**APPENDICES**

**APPENDIX A: RNA EXTRACTION**

A screenshot of a computer

Description automatically generated**A close-up of a test

Description automatically generated**

**Figure A:** Aliquot of 100 ng or 1µL RNA were run on 1% TAE agarose gel in 1×TAE buffer at 100 V for 60 minutes.

**Table A:** Nanodrop analysis RNA isolated from untreated (A1, A2 and A3) and MRSA treated with AgNPs-K (B1, B2, and B3)*.* Absorption values 260/280 and 260/230 indicate nucleic acid purity.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample Name** | **Organism** | **Spectrophotometer (water as blank)** | | | **LabChip Results** | | | | | | |
| **A260/280** | **A260/230** | **Concentration (ng/uL)** | | **rRNA Ratio** | **RIN** | **Concentration (ng/µL)** | **Volume (µL)** | **Total Amount (µg)** | **Comments** | |
| **A1** | **Bacteria** | **2.120** | **2.243** | **114.84** | | **0.4** | **4.0** | **197.00** | **55** | **10.48** | **After DNase treatment** | |
| **A2** | **Bacteria** | **2.095** | **2.209** | **56.72** | | **2.0** | **7.0** | **198.00** | **55** | **10.89** | **After DNase treatment** | |
| **A3** | **Bacteria** | **2.135** | **2.223** | **112.48** | | **4.1** | **6.0** | **108.00** | **55** | **5.94** | **After DNase treatment** | |
| **B1** | **Bacteria** | **2.144** | **2.037** | **57.12** | | **4.1** | **5.0** | **108.00** | **55** | **5.94** | **After DNase treatment** | |
| **B2** | **Bacteria** | **2.154** | **2.157** | **64.36** | | **2.5** | **6.0** | **132.00** | **45** | **5.94** | **After DNase treatment** | |
| **B3** | **Bacteria** | **2.098** | **0.351** | **34.16** | | **6.9** | **6.0** | **79.00** | **35** | **2.77** | **After DNase treatment** | |

Note: RNA is in water

**Appendix B: TRANCRIPTOMIC PROFILE ANALYSIS**

**Table B1:** Log2 fold change of downregulated gene of KEGG pathway of MRSA after treated with AgNPs-K (1.25 mg/mL).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene ID | log2FoldChange | pvalue | Gene name | Gene Description |
| IL357\_RS11625 | -1.927298917 | 9.59E-27 | rpsD | 30S ribosomal protein S4 && PF01479:S4 domain|PF00163:Ribosomal protein S4/S9 N-terminal domain |
| IL357\_RS03395 | -2.667732057 | 4.49E-26 | rplS | 50S ribosomal protein L19 && PF01245:Ribosomal protein L19 |
| IL357\_RS03380 | -1.503743875 | 4.38723E-21 | rpsP | 30S ribosomal protein S16 && - |
| IL357\_RS05890 | -1.293566613 | 3.51346E-20 | rplW | 50S ribosomal protein L23 && - |
| IL357\_RS05900 | -1.774910545 | 1.70069E-19 | rplC | 50S ribosomal protein L3 && PF00297:Ribosomal protein L3 |
| IL357\_RS05905 | -2.06872512 | 8.55452E-19 | rpsJ | 30S ribosomal protein S10 && PF00338:Ribosomal protein S10p/S20e |

**Table B2:** Log2 fold change of upregulated gene of KEGG pathway of MRSA after treated with AgNPs-K (1.25 mg/mL).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene ID | log2FoldChange | pvalue | Gene name | Gene Description |
| IL357\_RS08075 | 3.441962718 | 4.47E-39 | manA | mannose-6-phosphate isomerase%2C class I && PF01238:Phosphomannose isomerase type I |
| IL357\_RS00380 | 0.781577786 | 8.52E-11 | IL357\_RS00380 | phosphomevalonate kinase && PF00288:GHMP kinases N terminal domain|PF08544:GHMP kinases C terminal |
| IL357\_RS07545 | 0.992061481 | 1.16E-09 | IL357\_RS07545 | NAD(P)/FAD-dependent oxidoreductase && PF01593:Flavin containing amine oxidoreductase |
| IL357\_RS07540 | 0.973786197 | 1.87E-09 | crtQ | 4%2C4'-diaponeurosporenoate glycosyltransferase && PF00535:Glycosyl transferase family 2 |
| IL357\_RS10740 | 1.306997561 | 6.02E-09 | sph | sphingomyelin phosphodiesterase && PF03372:Endonuclease/Exonuclease/phosphatase family |

**Table B3:** Log2 fold change of downregulated genes in BP which involves primary metabolites process of MRSA after treated with AgNPs-K (1.25 mg/mL).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene\_ID | Log2foldchange | Pvalue | Gene\_Name | Gene\_Description |
| IL357\_RS03395 | -2.667732057 | 4.49E-26 | rplS | 50S ribosomal protein L19 && PF01245:Ribosomal protein L19 |
| IL357\_RS05175 | -1.557164395 | 6.60E-24 | IL357\_RS05175 | 16S rRNA (uracil(1498)-N(3))-methyltransferase && PF04452:RNA methyltransferase |
| IL357\_RS02025 | -1.663481435 | 1.42E-23 | clpB | ATP-dependent chaperone ClpB && PF07724:AAA domain (Cdc48 subfamily)|PF02861:Clp amino terminal domain, pathogenicity island component|PF10431:C-terminal, D2-small domain, of ClpB protein|PF00004:ATPase family associated with various cellular activities (AAA) |
| IL357\_RS05900 | -1.774910545 | 1.70E-19 | rplC | 50S ribosomal protein L3 && PF00297:Ribosomal protein L3 |
| IL357\_RS10080 | -1.907325181 | 6.11E-18 | metE | 5-methyltetrahydropteroyltriglutamate--homocysteine S-methyltransferase && PF08267:Cobalamin-independent synthase, N-terminal domain|PF01717:Cobalamin-independent synthase, Catalytic domain |
| Novel00181 | -2.291513033 | 3.76E-13 | - | PF00239:Resolvase, N terminal domain|PF02796:Helix-turn-helix domain of resolvase |

**Table B4:** Log2 fold change of downregulated genes in CC which involves cellular component of MRSA after treated with AgNPs-K (1.25 mg/mL).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene\_ID | Log2foldchange | Pvalue | Gene\_Name | Gene\_Description |
| IL357\_RS09970 | -4.075431035 | 5.58E-88 | *mepA* | multidrug efflux MATE transporter MepA && PF01554:MatE |
| IL357\_RS02970 | -2.444377553 | 2.51E-36 | *efb* | complement convertase inhibitor Efb && PF12199:Extracellular fibrinogen binding protein C terminal |
| IL357\_RS11625 | -1.927298917 | 9.59E-27 | *rpsD* | 30S ribosomal protein S4 && PF01479:S4 domain|PF00163:Ribosomal protein S4/S9 N-terminal domain |
| IL357\_RS02950 | -3.832990361 | 9.78E-27 | *ecb* | complement convertase inhibitor Ecb && PF12199:Extracellular fibrinogen binding protein C terminal |
| IL357\_RS02600 | -2.07187928 | 4.17E-26 | IL357\_RS02600 | cytochrome ubiquinol oxidase subunit I && PF01654:Cytochrome bd terminal oxidase subunit I |
| IL357\_RS03395 | -2.667732057 | 4.49E-26 | *rplS* | 50S ribosomal protein L19 && PF01245:Ribosomal protein L19 |
| IL357\_RS13225 | -3.733772836 | 1.76E-22 | IL357\_RS13225 | superantigen-like protein SSL11 && PF02876:Staphylococcal/Streptococcal toxin, beta-grasp domain|PF09199:Staphylococcal superantigen-like OB-fold domain |
| IL357\_RS05900 | -1.774910545 | 1.70E-19 | *rplC* | 50S ribosomal protein L3 && PF00297:Ribosomal protein L3 |
| IL357\_RS02605 | -1.399053696 | 3.15E-16 | IL357\_RS02605 | cytochrome d ubiquinol oxidase subunit II && PF02322:Cytochrome bd terminal oxidase subunit II |
| IL357\_RS05875 | -1.077588284 | 1.94E-12 | *rplV* | 50S ribosomal protein L22 && PF00237:Ribosomal protein L22p/L17e |

**Table B5:** Log2 fold change of downregulated genes in MF which involves nucleic acid binding of MRSA after treated with AgNPs-K (1.25 mg/mL).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene\_ID | Log2foldchange | Pvalue | Gene\_Name | Gene\_Description |
| IL357\_RS11625 | -1.927298917 | 9.59E-27 | *rpsD* | 30S ribosomal protein S4 && PF01479:S4 domain|PF00163:Ribosomal protein S4/S9 N-terminal domain |
| IL357\_RS02665 | -2.262550204 | 7.25E-22 | IL357\_RS02665 | XRE family transcriptional regulator && PF12844:Helix-turn-helix domain|PF07883:Cupin domain |
| IL357\_RS10130 | -1.373228625 | 5.54E-18 | *ssb* | single-stranded DNA-binding protein && PF00436:Single-strand binding protein family |
| IL357\_RS07610 | -2.023636762 | 5.39E-15 | IL357\_RS07610 | TetR/AcrR family transcriptional regulator && PF00440:Bacterial regulatory proteins, tetR family |
| Novel00181 | -2.291513033 | 3.76E-13 | - | PF00239:Resolvase, N terminal domain|PF02796:Helix-turn-helix domain of resolvase |
| IL357\_RS11320 | -1.807383355 | 1.24E-12 | IL357\_RS11320 | 3'-5' exonuclease && PF00929:Exonuclease |
| IL357\_RS13595 | -2.037065754 | 1.67E-12 | *infC* | translation initiation factor IF-3 && PF00707:Translation initiation factor IF-3, C-terminal domain|PF05198:Translation initiation factor IF-3, N-terminal domain |
| IL357\_RS03145 | -1.231772256 | 3.93E-12 | IL357\_RS03145 | RNA-binding protein && PF01479:S4 domain |
| IL357\_RS03735 | -1.759207471 | 6.68E-12 | IL357\_RS03735 | MerR family transcriptional regulator && PF13411:MerR HTH family regulatory protein |
| IL357\_RS12530 | -1.039240866 | 1.03E-11 | IL357\_RS12530 | DEAD/DEAH box helicase && PF00270:DEAD/DEAH box helicase|PF00271:Helicase conserved C-terminal domain |