**Supplementary material**

**Table S1.** RT-qPCR target genes and primer sequences

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Gene id. Bf  | MSa | Ref. | Description/Name | Cellular | Primer |  |  |
|  | 638R | FC |  |  | pathwayb | designation | sequence F(1)d | sequence R(2)d |
| 1 | BF638R\_0963 | 8,30 | [24] | FprA family A-type flavoprotein (*fprA*) | 1 | fprA1/2 | TAACGACCGTAACANGCACC | GATCAGTGCCACCATTTCAT |
| 2 | BF638R\_1663 | 10,55 | [24] | galactokinase (*galK*) | 1 | galK1/2 | TGGCAGANGCAAAAGCTGAT | YTCGTAAAGTTTGCTCATGC |
| 3 | BF638R\_1728 | -2,95 | [24] | exo-alpha-sialidase (*nanH*) | 2 | nanH1/2 | TGTGTGCTATACCGGTAGTT | GTCTGGCTCTCTTTAGCATC |
| 4 | BF638R\_3176 | 8,40 | [24] | BmeB15 (*acr15*) | 2 | act15-1/2 | AGTATCGATCACCTTCGGAA | TACTTGCTGTCGTTCAGTTC |
| 5 | BF638R\_3999 | 9,10 | [24] | BmeB5 (*acr5*) | 2 | acr51/2 | CCTATCGACTTGTATCCGGA | ATTCAGCGTATTCTCCAACG |
| 6 | BF638R\_2281 | 4,00 | [24] | RelA/SpoT family protein (*relA*) | 5 | relA\_1/2 | CGTGACCGTTATATTGCCAA | ACTGGCATTTCTGTTTCTTCA |
| 7 | BF638R\_1701 | 22,80 | [24] | 50S ribosomal protein L20 | 4 | L20\_31/2 | CGTGACCGTAGAAACAAGAA | AAGTCAGCCAAAACCTTACG |
| 8 | BF638R\_4035 | 15,10 | [24] | 30S ribosomal protein S3  | 4 | S3-1/2 | GTGGCCAGGAAGTTGATAAG | ACAATCACAGCATCCAGTTC |
| 9 | BF638R\_3828 | - | This study | malate dehydrogenase (*mdh*) | 1 | mdh-1/2 | GACTCGTGAAGAACTGATCG | AGTCATTGTATCCATCGGGT |
| 10 | BF638R\_1473 | - | [17] | lactate dehydrogenase (*ldh*) | 1 | ldh-1/2 | TCGGACCGTATCATNGACGA | TATTCTCCAGAGTCGTTGCT |
| 11 | BF638R\_3133 | - | This study | phospho-glycerate kinase (*pgk*) | 1 | pgk-1/2 | TTCGAAAACTTCACTCACGG | TACCCGGAAGAACTTTTCCT |
| 12 | BF638R\_1421 | - | [16] | *feoAB* | 3 | feoAB-1/2 | GCTTCCGTAAACGNATTGTG | GTCGNCGCAGAGAGATTTCA |
| 13 | BF638R\_2696 | 4,1 | [24] | flavodoxin (*fldA*) | 1 | fldA-1/2 | ATTTTACGGTTCCACAACGG | TCACTAACTCGTCGTTCAGT |
| 14 | BF638R\_4500 | - | This study | fumarate reductase (*frdA*) | 1 | frdA-1/2 | ATCTGGGTTCCGAAGAAGAT | GCGTTCCAGATAGAAGTCAC |
| 15 | BF638R\_4499 | 11,50 | [24] | succinate dehydrogenase/fumarate reductase cytochrome b subunit (*frdC*) | 1 | frdC-1/2 | TGGGCTAAAATGCAGTTACC | CAGAAACCATGAGTCAGGTG |
| 16 | BF638R\_2263 | 2,40 | [24] | Crp/Fnr family transcriptional regulator (*crpF*) | 5 | crpF-1/2 | CGTGATTTTGACGGAATCG | TAATCGATCATTTCTCCCCG |
| 17 | BF638R\_1544 | -3,20 | [24]c | GNAT family N-acetyltransferase (*gat*) | 5 | gatMZ-1/2c | TTCCCTATCGTAGCATACCC | TCTGGTGTCCACTCTGTATC |
| 18 | BF638R\_3194 | 43,12 | This studyc | pyruvate-flavodoxin/ferredoxin oxidoreductase (*por*) | 1 | porMZ-1/2c | ATGACATTCCGTATCCAGGT | GTTGTTTGCTCTTCACGTTG |
|  |  |  |  |  |  |  |  |  |
|  Endogeneous controls  |  |  |  |  |  |
| 19 | BF638R\_0945 |  | This study | glyceraldehyde-phosphate dehydrogenase (*gap*) | - | gap1/3BF | AGCCATTGTAGCAGCTTTTT | GAAGACGGGATGATGTTTC |
| 20 | 6 16S rRNA |  | This study | 16S rRNA (*rrn*) | - | rrnBF-1/2 | TCCTGTTTGATACCCACACT | GCTCAACCGTAAAATTGCAG |
| 21 | BF638R\_2767 |  | This study | *rpoD* | - | rpoD\_BF1/2 | CCAATCTTCGTTTCGTCGTA | TTCATCAAACTTCTCGGCAG |

a FC means fold change in the proteomic study. b The numbers designate the following pathways - 1: glycolysis, carbohydrate metabolism, redox processes, 2: cell-wall, efflux, virulence, 3: heme/Fe2+ metabolism, 4: protein synthesis, 5: nucleic acids, repair, stress, gene regulation. c These items were selected from the different paralog genes of these proteins in the B. fragilis genomes based on differential expressions, the pyruvate-ferredoxin oxidoreductase gene here was selected on its differential expression in RNASeq experiments (our unpublished results). d Forward (1) and reverse (2) orientations.

**Table S3.** Cross-correlation values of gene expressions metronidazole resistance for eight *nim*-positive *B. fragilis* strainsa

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | S3 | *acr5* | *acr15* | *crpF* | *frdC* | *feoAB* | *fldA* | *fprA* | *frdA* | *galK* | *gatMZ* | *ldh* | *mdh* | *nanH* | *porMZ* | *pgk* | *relA* | MICb |
| L20 | 0,476 | 0,429 | 0,762 | -0,167 | 0,31 | -0,333 | -0,214 | 0,286 | 0,881 | 0,762 | -0,247 | 0,143 | 0,119 | 0,429 | 0,381 | 0,5 | 0,357 | -0,334 |
|  | 0,207 | 0,26 | 0,0212 | 0,662 | 0,423 | 0,387 | 0,578 | 0,46 | 2E-07 | 0,0212 | 0,537 | 0,705 | 0,749 | 0,26 | 0,321 | 0,182 | 0,353 | 0,387 |
| S3 |  | 0,548 | 0,333 | -0,333 | 0,548 | 0,143 | 0,357 | 0,619 | 0,0952 | 0,738 | 0,577 | -0,119 | 0,429 | 0,0238 | 0,667 | 0,595 | 0,81 | -0,124 |
|  |  | 0,139 | 0,387 | 0,387 | 0,139 | 0,705 | 0,353 | 0,086 | 0,794 | 0,0287 | 0,12 | 0,749 | 0,26 | 0,931 | 0,0588 | 0,102 | 0,0096 | 0,749 |
| *acr5* |  |  | 0,286 | -0,0476 | 0,119 | -0,333 | -0,262 | 0,476 | 0,143 | 0,452 | 0,412 | -0,429 | 0,857 | -0,19 | 0,714 | 0,81 | 0,262 | 0,272 |
|  |  |  | 0,46 | 0,885 | 0,749 | 0,387 | 0,498 | 0,207 | 0,705 | 0,233 | 0,29 | 0,26 | 0,00178 | 0,619 | 0,0374 | 0,0096 | 0,498 | 0,498 |
| *acr15* |  |  |  | 0,0238 | 0,143 | -0,429 | 0,0238 | 0,5 | 0,81 | 0,5 | -0,247 | 0,476 | 0,19 | 0,786 | 0,262 | 0,357 | 0,119 | -0,136 |
|  |  |  |  | 0,931 | 0,705 | 0,26 | 0,931 | 0,182 | 0,0096 | 0,182 | 0,537 | 0,207 | 0,619 | 0,0149 | 0,498 | 0,353 | 0,749 | 0,705 |
| *crpF* |  |  |  |  | 0 | -0,619 | -0,119 | -0,452 | 0,0476 | 0,0952 | -0,577 | 0,333 | 0,214 | 0,381 | 0,0952 | 0,286 | 0 | 0,568 |
|  |  |  |  |  | 0,977 | 0,086 | 0,749 | 0,233 | 0,885 | 0,794 | 0,12 | 0,387 | 0,578 | 0,321 | 0,794 | 0,46 | 0,977 | 0,12 |
| *frdC* |  |  |  |  |  | 0,0238 | -0,0476 | 0,429 | 0,262 | 0,476 | 0,0825 | -0,167 | -0,167 | 0,0476 | 0,167 | 0,452 | 0,405 | -0,507 |
|  |  |  |  |  |  | 0,931 | 0,885 | 0,26 | 0,498 | 0,207 | 0,839 | 0,662 | 0,662 | 0,885 | 0,662 | 0,233 | 0,29 | 0,182 |
| *feoAB* |  |  |  |  |  |  | 0,619 | 0,0714 | -0,452 | -0,357 | 0,577 | 0,0476 | -0,357 | -0,548 | -0,5 | -0,619 | 0,0476 | -0,148 |
|  |  |  |  |  |  |  | 0,086 | 0,839 | 0,233 | 0,353 | 0,12 | 0,885 | 0,353 | 0,139 | 0,182 | 0,086 | 0,885 | 0,705 |
| *fldA* |  |  |  |  |  |  |  | 0,0952 | -0,333 | 0,0238 | 0,412 | 0,595 | 0,0238 | 0,0952 | -0,119 | -0,333 | 0,429 | 0,309 |
|  |  |  |  |  |  |  |  | 0,794 | 0,387 | 0,931 | 0,29 | 0,102 | 0,931 | 0,794 | 0,749 | 0,387 | 0,26 | 0,423 |
| *fprA* |  |  |  |  |  |  |  |  | 0,167 | 0,167 | 0,577 | -0,19 | 0,286 | 0,0952 | 0,286 | 0,357 | 0,0714 | -0,321 |
|  |  |  |  |  |  |  |  |  | 0,662 | 0,662 | 0,12 | 0,619 | 0,46 | 0,794 | 0,46 | 0,353 | 0,839 | 0,423 |
| *frdA* |  |  |  |  |  |  |  |  |  | 0,5 | -0,577 | 0,333 | -0,143 | 0,619 | 0,0476 | 0,286 | 0 | -0,383 |
|  |  |  |  |  |  |  |  |  |  | 0,182 | 0,12 | 0,387 | 0,705 | 0,086 | 0,885 | 0,46 | 0,977 | 0,321 |
| *galK* |  |  |  |  |  |  |  |  |  |  | -0,0825 | 0,0714 | 0,333 | 0,381 | 0,738 | 0,738 | 0,833 | -0,0865 |
|  |  |  |  |  |  |  |  |  |  |  | 0,839 | 0,839 | 0,387 | 0,321 | 0,0287 | 0,0287 | 0,00526 | 0,794 |
| *gatMZ* |  |  |  |  |  |  |  |  |  |  |  | -0,412 | 0,412 | -0,577 | 0,247 | 0,0825 | 0,247 | 0,0856 |
|  |  |  |  |  |  |  |  |  |  |  |  | 0,29 | 0,29 | 0,12 | 0,537 | 0,839 | 0,537 | 0,794 |
| *ldh* |  |  |  |  |  |  |  |  |  |  |  |  | -0,19 | 0,667 | -0,333 | -0,333 | 0,0714 | 0,272 |
|  |  |  |  |  |  |  |  |  |  |  |  |  | 0,619 | 0,0588 | 0,387 | 0,387 | 0,839 | 0,498 |
| *mdh* |  |  |  |  |  |  |  |  |  |  |  |  |  | -0,0714 | 0,738 | 0,69 | 0,31 | 0,667c |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,839 | 0,0287 | 0,0474 | 0,423 | 0,0588 |
| *nanH* |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,143 | 0,143 | 0,119 | -0,0618 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,705 | 0,705 | 0,749 | 0,839 |
| *porMZ* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,857 | 0,667 | 0,185 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,00178 | 0,0588 | 0,619 |
| *pgk* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,5 | 0,124 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,182 | 0,749 |
| *relA* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,0741 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,839 |

 a The colour-coding means the following: yellow – 0.5 < r < 0.7, 0.05 > p >0.001, orange: r > 0.7, p<0.001, , abbreviations in Table S1. b Metronidazole MIC. c Borderline significance

**Table S4.** Cross-correlation values of gene expressions metronidazole resistance for seven *nim*-negative *B. fragilis* strainsa

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | S3 | *acr5* | *acr15* | *crpF* | *frdC* | *feoAB* | *fldA* | *fprA* | *frdA* | *galK* | *gatMZ* | *ldh* | *mdh* | *nanH* | *por* | *pgk* | *relA* | MIC |
| L20 | 0,643 | -0,786 | -0,0357 | -0,536 | 0,821 | -0,198 | -0,786 | 0,143 | 0,679 | 0,464 | -0,018 | -0,464 | 0,643 | -0,821 | 0,0357 | 0,964 | 0,536 | 0,128 |
|  | 0,0956 | 0,0251 | 0,905 | 0,181 | 0,0145 | 0,602 | 0,0251 | 0,72 | 0,0735 | 0,255 | 0,905 | 0,255 | 0,0956 | 0,0145 | 0,905 | 2E-07 | 0,181 | 0,72 |
| S3 |  | -0,107 | 0,607 | -0,25 | 0,5 | -0,577 | -0,429 | -0,214 | 0,679 | 0,857 | 0,0541 | -0,0357 | 0,25 | -0,321 | 0 | 0,75 | 0,5 | 0,0551 |
|  |  | 0,781 | 0,121 | 0,545 | 0,217 | 0,15 | 0,297 | 0,602 | 0,0735 | 0,00609 | 0,843 | 0,905 | 0,545 | 0,438 | 0,968 | 0,0384 | 0,217 | 0,843 |
| *acr5* |  |  | 0,464 | 0,536 | -0,75 | -0,018 | 0,821 | -0,179 | -0,286 | 0,0714 | -0,234 | 0,714 | -0,679 | 0,893 | 0,179 | -0,714 | -0,214 | 0,147 |
|  |  |  | 0,255 | 0,181 | 0,0384 | 0,905 | 0,0145 | 0,66 | 0,491 | 0,843 | 0,545 | 0,0545 | 0,0735 | 2E-07 | 0,66 | 0,0545 | 0,602 | 0,72 |
| *acr15* |  |  |  | 0,429 | 0,143 | -0,18 | -0,0357 | -0,0357 | 0,5 | 0,321 | 0,252 | 0,571 | -0,0357 | 0,0714 | 0,321 | 0,0714 | 0,464 | -0,257 |
|  |  |  |  | 0,297 | 0,72 | 0,66 | 0,905 | 0,905 | 0,217 | 0,438 | 0,545 | 0,15 | 0,905 | 0,843 | 0,438 | 0,843 | 0,255 | 0,545 |
| *crpF* |  |  |  |  | -0,143 | 0,631 | 0,536 | 0,0357 | 0,214 | -0,357 | 0,27 | 0,857 | 0 | 0,464 | 0,643 | -0,607 | 0,25 | 0,0551 |
|  |  |  |  |  | 0,72 | 0,0956 | 0,181 | 0,905 | 0,602 | 0,388 | 0,491 | 0,00609 | 0,968 | 0,255 | 0,0956 | 0,121 | 0,545 | 0,843 |
| *frdC* |  |  |  |  |  | 0 | -0,786 | 0 | 0,75 | 0,143 | 0,432 | -0,321 | 0,643 | -0,857 | 0,107 | 0,75 | 0,5 | -0,128 |
|  |  |  |  |  |  | 0,968 | 0,0251 | 0,968 | 0,0384 | 0,72 | 0,297 | 0,438 | 0,0956 | 0,00609 | 0,781 | 0,0384 | 0,217 | 0,72 |
| *feoAB* |  |  |  |  |  |  | 0,252 | 0,595 | 0,18 | -0,667 | -0,0909 | 0,523 | 0,324 | 0,0541 | 0,775 | -0,396 | 0,36 | 0,232 |
|  |  |  |  |  |  |  | 0,545 | 0,121 | 0,66 | 0,0735 | 0,781 | 0,181 | 0,438 | 0,843 | 0,0251 | 0,341 | 0,388 | 0,602 |
| *fldA* |  |  |  |  |  |  |  | -0,214 | -0,393 | -0,0714 | -0,378 | 0,607 | -0,607 | 0,964 | 0,214 | -0,821 | -0,357 | 0,496 |
|  |  |  |  |  |  |  |  | 0,602 | 0,341 | 0,843 | 0,341 | 0,121 | 0,121 | 2E-07 | 0,602 | 0,0145 | 0,388 | 0,217 |
| *fprA* |  |  |  |  |  |  |  |  | 0,179 | -0,393 | -0,36 | 0,25 | 0,429 | -0,286 | 0,607 | 0,0714 | 0,571 | -0,0367 |
|  |  |  |  |  |  |  |  |  | 0,66 | 0,341 | 0,388 | 0,545 | 0,297 | 0,491 | 0,121 | 0,843 | 0,15 | 0,905 |
| *frdA* |  |  |  |  |  |  |  |  |  | 0,393 | 0,0901 | 0,286 | 0,607 | -0,464 | 0,607 | 0,607 | 0,857 | 0,239 |
|  |  |  |  |  |  |  |  |  |  | 0,341 | 0,781 | 0,491 | 0,121 | 0,255 | 0,121 | 0,121 | 0,00609 | 0,545 |
| *galK* |  |  |  |  |  |  |  |  |  |  | -0,252 | -0,0714 | 0 | 0,0357 | -0,143 | 0,571 | 0,214 | 0,385 |
|  |  |  |  |  |  |  |  |  |  |  | 0,545 | 0,843 | 0,968 | 0,905 | 0,72 | 0,15 | 0,602 | 0,341 |
| *gatMZ* |  |  |  |  |  |  |  |  |  |  |  | -0,144 | 0,306 | -0,306 | -0,27 | 0,0541 | -0,018 | -0,722c |
|  |  |  |  |  |  |  |  |  |  |  |  | 0,72 | 0,438 | 0,438 | 0,491 | 0,843 | 0,905 | 0,0545 |
| *ldh* |  |  |  |  |  |  |  |  |  |  |  |  | -0,0714 | 0,571 | 0,786 | -0,5 | 0,429 | 0,257 |
|  |  |  |  |  |  |  |  |  |  |  |  |  | 0,843 | 0,15 | 0,0251 | 0,217 | 0,297 | 0,545 |
| *mdh* |  |  |  |  |  |  |  |  |  |  |  |  |  | -0,643 | 0,357 | 0,607 | 0,75 | -0,11 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,0956 | 0,388 | 0,121 | 0,0384 | 0,781 |
| *nanH* |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,0714 | -0,786 | -0,393 | 0,349 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,843 | 0,0251 | 0,341 | 0,388 |
| *por* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | -0,107 | 0,75 | 0,404 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,781 | 0,0384 | 0,341 |
| *pgk* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,5 | -0,0184 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,217 | 0,905 |
| *relA* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,11 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0,781 |

a The colour-coding means the following: yellow – 0.5 < r < 0.7, 0.05 > p >0.001, orange: r > 0.7, p<0.001, abbreviations in Table S1. b Metronidazole MIC.

. c Borderline significance

**Fig. S1.** The tricarboxylic acid pathways of *B. fragilis*



**Fig. S2.** Examples of the Etest results on modified M9 medium

*B. fragilis* 638R (A) and *B. fragilis* 638R/*nimA* (B) on glucose-supplemented M9 medium, respectively.

 A B

  

**Table S5.** Effects of C4 dicarboxylic acid supplementation on metronidazole MICs of selected *nim*-positive and negative *B. fragilis* strains

|  |  |  |
| --- | --- | --- |
| Strain | *nim* | Media (MICs in μg/ml) |
|  |  | SCA | M9-Ga | M9-O | M9-M | M9-F | M9-S |
| 638R | - | 0,125 | 0.25 | 0,064 | 0,032 | 0,032 | 0,032 |
| 638R/*nimA* | *nimA* | 4 | 4 | 4 | 4 | 2 | 4 |
| KSB-R | *nimB* | 16 | >256b | 16 | 256 | 16 | >256 |
| 19811 | - | 32 | >256 | 64 | >256 | 16 | >256 |
| 18807i2 | - | (0,25-)>256c | 0,5 | 0,25 | 0,125 | 0,125 | 0,125 |
| Q5 | *nimE* | >256 | >256 | >256 | >256 | 256 | >256 |

a M9 media supplemented with glucose (G), oxaloacetic acid (O), malate (M), fumarate (F) and succinate (S).

b Significantly increased metronidazole resistance values are highlighted in yellow. c Heterogeneous resistance phenotype.