

## *Listeria Monocytogenes* La111 and *Klebsiella Pneumoniae* KCTC 2242: Shine-Dalgarno Sequences

Gholamreza Motaleb\*

Department of Biology, University of Zabol, Zabol, Iran.

Submitted 7 Nov 2013; Accepted 1 Jan 2014

*Listeria monocytogenes* can cause serious infection and recently, relapse of listeriosis has been reported in leukemia and colorectal cancer, and the patients with *Klebsiella pneumoniae* are at increased risk of colorectal cancer. Translation initiation codon recognition is basically mediated by Shine-Dalgarno (SD) and the anti-SD sequences at the small ribosomal RNA (ssu rRNA). In this research, Shine-Dalgarno sequences prediction in *Listeria monocytogenes* La111 and *Klebsiella pneumoniae* KCTC 2242 was investigated. The whole genomic sequence of *Listeria monocytogenes* La111 and *Klebsiella pneumoniae* KCTC 2242 were retrieved from <http://www.ncbi.nlm.nih.gov/> (*Listeria monocytogenes* La111 NCBI Reference sequence: NC\_020557; *Klebsiella pneumoniae* KCTC 2242 NCBI Reference sequence: CP002910) in order to be analyzed with DAMBE software and BLAST. The results showed that the consensus sequence for *Klebsiella pneumoniae* KCTC 2242 was CCCCCCUCCCCUCCCCUCCUCCUCCUUUUUAAAAAGGGGAAAAACC and for *Listeria monocytogenes* La111 was CCCCCCUCCCCUCCCCUCCUCCUCCUUAUUCUUAUAAAAGGGGGGGGUUCAC. The  $P_{SD}$  was higher in *Listeria monocytogenes* La111 compared to *Klebsiella pneumoniae* KCTC 2242 ( $0.9090 > 0.8618$ ). The results showed that Nm in *Listeria monocytogenes* La111 was higher than *Klebsiella pneumoniae* KCTC 2242 ( $4.5846 > 4.4862$ ). Accurate characterization of SD sequences may increase our knowledge on how an organism's transcriptome is related to its cellular proteome.

**Key words:** Molecular biology, genomics, microbiology, Shine-Dalgarno sequences

Possible correlation and associations of rare bacteria with serious disease, especially cancer and laboratory isolations of these organisms in these patients have initiated the studies of pathogenetic significance of the agent (1). *Listeria monocytogenes* is an aerobic, gram-positive bacillus that has become an important pathogen in the 21st century (2). Transplantation patients, persons with neoplastic disease, immunocompromised subjects,

pregnant women (2), and HIV patients (3) are at high risk. To our knowledge, infection relapse of *Listeria monocytogenes* is rare, but relapse of listeriosis has been reported in leukemia and colorectal cancer (2, 4). *Klebsiella pneumoniae* is a gram-negative, anaerobic, and rod shaped bacterium. Neoplastic diseases are common in patients with nosocomial *Klebsiella pneumoniae* bacteraemia (5). Henao-Martínez et al. reported that

\* Corresponding author: Department of Biology, University of Zabol, Zabol, Iran. Email: reza.motaleb@uoz.ac.ir

*E. coli* and *Klebsiella pneumoniae* are especially prevalent in patients with gastrointestinal (GI) and lung cancers (6). Due to their abilities to cause basic cellular functional changes and attack host defense mechanisms, these bacteria have become a model for host pathogen interactions (7).

A molecular machine like ribosome translates the genetic code from messenger RNA into an amino acid sequence by RNA selection, peptide bond formation and translocation (8). Protein synthesis by ribosomes takes place on a linear substrate but at variable speeds. Transient pausing of ribosomes can impact a variety of co-translational processes, including protein targeting and folding. These pauses are influenced by the sequence of the mRNA. Thus, redundancy in the genetic code allows the same protein to be translated at different rates (9). mRNA sequences contain many AUG. How does the translation machinery distinguish which one is the initiation codon? Initial positioning of the ribosome on mRNA involves the recognition of a purine rich sequence, known as the Shine Dalgarno (SD) sequence, located upstream of the AUG initiation codon on the mRNA (8).

In 1974, Shine and Dalgarno sequenced the 3' end of *Escherichia coli*'s 16S ribosomal RNA (rRNA) and observed that part of the sequence, 5'-ACCUCC-3', was complementary to a motif, 5'-GGAGGU-3', located 5' of the initiation codons in several messenger RNAs (mRNAs) (9). They combined this observation with previously published experimental evidences and suggested that complementarity between the 3' tail of the 16S rRNA and the region 5' of the start codon on the mRNA was sufficient to create a stable, double-stranded structure that could position the ribosome correctly on the mRNA during translation initiation. The motif on the mRNAs, 5'-GGAGGU-3', and variations on it that are also complementary to parts of the 3' 16S rRNA tail, have since been referred to as the Shine-Dalgarno (SD) sequence. Shine and Dalgarno's theory was bolstered by Steitz and Jakes

in 1975 (10) and eventually experimentally verified in 1987, by Hui and de Boer (11) and Jacob et al. (12). The SD sequence has been established by experimental evidence that came from mutation studies. Unfortunately, experiments are tedious and only a few mutated SD sequences have been examined. Biopharmaceutical studies are highly interested in improving translation efficiency (13). In the present study, we tried to find the best possible SD for translation in *Listeria monocytogenes* La111, and *Klebsiella pneumoniae* KCTC 2242 through DAMBE software and BLAST analyzes.

## Materials and methods

This research started in Spring 2013 and data analyses were performed at bioinformatics facility of Faculty of Sciences in Zabol University, Iran. *Listeria monocytogenes* La111 (NCBI Reference sequence: NC\_020557) and *Klebsiella pneumoniae* KCTC 2242 (NCBI Reference sequence: CP002910) genome sequences were retrieved from <http://www.ncbi.nlm.nih.gov>. Fifty nucleotides upstream of the initiation coding sequences from each gene were extracted and position weight matrix (PWM) was employed to determine the SD sequence and location by the FASTA algorithm using DAMBE (14, 15). PWM is computed as:  $PWM_{ij} = \log_2 \left( \frac{p_{ij}}{p_i} \right)$  (1) where  $i = 1, 2, 3$  and 4 refer to A, C, G and U, respectively, and  $j$  is the site index, and  $p_i$  is the background frequency of nucleotide  $i$ , and  $p_{ij}$  is the site specific nucleotide frequency for nucleotide  $i$  at site  $j$ .

## Results

The position and sequence of Shine-Dalgarno as a functional motif was investigated in *Listeria monocytogenes* La111 and *Klebsiella pneumoniae* KCTC 2242 in order to find genetic motifs by DAMBE. SD sequence is often characterized by altered nucleotide frequencies (15). Table 1 and Figure 1 show the site specific frequency for

**Table 1.** Site specific frequencies analysis of *Klebsiella pneumoniae* KCTC 2242.

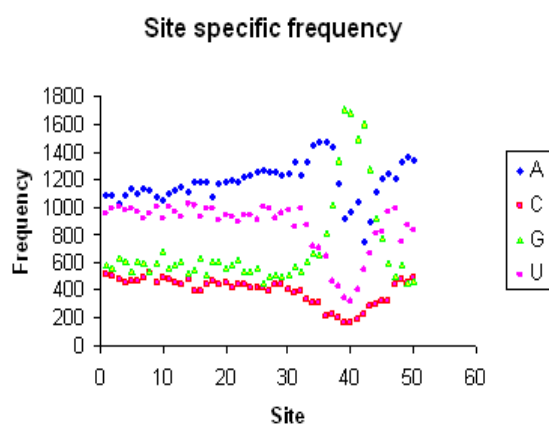
| Site | A    | C    | G    | U    |
|------|------|------|------|------|
| 1    | 1153 | 1289 | 1333 | 1148 |
| 2    | 1143 | 1313 | 1253 | 1214 |
| 3    | 1152 | 1247 | 1334 | 1190 |
| 4    | 1187 | 1222 | 1311 | 1203 |
| 5    | 1199 | 1247 | 1243 | 1234 |
| 6    | 1151 | 1269 | 1289 | 1214 |
| 7    | 1182 | 1299 | 1251 | 1191 |
| 8    | 1245 | 1174 | 1256 | 1248 |
| 9    | 1106 | 1301 | 1290 | 1226 |
| 10   | 1212 | 1237 | 1345 | 1129 |
| 11   | 1187 | 1286 | 1163 | 1287 |
| 12   | 1108 | 1274 | 1250 | 1291 |
| 13   | 1196 | 1243 | 1280 | 1204 |
| 14   | 1275 | 1204 | 1128 | 1316 |
| 15   | 1161 | 1295 | 1203 | 1264 |
| 16   | 1168 | 1321 | 1260 | 1174 |
| 17   | 1225 | 1290 | 1125 | 1283 |
| 18   | 1213 | 1235 | 1226 | 1249 |
| 19   | 1177 | 1281 | 1230 | 1235 |
| 20   | 1194 | 1248 | 1128 | 1353 |
| 21   | 1097 | 1299 | 1229 | 1298 |
| 22   | 1231 | 1308 | 1168 | 1216 |
| 23   | 1240 | 1216 | 1145 | 1322 |
| 24   | 1184 | 1314 | 1134 | 1291 |
| 25   | 1271 | 1303 | 1128 | 1221 |
| 26   | 1324 | 1201 | 1039 | 1359 |
| 27   | 1185 | 1335 | 1060 | 1343 |
| 28   | 1247 | 1291 | 1115 | 1270 |
| 29   | 1325 | 1232 | 992  | 1374 |
| 30   | 1258 | 1230 | 1011 | 1424 |
| 31   | 1368 | 1264 | 992  | 1299 |
| 32   | 1473 | 1169 | 911  | 1370 |
| 33   | 1339 | 1138 | 1052 | 1394 |
| 34   | 1507 | 1158 | 1007 | 1251 |
| 35   | 1541 | 1121 | 965  | 1296 |
| 36   | 1478 | 1035 | 1157 | 1253 |
| 37   | 1674 | 997  | 1178 | 1074 |
| 38   | 1734 | 962  | 1183 | 1044 |
| 39   | 1723 | 807  | 1562 | 831  |
| 40   | 1702 | 611  | 2053 | 557  |
| 41   | 1552 | 397  | 2456 | 518  |
| 42   | 1371 | 352  | 2738 | 462  |
| 43   | 1738 | 472  | 2084 | 629  |
| 44   | 1775 | 659  | 1640 | 849  |
| 45   | 1594 | 804  | 1430 | 1095 |
| 46   | 1541 | 1069 | 1122 | 1191 |
| 47   | 1556 | 1205 | 1082 | 1080 |
| 48   | 1929 | 942  | 1315 | 737  |
| 49   | 1088 | 1544 | 765  | 1526 |
| 50   | 1040 | 1484 | 993  | 1406 |

\* Site-specific counts with a window of 50 bases. A: adenine; C: cytosine; G: guanine; U: uracil. For example at site 1, A, 1153 times, C, 1289 times has been replicated and so on. The site specific frequencies can be used to derive a PWM to rapidly scan other sequences.

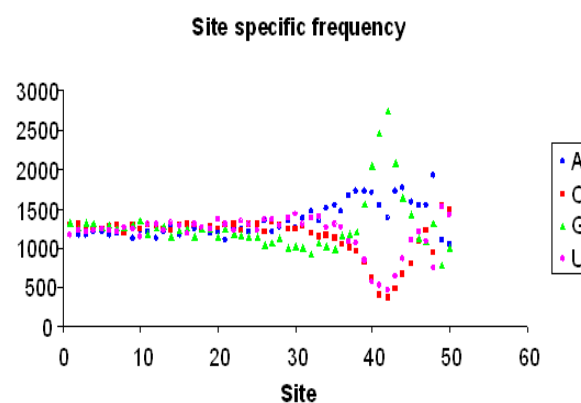
**Table 2.** Site specific frequencies analysis of *Listeria monocytogenes* La111.

| Site | A    | C   | G    | U    |
|------|------|-----|------|------|
| 1    | 1082 | 509 | 586  | 954  |
| 2    | 1079 | 495 | 563  | 994  |
| 3    | 1020 | 482 | 629  | 1000 |
| 4    | 1090 | 453 | 609  | 979  |
| 5    | 1136 | 469 | 536  | 990  |
| 6    | 1092 | 466 | 612  | 961  |
| 7    | 1135 | 491 | 592  | 913  |
| 8    | 1123 | 520 | 538  | 950  |
| 9    | 1075 | 455 | 600  | 1001 |
| 10   | 1051 | 492 | 675  | 913  |
| 11   | 1099 | 472 | 559  | 1001 |
| 12   | 1125 | 452 | 587  | 967  |
| 13   | 1150 | 447 | 604  | 930  |
| 14   | 1105 | 475 | 525  | 1026 |
| 15   | 1177 | 390 | 552  | 1012 |
| 16   | 1182 | 391 | 633  | 925  |
| 17   | 1183 | 441 | 512  | 995  |
| 18   | 1077 | 466 | 604  | 984  |
| 19   | 1172 | 447 | 605  | 907  |
| 20   | 1175 | 452 | 565  | 939  |
| 21   | 1190 | 422 | 589  | 930  |
| 22   | 1181 | 445 | 615  | 890  |
| 23   | 1210 | 442 | 537  | 942  |
| 24   | 1231 | 418 | 538  | 944  |
| 25   | 1253 | 414 | 555  | 909  |
| 26   | 1263 | 415 | 457  | 996  |
| 27   | 1246 | 391 | 503  | 991  |
| 28   | 1252 | 442 | 518  | 919  |
| 29   | 1232 | 437 | 504  | 958  |
| 30   | 1244 | 402 | 513  | 972  |
| 31   | 1321 | 380 | 572  | 858  |
| 32   | 1223 | 391 | 531  | 986  |
| 33   | 1320 | 335 | 603  | 873  |
| 34   | 1446 | 308 | 667  | 710  |
| 35   | 1461 | 313 | 659  | 698  |
| 36   | 1467 | 212 | 813  | 639  |
| 37   | 1430 | 228 | 1013 | 460  |
| 38   | 1171 | 199 | 1330 | 431  |
| 39   | 918  | 164 | 1708 | 341  |
| 40   | 961  | 172 | 1680 | 318  |
| 41   | 1039 | 194 | 1492 | 406  |
| 42   | 752  | 224 | 1602 | 553  |
| 43   | 898  | 283 | 1279 | 671  |
| 44   | 1108 | 302 | 913  | 808  |
| 45   | 1204 | 325 | 778  | 824  |
| 46   | 1241 | 325 | 598  | 967  |
| 47   | 1204 | 443 | 498  | 986  |
| 48   | 1321 | 482 | 581  | 747  |
| 49   | 1354 | 448 | 453  | 876  |
| 50   | 1341 | 487 | 463  | 840  |

\* Site-specific counts with a window of 50 bases. A: adenine; C: cytosine; G: guanine; U: uracil. For example at site 1, A, 1082 times, C, 509 times has been replicated and so on. The site specific frequencies can be used to derive a PWM to rapidly scan other sequences.



**Fig 1.** Site specific frequency scatter diagram of *Klebsiella pneumoniae* KCTC 2242.



**Fig 2.** Site specific frequency scatter diagram of *Listeria monocytogenes* La111.

*Klebsiella pneumoniae* KCTC 2242. Also Table 2 and Figure 2 show the site specific frequency for *Listeria monocytogenes* La111. PWM analysis showed that the consensus sequence for *Klebsiella pneumoniae* KCTC 2242 was CCCCCCUCUCCUCCUUUUUAAAAAGGGGAAAACC (Table 3) and for *Listeria monocytogenes* La111 was CCCCCCUCUCCCUUUCCCUCUAUUCUUAUAAAAGGGGGGGG-UUCAC (Table 4). FASTA algo-rithm analysis search output for *Klebsiella pneumoniae* KCTC 2242 and *Listeria monocytogenes* La111 has been shown in Tables 5 and 6. The results showed that the PSD was higher in *Listeria monocytogenes* La111 compared to *Klebsiella pneumoniae* KCTC 2242 ( $0.9090 > 0.8618$ ) (Table. 7). In *Listeria monocytogenes* La111, 2600 genes and in *Klebsiella pneumoniae* KCTC 2242, 3830 genes have Nm (the number of matched sites)  $\geq 3$  and Sm (the start of the match) within the range of 30 and 45 (NSD) and the proportion of 50 mers with the SD sequences is  $PSD = NSD/N$  (Table 7). In Tables 5 and 6, the second column being Nm or number of matched sites between the SD sequences and the 50 mers and third column is Sm or start of the match.

## Discussion

This study was conducted in order to find SD sequences in bacterial species by focusing on *Klebsiella pneumoniae* KCTC 2242 (representing gram negative bacteria) and *Listeria monocytogenes* La111 (representing gram positive bacteria) by performing a simulation study. Fifty nucleotides upstream of the CDS or coding sequences were extracted from each gene, then position weight matrix or PWM was used in order to find the SD sequence location (15). We studied and focused on those results or signals limited to the 20 nucleotides upstream of the CDSs. After that, the 16S rRNA or small subunit rRNA was extracted from the genome and we used the last 8 nucleotides in order to find the best match to the upstream sequence by the FASTA algorithm to rank SDs by the number of matched sites (matching strength or simply MS). Translation initiation is the limiting step and a main phase in gene expression in bacteria (16, 17).

As messenger RNAs has many AUG sequences, the main question is: How does the translational machinery knows which one is the initiation codon? In eukaryotes, this is accomplished by the scanning of the small ribosomal subunit which finds the first AUG and in prokaryotes, mainly through the matching between the Shine Dalgarno (SD) sequences located about 9 nucleotides upstream of the translation initiation

**Table 3.** PWM analysis of *K. pneumoniae* KCTC 2242. The consensus sequence is: CCCCCCUC-CCGCCUCCCCUCCUCCUCCUUUUUAAAAAAGGGGAAAAACC

| Site | A       | C       | G       | U       |
|------|---------|---------|---------|---------|
| 1    | 0.2105- | 0.1726  | 0.0677  | 0.0304- |
| 2    | 0.2230- | 0.1992  | 0.0215- | 0.0502  |
| 3    | 0.2117- | 0.1248  | 0.0688  | 0.0214  |
| 4    | 0.1685- | 0.0956  | 0.0437  | 0.0371  |
| 5    | 0.1540- | 0.1248  | 0.0331- | 0.0738  |
| 6    | 0.2130- | 0.1500  | 0.0193  | 0.0502  |
| 7    | 0.1746- | 0.1837  | 0.0238- | 0.0227  |
| 8    | 0.0997- | 0.0378  | 0.0181- | 0.0901  |
| 9    | 0.2705- | 0.1859  | 0.0204  | 0.0644  |
| 10   | 0.1385- | 0.1132  | 0.0807  | 0.0545- |
| 11   | 0.1685- | 0.1692  | 0.1290- | 0.1345  |
| 12   | 0.2679- | 0.1557  | 0.0250- | 0.1389  |
| 13   | 0.1576- | 0.1201  | 0.0092  | 0.0383  |
| 14   | 0.0654- | 0.0742  | 0.1731- | 0.1666  |
| 15   | 0.2005- | 0.1792  | 0.0803- | 0.1085  |
| 16   | 0.1918- | 0.2079  | 0.0135- | 0.0019  |
| 17   | 0.1231- | 0.1737  | 0.1770- | 0.1300  |
| 18   | 0.1373- | 0.1108  | 0.0529- | 0.0912  |
| 19   | 0.1807- | 0.1636  | 0.0483- | 0.0750  |
| 20   | 0.1601- | 0.1259  | 0.1731- | 0.2066  |
| 21   | 0.2823- | 0.1837  | 0.0494- | 0.1467  |
| 22   | 0.1160- | 0.1937  | 0.1229- | 0.0526  |
| 23   | 0.1055- | 0.0885  | 0.1515- | 0.1732  |
| 24   | 0.1722- | 0.2003  | 0.1655- | 0.1389  |
| 25   | 0.0699- | 0.1881  | 0.1731- | 0.0585  |
| 26   | 0.0110- | 0.0706  | 0.2917- | 0.2130  |
| 27   | 0.1710- | 0.2231  | 0.2628- | 0.1959  |
| 28   | 0.0974- | 0.1748  | 0.1898- | 0.1153  |
| 29   | 0.0099- | 0.1073  | 0.3584- | 0.2288  |
| 30   | 0.0847- | 0.1050  | 0.3311- | 0.2804  |
| 31   | 0.0362  | 0.1443  | 0.3584- | 0.1479  |
| 32   | 0.1428  | 0.0316  | 0.4813- | 0.2246  |
| 33   | 0.0053  | 0.0072- | 0.2737- | 0.2497  |
| 34   | 0.1757  | 0.0180  | 0.3368- | 0.0935  |
| 35   | 0.2079  | 0.0289- | 0.3982- | 0.1445  |
| 36   | 0.1477  | 0.1440- | 0.1365- | 0.0959  |
| 37   | 0.3273  | 0.1980- | 0.1106- | 0.1265- |
| 38   | 0.3781  | 0.2495- | 0.1044- | 0.1674- |
| 39   | 0.3690  | 0.5029- | 0.2964  | 0.4965- |
| 40   | 0.3513  | 0.9042- | 0.6907  | 1.0734- |
| 41   | 0.2182  | 1.5259- | 0.9493  | 1.1781- |
| 42   | 0.0393  | 1.6994- | 1.1060  | 1.3431- |
| 43   | 0.3815  | 1.2764- | 0.7123  | 0.8981- |
| 44   | 0.4118  | 0.7951- | 0.3667  | 0.4656- |
| 45   | 0.2567  | 0.5083- | 0.1691  | 0.0986- |
| 46   | 0.2079  | 0.0974- | 0.1808- | 0.0227  |
| 47   | 0.2219  | 0.0754  | 0.2332- | 0.1185- |
| 48   | 0.5319  | 0.2798- | 0.0481  | 0.6696- |
| 49   | 0.2942- | 0.4329  | 0.7332- | 0.3802  |
| 50   | 0.3592- | 0.3758  | 0.3570- | 0.2620  |

\* PWM sequences scanning with a window of 50 bases, e.g., from site 1 to site 50, from site 2 to site 50, and so on. A: adenine; C: cytosine; G: guanine; U: uracil.

**Table 4.** PWM analysis of *Listeria monocytogenes* La111. The consensus sequence is: CCCCCCCU-CCCCCUUCCCUCCUAUUCUUAUAAAAGG-GGGGGGUUCAC

| Site | A       | C       | G       | U       |
|------|---------|---------|---------|---------|
| 1    | 0.1200- | 0.3692  | 0.2661- | 0.1573  |
| 2    | 0.1240- | 0.3289  | 0.3239- | 0.2165  |
| 3    | 0.2051- | 0.2906  | 0.1640- | 0.2252  |
| 4    | 0.1093- | 0.2011  | 0.2106- | 0.1946  |
| 5    | 0.0497- | 0.2511  | 0.3947- | 0.2107  |
| 6    | 0.1067- | 0.2419  | 0.2035- | 0.1678  |
| 7    | 0.0510- | 0.3172  | 0.2514- | 0.0939  |
| 8    | 0.0663- | 0.4000  | 0.3894- | 0.1512  |
| 9    | 0.1293- | 0.2074  | 0.2321- | 0.2267  |
| 10   | 0.1619- | 0.3202  | 0.0622- | 0.0939  |
| 11   | 0.0975- | 0.2603  | 0.3342- | 0.2267  |
| 12   | 0.0638- | 0.1979  | 0.2637- | 0.1768  |
| 13   | 0.0321- | 0.1818  | 0.2225- | 0.1205  |
| 14   | 0.0896- | 0.2695  | 0.4246- | 0.2622  |
| 15   | 0.0014  | 0.0149- | 0.3523- | 0.2424  |
| 16   | 0.0075  | 0.0112- | 0.1549- | 0.1128  |
| 17   | 0.0088  | 0.1623  | 0.4608- | 0.2180  |
| 18   | 0.1266- | 0.2419  | 0.2225- | 0.2020  |
| 19   | 0.0047- | 0.1818  | 0.2201- | 0.0844  |
| 20   | 0.0010- | 0.1979  | 0.3188- | 0.1344  |
| 21   | 0.0173  | 0.0988  | 0.2588- | 0.1205  |
| 22   | 0.0063  | 0.1754  | 0.1965- | 0.0571  |
| 23   | 0.0413  | 0.1656  | 0.3921- | 0.1390  |
| 24   | 0.0661  | 0.0851  | 0.3894- | 0.1421  |
| 25   | 0.0917  | 0.0712  | 0.3445- | 0.0876  |
| 26   | 0.1031  | 0.0747  | 0.6247- | 0.2194  |
| 27   | 0.0836  | 0.0112- | 0.4864- | 0.2122  |
| 28   | 0.0905  | 0.1656  | 0.4440- | 0.1034  |
| 29   | 0.0673  | 0.1492  | 0.4835- | 0.1633  |
| 30   | 0.0813  | 0.0288  | 0.4580- | 0.1843  |
| 31   | 0.1679  | 0.0524- | 0.3010- | 0.0043  |
| 32   | 0.0567  | 0.0112- | 0.4083- | 0.2049  |
| 33   | 0.1668  | 0.2342- | 0.2249- | 0.0293  |
| 34   | 0.2983  | 0.3553- | 0.0794- | 0.2687- |
| 35   | 0.3132  | 0.3321- | 0.0968- | 0.2933- |
| 36   | 0.3191  | 0.8939- | 0.2061  | 0.4207- |
| 37   | 0.2822  | 0.7890- | 0.5233  | 0.8946- |
| 38   | 0.0060- | 0.9852- | 0.9160  | 0.9885- |
| 39   | 0.3570- | 1.2641- | 1.2768  | 1.3262- |
| 40   | 0.2910- | 1.1954- | 1.2530  | 1.4268- |
| 41   | 0.1784- | 1.0219- | 1.0818  | 1.0747- |
| 42   | 0.6446- | 0.8145- | 1.1844  | 0.6291- |
| 43   | 0.3888- | 0.4774- | 0.8596  | 0.3502- |
| 44   | 0.0857- | 0.3837- | 0.3734  | 0.0823- |
| 45   | 0.0341  | 0.2779- | 0.1426  | 0.0540- |
| 46   | 0.0778  | 0.2779- | 0.2369- | 0.1768  |
| 47   | 0.0341  | 0.1689  | 0.5008- | 0.2049  |
| 48   | 0.1679  | 0.2906  | 0.2785- | 0.1955- |
| 49   | 0.2035  | 0.1850  | 0.6374- | 0.0343  |
| 50   | 0.1896  | 0.3054  | 0.6059- | 0.0262- |

\* PWM sequences scanning with a window of 50 bases, e.g., from site 1 to site 50, from site 2 to site 50, and so on. A: adenine; C: cytosine; G: guanine; U: uracil.

**Table 5.** FASTA algorithm representative output of *Klebsiella pneumoniae* KCTC 2242 in some of target sequences.

| Target name          | Max match | Shift |
|----------------------|-----------|-------|
| KPN2242_00005 C803   | 5         | 40    |
| KPN2242_00010 960    | 5         | 34    |
| KPN2242_00015 2150   | 5         | 38    |
| KPN2242_00020 3831   | 4         | 36    |
| KPN2242_00025 5238   | 4         | 38    |
| KPN2242_00030 C7902  | 5         | 39    |
| KPN2242_00035 8097   | 4         | 43    |
| KPN2242_00045 C14438 | 6         | 5     |
| KPN2242_00050 C15663 | 5         | 41    |
| KPN2242_00055 C16560 | 4         | 39    |
| KPN2242_00060 C17372 | 5         | 19    |
| KPN2242_00065 C17932 | 6         | 37    |
| KPN2242_00070 C18339 | 6         | 40    |
| KPN2242_00075 C19152 | 4         | 39    |
| KPN2242_00080 C20170 | 5         | 32    |
| KPN2242_00085 20520  | 6         | 40    |

\*First column is some of sequences name; second column is the number of matched sites (Nm) between the SD sequence and the 50mer, and the last column is the start of the match (Sm).

**Table 6.** FASTA algorithm output of *Listeria monocytogenes* La111 in some of target sequences.

| Target name       | Max Match | Shift |
|-------------------|-----------|-------|
| BN418_0001 318    | 5         | 39    |
| BN418_0002 1867   | 4         | 39    |
| BN418_0003 3121   | 5         | 34    |
| BN418_0005 4869   | 4         | 34    |
| BN418_0006 6030   | 4         | 37    |
| BN418_0007 8065   | 5         | 34    |
| BN418_0008 10728  | 4         | 36    |
| BN418_0009 12090  | 5         | 39    |
| BN418_0010 12750  | 4         | 42    |
| BN418_0011 13675  | 4         | 40    |
| BN418_0012 14636  | 4         | 35    |
| BN418_0013 16051  | 5         | 37    |
| BN418_0015 17154  | 5         | 35    |
| BN418_0016 19121  | 6         | 38    |
| BN418_0017 19734  | 5         | 35    |
| BN418_0019 C21231 | 5         | 38    |
| BN418_0020 21457  | 4         | 36    |

\*First column is some of sequences name; second column is the number of matched sites (Nm) between the SD sequence and the 50mer, and the last column is the start of the match (Sm).

**Table 7.** Statistical analysis of the SD sequences in *Listeria monocytogenes* La111 and *Klebsiella pneumoniae* KCTC 2242.

| <i>Klebsiella pneumoniae</i><br>KCTC 2242 | <i>Listeria monocytogenes</i><br>La111 | Statistical analysis        |
|-------------------------------------------|----------------------------------------|-----------------------------|
| 4444                                      | 2860                                   | $N_{\text{gene}}$           |
| 3830                                      | 2600                                   | $N_{\text{SD}}$             |
| 0.8618                                    | 0.9090                                 | $P_{\text{SD}}$             |
| 4.4862                                    | 4.5846                                 | Average of $N_m$            |
| 0.5736                                    | 0.5897                                 | Standard deviation of $N_m$ |

\*First column is some of sequences name; second column is the number of matched sites (Nm) between the SD sequence and the 50mer, and the last column is the start of the match (Sm).

### Shine Dalgarno Sequences in Bacteria

codon and the anti-SD sequences at the 3' end of ssu rRNA or small ribosomal RNA (18). The SD sequence may be defined by experimental tests showing the SD sequences related to the most accurate positioning of the ribosome at the translation initiation site and the best documents correspond to mutagenesis studies. On the other hand, first, many genes in bacteria (gram negative) have not any short or even trace of a SD sequence and second, when SD sequences are present, their location is very often variable. The ribosomal protein S1 in gram negative bacteria helps to locate TIC or translation initiation codon by binding to AU-rich sequences located 15-30 nucleotides upstream of start codon (18). We called it as S1 hypothesis. For efficient translation initiation, Nm should be four or more and SD sequence may be defined as one with  $Nm \geq 3$  and  $31 \leq Sm \leq 45$  (11, 14). For mRNAs that have a weak or no SD sequence, the S1 protein is necessary to recognize the initiation codon and therefore, reduces the importance of a strong SD sequence and may allow the SD sequence to degrade. In gram-positive bacteria, either they do not have the S1 protein, or have an "S1 protein" that is not conserved and probably is not used to recognize the initiation codon. This important fact suggests that in gram positive bacteria TIC localization may be more dependent on the SD sequence than in the gram negative bacteria (18). Therefore if an essential protein-coding gene in *Listeria monocytogenes* La111 had lost the SD sequence, so it may not be properly translated and the mutant will be selected against and in *Klebsiella pneumoniae* KCTC, genes may be more tolerant to mutations obliterating the SD sequence (18). These important facts caused to lead us to test two predictions: (1) the presence of a greater proportion of SD-containing protein-coding genes in *Listeria monocytogenes* La111 than in *Klebsiella pneumoniae* KCTC, and (2) the existence of better matches between the SD

sequence in mRNA and the anti-SD sequence in *Listeria monocytogenes* La111 than in *Klebsiella pneumoniae* KCTC. The results showed that the  $P_{SD}$  is greater in *Listeria monocytogenes* La111 than in *Klebsiella pneumoniae* KCTC 2242 and this agreed with one of our predictions that *Listeria monocytogenes* La111 genes should more likely have the SD sequence than those in *Klebsiella pneumoniae* KCTC 2242. The second of our prediction was that Nm should be higher for *Listeria monocytogenes* La111 genes than *Klebsiella pneumoniae* KCTC 2242 genes. Our results confirmed this hypothesis ( $4.5846 > 4.4862$ ). Thus, it can be concluded that accurate characterization of SD sequences may increase our knowledge on how an organism's transcriptome is related to its cellular proteome.

### Conflict of interest

The author declared no conflicts of interest.

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