis thaliana*

The codon usage bias among PcD6FAE gene with genome of *E.coli*, yeast, *Synechocystis sp.* PCC 6803 and *Arabidopsis thaliana* were compared based on the codon usage database (<http://www.kazusa.or.jp/codon>) and the CUSP program in the EMBOSS software suite.

## 3. Results and Discussion

### 3.1 Synonymous codon usage variation in D6FAE genes

The codon usage of PcD6FAE was presented in Table 1. The ENc value of PcD6FAE was 44.745.

Generally speaking, the gene is thought to possess strong codon bias if the ENc value of a gene is 35 or less and weak codon bias if the ENc value of a gene is 50 or more (Wright, 1990). The result shows that the PcD6FAE gene doesn't have a strong codon usage bias according to its values as shown in Table 1.

Table 1 Condon bias of PcD6FAE gene analyzed by CUSP and CodonW program

| GenBank accession No. | ENc value | GC3s | GC   | Length/bp |
|-----------------------|-----------|------|------|-----------|
| GQ202034              | 44.75     | 0.64 | 0.54 | 867       |

Base composition has been found to influence both codon usage and gene function (Fadiel et al., 2001). The GC contents of the overall and the third codon position (GC3s) were 0.542 and 0.635, respectively, indicating that G+C content was higher than A+T content. Since this species has a moderately high GC content, it is expected that G or C ending codons would predominate.

The results calculated by CUSP and CodonW online program shows that 25 codon RSCU value is greater than 1 in PcD6FAE gene, in which there are 22 codons are G-end or C-end (that are GCG, UGC, GAC, GAG, UUC, GGC, CAC, AUC, AAG, CUG, CUC, AAC, CCC, CAG, CGC, AGC, UCC, ACG, ACC, GUG, GUC, UAC). The fraction values corresponding to these codons are also higher. The results mean that the used degenerate codons are found to be C or G ending. This supports the argument of mutational bias presented in Table 1.

Table 2 codon usage bias of PcD6FAE gene analyzed by CUSP and CodonW program

| Amino acid | Codon | Fraction | RSCU        | Amino acid | Codon | Fraction    | RSCU        |
|------------|-------|----------|-------------|------------|-------|-------------|-------------|
| Ala        | GCG   | 0.46     | <u>1.86</u> | Asn        | AAU   | 0.00        | 0.00        |
|            | GCA   | 0.14     | 0.57        |            | AAC   | 1.00        | <u>2.00</u> |
|            | GCU   | 0.18     | 0.71        | Pro        | CCG   | 0.15        | 0.62        |
|            | GCC   | 0.21     | 0.86        |            | CCA   | 0.00        | 0.00        |
| Cys        | UGU   | 0.00     | 0.00        |            | CCU   | 0.39        | <u>1.54</u> |
|            | UGC   | 1.00     | <u>2.00</u> | CCC        | 0.46  | <u>1.85</u> |             |
| Asp        | GAU   | 0.14     | 0.29        | Gln        | CAG   | 0.91        | <u>1.82</u> |
|            | GAC   | 0.86     | <u>1.71</u> |            | CAA   | 0.09        | 0.18        |
| Glu        | GAG   | 1.00     | <u>2.00</u> | Arg        | AGG   | 0.00        | 0.00        |
|            | GAA   | 0.00     | 0.00        |            | AGA   | 0.00        | 0.00        |
| Phe        | UUU   | 0.05     | 0.11        |            | CGG   | 0.00        | 0.00        |
|            | UUC   | 0.95     | <u>1.89</u> |            | CGA   | 0.00        | 0.00        |
| Gly        | GGG   | 0.14     | 0.57        |            | CGU   | 0.00        | 0.00        |
|            | GGA   | 0.07     | 0.29        | CGC        | 1.00  | <u>6.00</u> |             |
|            | GGU   | 0.29     | <u>1.14</u> | Ser        | AGU   | 0.11        | 0.63        |
| His        | GGC   | 0.50     | <u>2.00</u> |            | AGC   | 0.32        | <u>1.89</u> |
|            | CAU   | 0.43     | 0.86        |            | UCG   | 0.11        | 0.63        |
| Ile        | CAC   | 0.57     | <u>1.14</u> |            | UCA   | 0.05        | 0.32        |
|            | AUA   | 0.07     | 0.20        |            | UCU   | 0.21        | <u>1.26</u> |
|            | AUU   | 0.33     | 1.00        | UCC        | 0.21  | <u>1.26</u> |             |

|     |     |      |             |     |     |      |             |
|-----|-----|------|-------------|-----|-----|------|-------------|
|     | AUC | 0.60 | <u>1.80</u> |     | ACG | 0.36 | <u>1.45</u> |
| Lys | AAG | 0.94 | <u>1.88</u> | Thr | ACA | 0.18 | 0.73        |
|     | AAA | 0.06 | 0.12        |     | ACU | 0.00 | 0.00        |
|     | UUG | 0.09 | 0.53        |     | ACC | 0.46 | <u>1.82</u> |
| Leu | UUA | 0.00 | 0.00        | Val | GUG | 0.44 | <u>1.74</u> |
|     | CUG | 0.38 | <u>2.29</u> |     | GUA | 0.13 | 0.52        |
|     | CUA | 0.03 | 0.18        |     | GUU | 0.04 | 0.17        |
|     | CUU | 0.15 | 0.88        |     | GUC | 0.39 | <u>1.57</u> |
|     | CUC | 0.35 | <u>2.12</u> |     | UAU | 0.13 | 0.25        |
|     |     |      |             |     |     | UAC  | 0.88        |
|     |     |      |             | Tyr |     |      |             |

The data with underline mean that the value of RSCU>1

### 3.2 Comparison of codon usage bias among PcD6FAE, *E.coli*, yeast, *Synechocystis sp.* PCC 6803 and *Arabidopsis thaliana*

Generally, the codon usage bias in a gene remains conserved to a certain degree across species (Jia, 2009). *E.coli* and *Synechocystis sp.* PCC 6803 as prokaryotic expression systems, yeast and *Arabidopsis* as eukaryotic expression systems are widely used to express target genes. The PcD6FAE gene was compared with genome of *E.coli*, yeast, *Synechocystis sp.* PCC 6803 and *Arabidopsis thaliana* to see which will be the suitable host for the optimal expression of PcD6FAE gene. The ratio of codon usage frequency is one of the indicators to measure codon usage bias of different species. The ratio higher than 2 or lower 0.5 indicates that the codon usage preference differs, and

vice versa (Jia, 2009).

Data in Table 3 reveals there are 17 codons showing distinct usage differences between the PcD6FAE gene and *E.coli* genome; 26, between the PcD6FAE gene and yeast genome, 23, between the PcD6FAE gene and *Synechocystis sp.* PCC 6803 genome and 25, between the PcD6FAE gene and *Arabidopsis thaliana* genome, suggesting that codon usage of the PcD6FAE gene more closely resembles that of *E.coli* and *Synechocystis sp.* PCC 6803 genes than that of yeast and *Arabidopsis thaliana* genes. All of these may suggest that expressing D5FAD gene is more efficiently in *E.coli* or *Synechocystis sp.* PCC 6803 which will lay the foundation for prokaryotic expression of the PcD6FAE gene to enhance production of long chain unsaturated fatty acids.

Table 3 Comparison of codon preferences between the D6FAE gene and *E.coli*, yeast and *Synechocystis sp.* PCC 6803 (Sy) and *Arabidopsis thaliana* (A.th)

| Codon | P     | E     | S     | Sy    | A.th  | P/E         | P/S         | P/Sy        | P/A.th      |
|-------|-------|-------|-------|-------|-------|-------------|-------------|-------------|-------------|
| GCG   | 44.98 | 32.32 | 6.18  | 15.20 | 9.00  | 1.39        | <u>7.28</u> | <u>2.96</u> | <u>5.00</u> |
| GCA   | 13.84 | 20.69 | 16.21 | 10.90 | 17.50 | 0.67        | 0.85        | 1.27        | 0.79        |
| GCU   | 17.30 | 15.42 | 21.17 | 20.00 | 28.30 | 1.12        | 0.82        | 0.87        | 0.61        |
| GCC   | 20.76 | 25.18 | 12.60 | 37.50 | 10.30 | 0.82        | 1.65        | 0.55        | <u>2.02</u> |
| UGU   | 0.00  | 5.25  | 8.10  | 6.30  | 10.50 | 0.00        | 0.00        | 0.00        | 0.00        |
| UGC   | 6.92  | 6.38  | 4.76  | 3.90  | 7.20  | 1.08        | 1.45        | 1.77        | 0.96        |
| GAU   | 3.46  | 32.84 | 37.59 | 32.50 | 36.60 | <u>0.11</u> | <u>0.09</u> | <u>0.11</u> | <u>0.09</u> |
| GAC   | 20.76 | 19.21 | 20.21 | 17.80 | 17.20 | 1.08        | 1.03        | 1.17        | 1.21        |
| GAG   | 24.22 | 18.71 | 19.24 | 16.10 | 32.20 | 1.29        | 1.26        | 1.50        | 0.75        |
| GAA   | 0.00  | 39.30 | 45.60 | 44.70 | 34.30 | 0.00        | 0.00        | 0.00        | 0.00        |
| UUU   | 3.46  | 22.24 | 26.12 | 29.40 | 21.50 | <u>0.16</u> | <u>0.13</u> | <u>0.12</u> | <u>0.16</u> |
| UUC   | 62.28 | 15.86 | 18.44 | 10.50 | 20.70 | <u>3.93</u> | <u>3.38</u> | <u>5.93</u> | <u>3.01</u> |
| GGG   | 6.92  | 11.76 | 6.02  | 17.60 | 10.20 | 0.59        | 1.15        | <u>0.39</u> | 0.68        |
| GGA   | 3.46  | 8.87  | 10.90 | 12.90 | 24.20 | <u>0.39</u> | <u>0.32</u> | <u>0.27</u> | <u>0.14</u> |
| GGU   | 13.84 | 24.19 | 23.89 | 19.80 | 22.20 | 0.57        | 0.58        | 0.70        | 0.62        |
| GGC   | 24.22 | 28.06 | 9.78  | 22.30 | 9.20  | 0.86        | <u>2.48</u> | 1.09        | <u>2.63</u> |
| CAU   | 10.38 | 12.80 | 13.62 | 32.50 | 13.80 | 0.81        | 0.76        | <u>0.32</u> | 0.75        |
| CAC   | 13.84 | 9.38  | 7.77  | 17.80 | 8.70  | 1.48        | 1.78        | 0.78        | 1.59        |
| AUA   | 3.46  | 5.48  | 17.79 | 4.90  | 12.60 | 0.63        | <u>0.19</u> | 0.71        | <u>0.27</u> |
| AUU   | 17.30 | 29.69 | 30.13 | 40.00 | 21.50 | 0.58        | <u>0.57</u> | <u>0.43</u> | 0.80        |
| AUC   | 31.14 | 23.89 | 17.17 | 17.80 | 18.50 | 1.30        | 1.81        | 1.75        | 1.68        |
| AAG   | 55.36 | 11.00 | 30.82 | 12.80 | 32.70 | <u>5.03</u> | 1.80        | <u>4.33</u> | 1.69        |

|     |       |       |       |       |       |              |             |             |             |
|-----|-------|-------|-------|-------|-------|--------------|-------------|-------------|-------------|
| AAA | 3.46  | 33.96 | 41.87 | 30.10 | 30.80 | <u>0.10</u>  | <u>0.08</u> | <u>0.11</u> | <u>0.11</u> |
| UUG | 10.38 | 12.97 | 27.17 | 28.90 | 20.90 | 0.80         | <u>0.38</u> | <u>0.36</u> | 0.50        |
| UUA | 0.00  | 13.85 | 26.15 | 26.40 | 12.70 | 0.00         | 0.00        | 0.00        | 0.00        |
| CUG | 44.98 | 51.07 | 10.48 | 20.00 | 9.80  | 0.88         | <u>4.29</u> | <u>2.25</u> | <u>4.59</u> |
| CUA | 3.46  | 3.88  | 13.41 | 14.00 | 9.90  | 0.89         | <u>0.26</u> | <u>0.25</u> | <u>0.35</u> |
| CUU | 17.30 | 11.40 | 12.25 | 10.20 | 24.10 | 1.52         | 1.41        | 1.70        | 0.72        |
| CUC | 41.52 | 10.47 | 5.44  | 13.90 | 16.10 | <u>3.97</u>  | <u>7.63</u> | <u>2.99</u> | <u>2.58</u> |
| AUG | 55.36 | 27.21 | 20.94 | 19.30 | 24.50 | <u>2.03</u>  | <u>2.64</u> | <u>2.87</u> | <u>2.26</u> |
| AAU | 0.00  | 19.15 | 35.68 | 25.70 | 22.30 | 0.00         | 0.00        | 0.00        | 0.00        |
| AAC | 31.14 | 21.66 | 24.82 | 15.00 | 20.90 | 1.44         | 1.25        | <u>2.08</u> | 1.49        |
| CCG | 6.92  | 22.45 | 5.29  | 8.30  | 8.60  | <u>0.31</u>  | 1.31        | 0.83        | 0.80        |
| CCA | 0.00  | 8.38  | 18.31 | 8.10  | 16.10 | 0.00         | 0.00        | 0.00        | 0.00        |
| CCU | 17.30 | 7.23  | 13.51 | 10.00 | 18.70 | <u>2.39</u>  | 1.28        | 1.73        | 0.93        |
| CCC | 20.76 | 5.56  | 6.78  | 24.50 | 5.30  | <u>3.73</u>  | <u>3.06</u> | 0.85        | <u>3.92</u> |
| CAG | 34.60 | 29.37 | 12.11 | 21.20 | 15.20 | 1.18         | <u>2.86</u> | 1.63        | <u>2.28</u> |
| CAA | 3.46  | 14.71 | 27.28 | 34.00 | 19.40 | <u>0.24</u>  | <u>0.13</u> | <u>0.10</u> | <u>0.18</u> |
| AGG | 0.00  | 1.81  | 9.23  | 4.80  | 11.00 | 0.00         | 0.00        | 0.00        | 0.00        |
| AGA | 0.00  | 2.89  | 21.28 | 4.60  | 19.00 | 0.00         | 0.00        | 0.00        | 0.00        |
| CGG | 0.00  | 6.18  | 1.74  | 13.40 | 4.90  | 0.00         | 0.00        | 0.00        | 0.00        |
| CGA | 0.00  | 3.83  | 2.99  | 5.40  | 6.30  | 0.00         | 0.00        | 0.00        | 0.00        |
| CGU | 0.00  | 20.22 | 6.40  | 10.30 | 9.00  | 0.00         | 0.00        | 0.00        | 0.00        |
| CGC | 13.84 | 20.82 | 2.60  | 12.20 | 3.80  | 0.66         | <u>5.32</u> | 1.13        | <u>3.64</u> |
| AGU | 6.92  | 9.38  | 14.15 | 15.10 | 14.00 | 0.74         | <u>0.49</u> | <u>0.46</u> | <u>0.49</u> |
| AGC | 20.76 | 16.01 | 9.75  | 10.30 | 11.30 | 1.30         | <u>2.13</u> | <u>2.02</u> | 1.84        |
| UCG | 6.92  | 8.82  | 8.56  | 4.10  | 9.30  | 0.78         | 0.81        | 1.69        | 0.74        |
| UCA | 3.46  | 8.14  | 18.67 | 4.30  | 18.30 | <u>0.43</u>  | <u>0.19</u> | 0.80        | <u>0.19</u> |
| UCU | 13.84 | 8.70  | 23.50 | 9.00  | 25.20 | 1.59         | 0.59        | 1.54        | 0.55        |
| UCC | 13.84 | 8.95  | 14.22 | 15.80 | 11.20 | 1.55         | 0.97        | 0.88        | 1.24        |
| ACG | 13.84 | 14.97 | 7.96  | 7.80  | 7.70  | 0.92         | 1.74        | 1.77        | 1.80        |
| ACA | 6.92  | 8.09  | 17.76 | 7.00  | 15.70 | 0.86         | <u>0.39</u> | 0.99        | <u>0.44</u> |
| ACU | 0.00  | 9.08  | 20.28 | 13.90 | 17.50 | 0.00         | 0.00        | 0.00        | 0.00        |
| ACC | 17.30 | 22.79 | 12.73 | 26.00 | 10.30 | 0.76         | 1.36        | 0.67        | 1.68        |
| GUG | 34.60 | 26.22 | 10.76 | 28.00 | 17.40 | 1.32         | <u>3.22</u> | 1.24        | 1.99        |
| GUA | 10.38 | 10.90 | 11.77 | 10.50 | 9.90  | 0.95         | 0.88        | 0.99        | 1.05        |
| GUU | 3.46  | 18.12 | 22.07 | 16.80 | 27.20 | <u>0.19</u>  | <u>0.16</u> | <u>0.21</u> | <u>0.13</u> |
| GUC | 31.14 | 14.81 | 11.78 | 11.20 | 12.80 | <u>2.10</u>  | <u>2.64</u> | <u>2.78</u> | <u>2.43</u> |
| UGG | 27.68 | 15.30 | 10.37 | 15.50 | 12.50 | 1.81         | <u>2.67</u> | 1.79        | <u>2.21</u> |
| UAU | 10.38 | 16.54 | 18.78 | 17.40 | 14.60 | 0.63         | 0.55        | 0.60        | 0.71        |
| UAC | 72.66 | 12.27 | 14.78 | 11.90 | 13.70 | <u>5.92</u>  | <u>4.92</u> | <u>6.11</u> | <u>5.30</u> |
| UGA | 0.00  | 1.09  | 0.68  | 0.60  | 1.20  | 0.00         | 0.00        | 0.00        | 0.00        |
| UAG | 3.46  | 0.27  | 0.51  | 1.10  | 0.50  | <u>12.81</u> | <u>6.78</u> | <u>3.15</u> | <u>6.92</u> |
| UAA | 0.00  | 1.96  | 1.06  | 1.40  | 0.90  | 0.00         | 0.00        | 0.00        | 0.00        |

Data with underline: There are obvious differences of values ( $\leq 0.5$ ,  $\geq 2$ ) between the codons of two species

#### Acknowledgments

This work was supported by National Natural Science Foundation of China (No. 31270102) and Shandong Province "Taishan Scholar" Foundation (No.tshw20091014).

#### \*Corresponding author

Qing Fang He  
High-Tech Research Center, Shandong Academy of Agricultural Science, Key Laboratory for Genetic

Improvement of Crop, Animal and Poultry of Shandong Province, Key Laboratory of Crop Genetic Improvement and Biotechnology, Huanghuaihai, Ministry of Agriculture, Jinan, Shandong, 250100, PR China.

Department of Applied Science, University of Arkansas, Little Rock, Arkansas, 72204, US.  
Tel: +86-531-83179149 or 501-569-8033  
E-mail: [qfhe@ualr.edu](mailto:qfhe@ualr.edu)

**References**

1. Biro JC. "Does codon bias have an evolutionary origin?". *Theor Biol Med Model* 2008; 5(16):1-16.
2. Dittmar KA, Goodenbour JM, Pan T. Tissue-specific differences in human transfer RNA expression. *PLoS Genet.* 2006; 2(12): 2107–2115.
3. Elhaik E, Landan G, Graur D. Can GC content at third-codon positions be used as a proxy for isochore composition? *Mol Biol Evol* 2009; 26(8):1829–1833.
4. Fadiel A, Lithwick S, Wanas MQ, Cuticchia AJ. Influence of intercodon and base frequencies on codon usage in *filarial parasites*. *Genomics* 2001; 74: 197-210.
5. Gupta SK, Ghosh TC. Gene expressivity is the main factor in dictating the codon usage variation among the genes in *Pseudomonas aeruginosa*. *Gene* 2001; 273(1):63-70.
6. Jia R, Cheng A, Wang M, Xin H, Guo Y, Zhu D, Qi X, Zhao L, Ge H, Chen X. Analysis of synonymous codon usage in the UL24 gene of duck enteritis virus. *Virus Genes* 2009; 38(1):96-103
7. Levin DB, Whittome B. Codon usage in nucleopolyhedroviruses. *J Gen Virol* 2000; 81(9):2313-25
8. Li M, Zhao Z, Chen J, Wang B, Li Z, Li J, Cai M. Characterization of Synonymous Codon Usage Bias in the *Pseudorabies Virus* US1 Gene. *ViroSin* 2012; 27 (5):303-315.
9. Petrie JR, Liu Q, Mackenzie AM, Shrestha P, Mansour MP, Robert SS, Frampton DF, Blackburn SI, Nichols PD, Singh SP. Isolation and characterization of a high-efficiency desaturase and elongases from microalgae for transgenic LC-PUFA production. *Mar Biotechnol* (NY) 2010; 12(4):430-8
10. Saito R, Matsuzaka T, Karasawa T, Sekiya M, Okada N, Igarashi M, Matsumori R, Ishii K, Nakagawa Y, Iwasaki H, Kobayashi K, Yatoh S, Takahashi A, Sone H, Suzuki H, Yahagi N, Yamada N, Shimano H. Macrophage Elov16 deficiency ameliorates foam cell formation and reduces atherosclerosis in low-density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 2011; 31(9):1973-9.
11. Sharp PM, Tuohy TMF, Mosurski KR. Codon usage in yeast: cluster analysis clearly differentiates highly and lowly expressed genes. *Nucleic Acids Res* 1986; 14(13):5125-43
12. Tonon T, Harvey D, Larson TR, Graham IA. Identification of a very long chain polyunsaturated fatty acid delta4 desaturase from the microalga *Pavlova lutheri*. *FEBS letters* 2003; 553(3):440-444
13. Wright F. The 'effective number of codons' used in a gene. *Gene* 1990; 87(1): 23–29.
14. Zhou T, Cheng AC, Wang MS, Zhu D, Jia R. Characterization of Codon Usage Bias in the Newly Identified DEVUL 10 Gene. *Proceeding BIOCOMPUCHEM'09 Proceedings of the 3rd WSEAS International Conference on Computational Chemistry, 2009,132-142.*

24/6/2013