## SUPPLEMENTARY MATERIALS

# A Meta-Analysis Approach to Understanding Stress Response and Cross-Protection in *Escherichia coli*

Maria Genesis M. Acosta and Gil M. Penuliar*

Institute of Biology, National Science Complex, College of Science, University of the Philippines Diliman, Diliman, 1101 Quezon City, Philippines

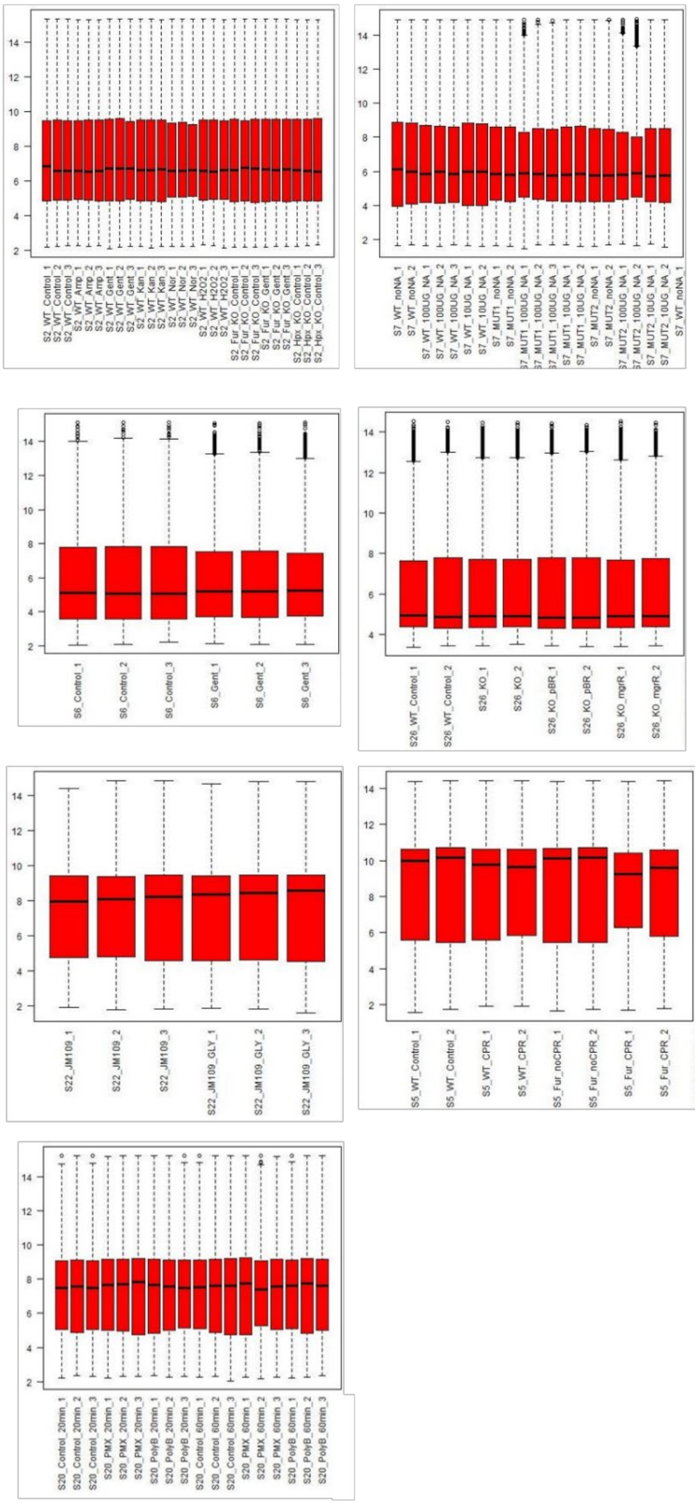
### Appendix A: Summary of microarray studies acquired from Gene Expression Omnibus. The table below summarizes published journal articles related to microarray deposited in GEO.

| Accession no. | Affymetrix platform | Journal article | No. of datasets |
|---|---|---|---|
| GSE56133 | GPL3154 [E_coli_2] Affymetrix E. coli Genome 2.0 array | Dwyer D J, Belenky P A, Yang J H, MacDonald I C, Martell J D, Takahashi N, Collins J J, et al. (2014). Antibiotics induce redox-related physiological alterations as part of their lethality. *Proceedings of the National Academy of Sciences* 111(20): E2100–E2109. | 27 |
| GSE56251 | GPL3154 [E_coli_2] Affymetrix E. coli Genome 2.0 array | Erental A, Kalderon Z, Saada A, Smith Y and Engelberg-Kulka H. (2014). Apoptosis-like death, an extreme SOS response in Escherichia coli. *MBio* 5(4): e01426-14. https://doi.org/10.1128/mBio.01426-14 | 20 |
| GSE124807 | GPL3154 [E_coli_2] Affymetrix E. coli Genome 2.0 Array | Hu G, Hu T, Zhan Y, Lu W, Lin M, Huang Y and Yan Y. (2019). NfiS, a species-specific regulatory noncoding RNA of *Pseudomonas stutzeri*, enhances oxidative stress tolerance in Escherichia coli. *AMB Express* 9(1): 1–10. https://doi.org/10.1186/s13568-019-0881-7 | 6 |
| GSE44211 | GPL3154 [E_coli_2] Affymetrix E. coli Genome 2.0 Array | Kashyap D R, Rompca A, Gaballa A, Helmann J D, Chan J, Chang C J, Dziarski, R et al. (2014). Peptidoglycan recognition proteins kill bacteria by inducing oxidative, thiol, and metal stress. *PLoS Pathogens* 10(7): e1004280. | 6 |
| GSE30838 | GPL3154 [E_coli_2] Affymetrix E. coli Genome 2.0 Array | Lu W, Li L, Chen M, Zhou Z, Zhang W, Ping S, Lin M et al. (2013). Genome-wide transcriptional responses of *Escherichia coli* to glyphosate, a potent inhibitor of the shikimate pathway enzyme 5-enolpyruvylshikimate-3-phosphate synthase. *Molecular BioSystems* 9(3): 522-530. | 6 |
| GSE55662 | GPL3154 | Méhi O, Bogos B, Csörgő B, Pál F, Nyerges Á, Papp B and Pál C. (2014). Perturbation of iron homeostasis promotes the evolution of | 8 |

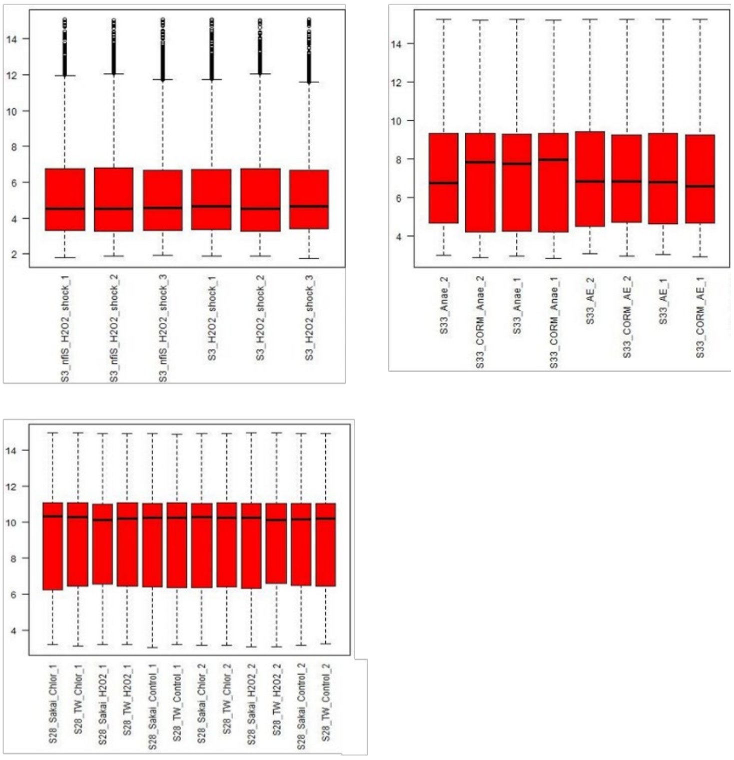
| | | | |
|---|---|---|---|
| | [E_coli_2] Affymetrix E. coli Genome 2.0 Array | antibiotic resistance. *Molecular Biology and Evolution* 31(10): 2793–2804. | |
| GSE31140 | GPL3154 [E_coli_2] Affymetrix E. coli Genome 2.0 Array | Mensa B, Kim Y H, Choi S, Scott R, Caputo G A and DeGrado W F (2011). Antibacterial mechanism of action of arylamide foldamers. *Antimicrobial Agents and Chemotherapy* 55(11): 5043–5053. https://doi.org/10.1128/AAC.05009- 11 | 18 |
| GSE18935 | GPL3154 [E_coli_2] Affymetrix E. coli Genome 2.0 Array | Moon K and Gottesman S. (2009). A PhoQ/P-regulated small RNA regulates sensitivity of *Escherichia coli* to antimicrobial peptides. *Molecular Microbiology* 74(6): 1314–1330. https://doi.org/10.1111/j.1365-2958.2009.06944.x | 8 |
| GSE13982 | GPL3154 [E_coli_2] Affymetrix E. coli Genome 2.0 Array | Nobre L S, Al-Shahrour F, Dopazo J and Saraiva L M (2009). Exploring the antimicrobial action of a carbon monoxide-releasing compound through whole-genome transcription profiling of *Escherichia coli. Microbiology* 155(3): 813–824. | 8 |
| GSE28399 | GPL3154 [E_coli_2] Affymetrix E. coli Genome 2.0 Array | Withman B, Gunasekera T S, Beesetty P, Agans R and Paliy O. (2013). Transcriptional responses of uropathogenic *Escherichia coli* to increased environmental osmolality caused by salt or urea. *Infection and Immunity*, 81(1): 80–89. | 9 |
| GSE3665 | GPL3154 | Alper H and Stephanopoulos, G. (2007). Global transcription machinery engineering: a new approach for improving cellular phenotype. *Metabolic Engineering* 9(3): 258–267. | 20 |

**Appendix B: Box-plot expression of all stress conditions. (A) antibiotic; B) oxidative; C) osmotic; D. alcohol. The x-axes show the samples or microarray datasets. The y-axes display the gene expression values. GEO Series or GSExxx stands for the identification of original record that provides a brief background of the study or data**

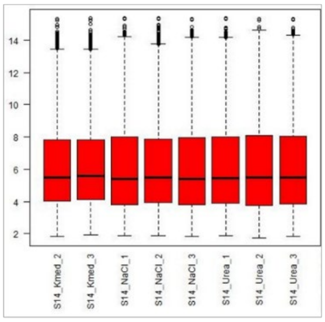
A.



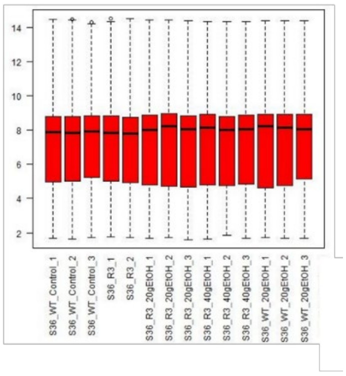
B.


2
4
6
8
10
12
14
S3_nflS_H2O2_shock_1
S3_nflS_H2O2_shock_2
S3_nflS_H2O2_shock_3
S3_H2O2_shock_1
S3_H2O2_shock_2
S3_H2O2_shock_3
4
6
8
10
12
14
S33_Anae_2
S33_CORM_Anae_2
S33_Anae_1
S33_CORM_Anae_1
S33_AE_2
S33_CORM_AE_2
S33_AE_1
S33_CORM_AE_1
4
6
8
10
12
14
S28_Sakai_Chlor_1
S28_TW_Chlor_1
S28_Sakai_H2O2_1
S28_TW_H2O2_1
S28_Sakai_Control_1
S28_TW_Control_1
S28_Sakai_Chlor_2
S28_TW_Chlor_2
S28_Sakai_H2O2_2
S28_TW_H2O2_2
S28_Sakai_Control_2
S28_TW_Control_2

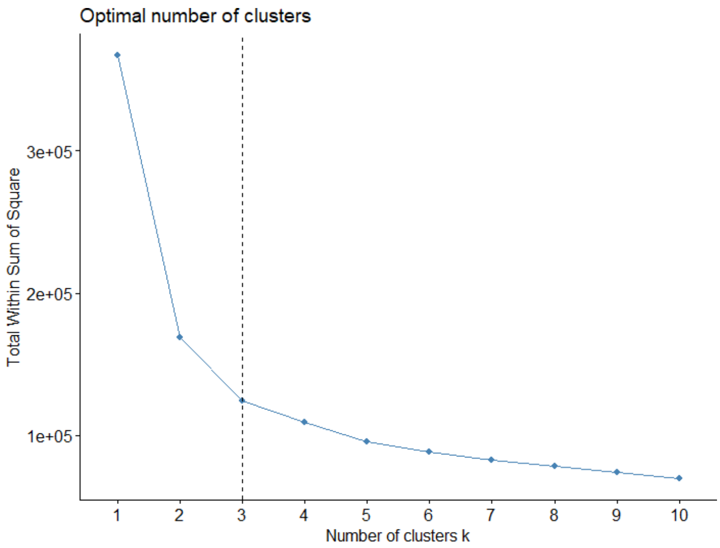
C.


2
4
6
8
10
12
14
S14_Kmed_2
S14_Kmed_3
S14_NaCl_1
S14_NaCl_2
S14_NaCl_3
S14_Urea_1
S14_Urea_2
S14_Urea_3

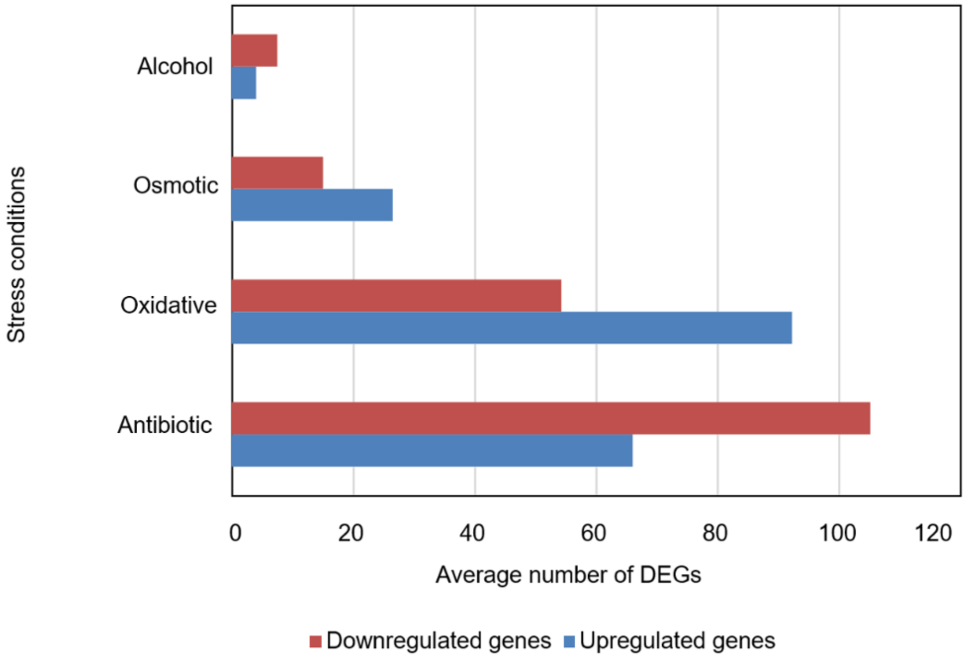
D.


2
4
6
8
10
12
14
S36_WT_Control_1
S36_WT_Control_2
S36_WT_Control_3
S36_R3_1
S36_R3_2
S36_R3_20gEtOH_1
S36_R3_20gEtOH_2
S36_R3_20gEtOH_3
S36_R3_40gEtOH_1
S36_R3_40gEtOH_2
S36_R3_40gEtOH_3
S36_WT_20gEtOH_1
S36_WT_20gEtOH_2
S36_WT_20gEtOH_3

**Appendix C: The optimum number of *k*-clusters is based on the elbow method. The elbow plot demonstrates y-axis as the total within sum of squares by x-axis as the number of clusters (k). The broken line shows the optimum number of *k*-clusters.**



**Appendix D: Overview of the average number of DEGs per stress condition.**

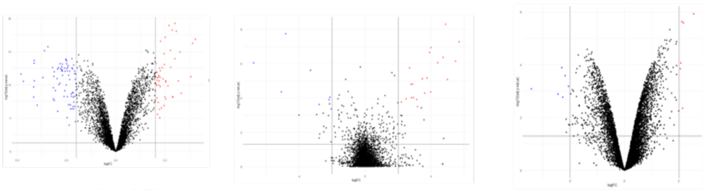


### Appendix E: Absolute number of differentially expressed genes per *E. coli* strain.

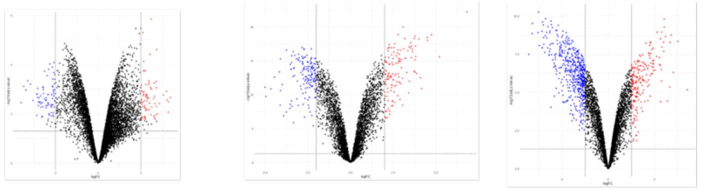
| Strains | Count of stress conditions | Ave. of UP-DEGS | Ave. of DOWN-DEGS |
|---|---|---|---|
| *E. coli CFT073 (UPEC)* | 2 | 91.5 | 34.0 |
| *E. coli DH5-alpha* | 3 | 2.7 | 6.0 |
| *E. coli JM109* | 1 | 25.0 | 18.0 |
| *E. coli K-12 BW25113* | 2 | 41.0 | 41.0 |
| *E. coli K-12 MG1655* | 10 | 76.7 | 117.9 |
| *E. coli MC4100relA+* | 6 | 73.8 | 137.5 |
| *E. coli O157:H7 (UHEC)* | 1 | 43.0 | 3.0 |
| *E. coli Trans10-nfiS* | 1 | 157.0 | 165.0 |
| *E. coli D31* | 2 | 21.0 | 2.5 |

### Appendix F: Volcano plots of specific stress conditions displaying significance vs. gene expression on the y and x axes. A. antibiotic; B. oxidative; C. osmotic; D. alcohol. Blue dots are downregulated genes, red dots are upregulated genes, and black dots are not differentially expressed genes.

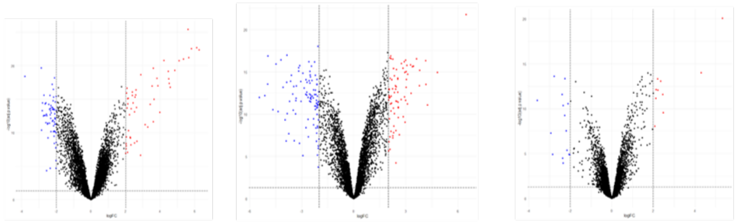
A.



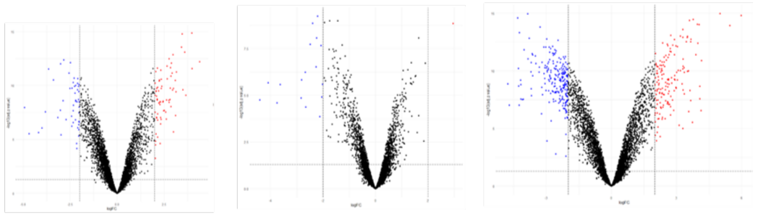
5 ug/ml ampicillin    100 ug/ml M2_ampicillin    100 ng/ml ciprofloxacin



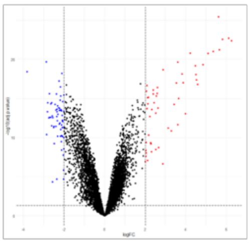
100 ng/ml M1_ciprofloxacin    5 ug/ml gentamicin_1    5 ug/ml gentamicin_2



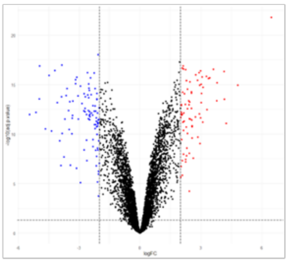
5 ug/ml M1_gentamicin    5 ug/ml kanamycin    250 ng/ml norfloxacin



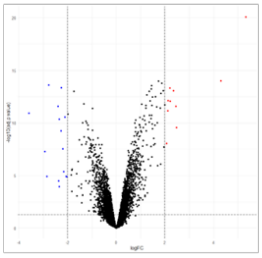
100 µg/ml nalidixic acid    10 µg/ml nalidixic acid    100 µg/ml M1_nalidixic acid



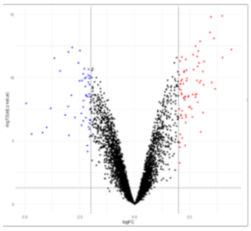
5 ug/ml M1_gentamicin



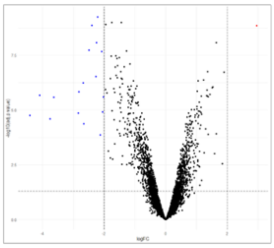
5 ug/ml kanamycin



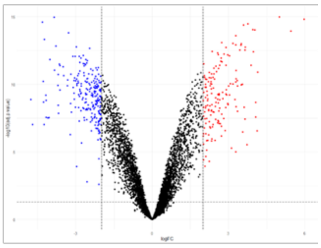
250 ng/ml norfloxacin



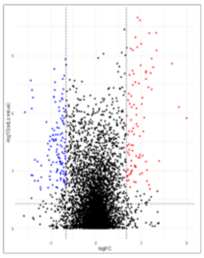
100 µg/ml nalidixic acid



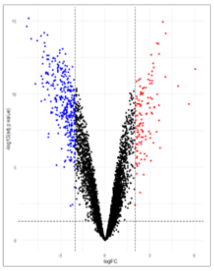
10 µg/ml nalidixic acid



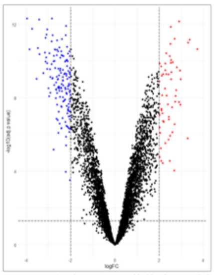
100 µg/ml M1_nalidixic acid



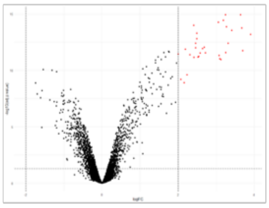
10 µg/ml M1_nalidixic acid



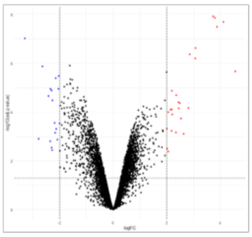
100 µg/ml M2_nalidixic acid



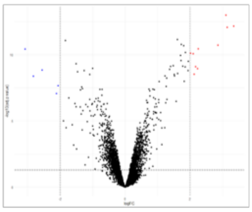
10 µg/ml M2_nalidixic acid



0.39 µg/ml polymyxin B sulfate_1

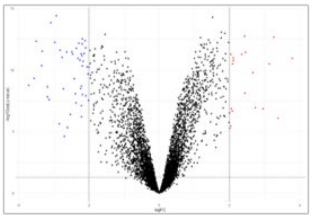


0.39 µg/ml polymyxin B sulfate_2

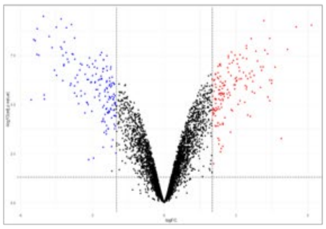


200 mM glyphosate

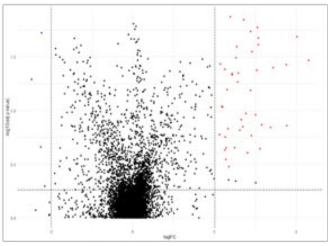
**B.**



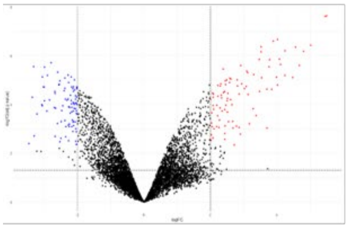
10 µM hydrogen peroxide



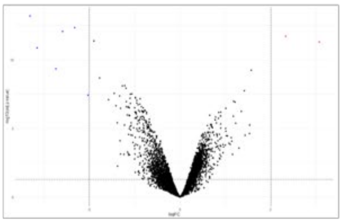
20 µM M1_hydrogen peroxide



5 µl/ml chlorine

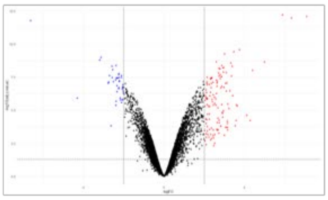


250 µM CORM_1

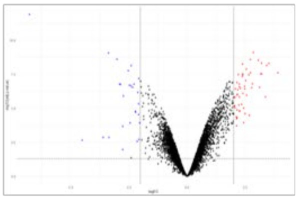


250 µM CORM_2

**C.**

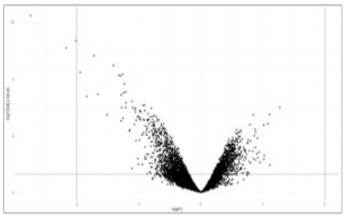


0.3M sodium chloride

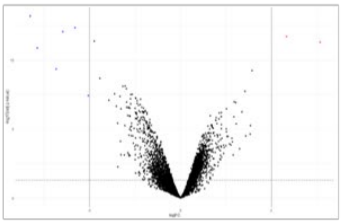


0.6M urea

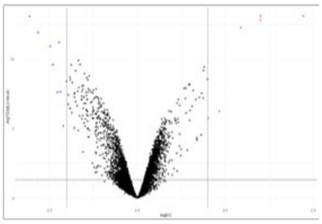
**D.**



20g/L M1_ethanol



40g/L M1_ethanol



20g/L ethanol

## Appendix G: Common upregulated genes expressed in different stress conditions.

| Treatments | Number of genes | Gene names |
|---|---|---|
| Antibiotic, Osmotic, Oxidative | 1 | *ghoS* |
| Antibiotic, Oxidative | 51 | *hslJ, hslR, eptB, ygfJ, sodA, zraS, malE, gpt, pspA, bdm, ybeD, lexA, ydeT, ldtC, yjhQ, pspD, gntP, degP, rbsB, stpA, mgrB, tqsA, wcaE, mqsA, ibpA, rbsA, nfeR, arsR, yccV, uhpT, upp, uxuR, pspG, marR, trhO, dnaJ, yfdL, ibpB, lipB, frmR, pspB, pspC, bhsA, ycfJ, nemR, pspE, marB, cpxP, lacI, yjaA, puuR* |
| Antibiotic, Osmotic | 11 | *ydjR, ynaE, cspG, yjdK, ecnA, higB, ghoT, punC, nirD, cspF, yjjI* |
| Antibiotic | 55 | *ytfK, umuC, omrA, recX, hyaF, hycB, gadY, rihA, mutM, aaeB, umuD, fumC, fadD, pyrL, sulA, yhcN, citD, dinF, sgrS, ybiJ, osmB, queG, rydB, rprA, dinD, deoA, hycC, citE, "yebG", recN, dinB, symE, citC, fumB, dinI, omrB, ybfE, polB, glmZ, nrdB, yqhD, yojI, rttR, add, aaeA, fadE, micF, nrdA, fdoG, mcrC, oxyS, osmY, macB, yciA, hypE* |
| Oxidative | 6 | *sbp, cysA, cysU, cysW, cysP, yeeE* |

## Appendix H: Common downregulated genes expressed in different stress conditions.

| Treatments | Number of genes | Gene names |
|---|---|---|
| Antibiotic, oxidative | 36 | *sufD, nrfA, nrdE, cysQ, cysD, focA, ghoT, nrdA, sapF, purU, pyrI, glnD, sufC, cysC, yfcI, sad, narG, purD, sufS, guaB, cysI, nirB, cysH, hsdS, sufE, narK, nrdF, rluC, sufB, yjdK, napA, cysJ, dcuC, hsdR, cysN, pyrC* |
| Osmotic, oxidative | 5 | *cysP, yeeE, sbp, cysA, cysW* |
| Oxidative | 15 | *ltaE, gadA, dcp, dadX, astD, dadA, hinT, gcvP, astA, ansB, grcA, gcvT, gadB, tdcB, glsB* |
| Antibiotic | 276 | *gstB, slp, psiE, gcvR, wrbA, yiaG, alsA, thrL, murE, ydfG, dhaL, poxB, tyrB, mcbR, yjiT, btuD, yohC, cydC, yjgM, dppA, ygjR, ycaN, blr, chaB, hypC, zinT, ydgD, purL, yeaG, csgA, oppF, ycaD, artP, mraY, ydiJ, yodD, pheS, carA, malF, otsB, artI, zapC, aroH, tar, aroD, amyA, nth, sdhD, rnlA, lgoR, yphA, pdeR, ravA, uraA, tyrP, cobS, yciA, garR, pphA, hdeB, appY, ydeI, talA, nlpA, yedL, speD, melA, ilvH, gnd, livG, ppc, cspG, malE, pyrB, rimL, tcyJ, napD, gdhA, gudP, ldtE, hnr, minE, rstA, lamB, ynfE, ynjE, cobU, npr, rffH, blc, oppB, trpA, pgl, mocA, minD, btsT, mepH, gldA, artJ, ghrB, malY, asr, rstB, katE, elaB, gatD, ribC, gltD, waaL, lsrR, bioD, yddB, ycgK, osmE, feoA, tsuB, cysB, serA, speG, ydcK, carB, gsiA, yedE, ptsA, metA, potD, alsB, cdaR, codA,* |

| | |
|---|---|
| | *gadW, alsR, treF, artQ, dgcM, yodB, bioA, opgD, mpaA, bioB, garK, ydhY, yhhY, dhaM, yahK, glgB, ychH, rne, ydiK, sdhC, sppA, eptC, cobT, gadC, yiaI, atoS, aroP, mdtM, murF, ybhC, ppnN, tehB, dtpB, dtpA, malP, atoC, ybjE, gatZ, efeB, yegP, dgsA, tauB, oppC, otsA, potC, yehD, srlA, feoB, minC, dhaK, pheT, dacC, msrC, cbl, cysM, ybdK, ppsR, ybaT, glsA, serC, yeeO, bluF, malK, putA, abrB, alaC, pdxH, mipA, gltB, folM, nadE, yddG, pheA, nac, hisL, purR, yncG, osmF, malM, ilvI, galU, elyC, napF, yniA, dps, proV, yeaH, murD, ribB, tauA, argD, csgD, btsR, mepM, tdcA, bcp, btuE, uspB, yciT, yciW, fhuF, RecD, iraP, shiA, yneJ, uspF, ytjA, gadX, fruB, feoC, garD, flhC, malG, mepK, bioF, fruK, aroG, melR, ycbX, zraP, tktB, adiY, yeaT, fhuE, elaA, yahN, hypB, lsrG, ldtA, garL, mppA, yjiM, hipB, aceB, aceA, dmlA, curA, cfa, mdtH, oppD, glnK, menE,potF, ycaC, yhbU, artM, tyrR* |

## Appendix I: Enriched biological process (BP) GO terms of upregulated CP- DEGs.

| Term ID | Term description | Observed gene count | Background gene count | Strength | FDR |
|---|---|---|---|---|---|
| GO:0006950 | Response to stress | 46 | 543 | 0.49 | 3.11E-09 |
| GO:0009432 | SOS response | 12 | 30 | 1.16 | 4.91E-07 |
| GO:0050896 | Response to stimulus | 56 | 913 | 0.35 | 4.91E-07 |
| GO:0009605 | Response to external stimulus | 19 | 138 | 0.7 | 1.20E-05 |
| GO:0033554 | Cellular response to stress | 28 | 363 | 0.45 | 0.00036 |
| GO:0009266 | Response to temperature stimulus | 13 | 83 | 0.75 | 0.00046 |
| GO:0006974 | Cellular response to DNA damage stimulus | 22 | 267 | 0.47 | 0.0013 |
| GO:0009408 | Response to heat | 10 | 58 | 0.8 | 0.0018 |
| GO:0009628 | Response to abiotic stimulus | 20 | 239 | 0.48 | 0.0022 |
| GO:0019219 | Regulation of nucleobase-containing compound metabolic process | 26 | 387 | 0.39 | 0.0036 |
| GO:0009271 | Phage shock | 4 | 4 | 1.56 | 0.0046 |
| GO:0060255 | Regulation of macromolecule metabolic process | 29 | 474 | 0.35 | 0.0049 |
| GO:0051252 | Regulation of rNA metabolic process | 25 | 376 | 0.38 | 0.005 |
| GO:0051716 | Cellular response to stimulus | 30 | 512 | 0.33 | 0.0068 |
| GO:0048519 | Negative regulation of biological process | 18 | 230 | 0.45 | 0.0083 |
| GO:0050789 | Regulation of biological process | 35 | 657 | 0.29 | 0.0083 |
| GO:0098661 | Inorganic anion transmembrane transport | 6 | 22 | 0.99 | 0.0083 |
| GO:0010468 | Regulation of gene expression | 26 | 432 | 0.34 | 0.0127 |

| | | | | | |
|---|---|---|---|---|---|
| GO:0010629 | Negative regulation of gene expression | 14 | 158 | 0.51 | 0.0151 |
| GO:1903506 | Regulation of nucleic acid-templated transcription | 23 | 362 | 0.36 | 0.0151 |
| GO:0007154 | Cell communication | 14 | 162 | 0.5 | 0.0174 |
| GO:0010605 | Negative regulation of macromolecule metabolic process | 15 | 183 | 0.47 | 0.0174 |
| GO:0050794 | Regulation of cellular process | 31 | 582 | 0.29 | 0.0177 |
| GO:0006281 | DNA repair | 10 | 88 | 0.61 | 0.0181 |
| GO:0010556 | Regulation of macromolecule biosynthetic process | 25 | 423 | 0.33 | 0.0181 |
| GO:0006355 | Regulation of transcription, DNA-templated | 22 | 356 | 0.35 | 0.0212 |
| GO:0019985 | Trans lesion synthesis | 3 | 3 | 1.56 | 0.0212 |
| GO:1903507 | Negative regulation of nucleic acid-templated transcription | 12 | 129 | 0.53 | 0.0212 |
| GO:0065007 | Biological regulation | 37 | 772 | 0.24 | 0.0215 |
| GO:0010558 | Negative regulation of macromolecule biosynthetic process | 13 | 155 | 0.48 | 0.0256 |
| GO:0031327 | Negative regulation of cellular biosynthetic process | 13 | 156 | 0.48 | 0.0265 |
| GO:0031326 | Regulation of cellular biosynthetic process | 24 | 425 | 0.31 | 0.03 |
| GO:0045892 | Negative regulation of transcription, DNA-templated | 11 | 121 | 0.52 | 0.0323 |
| GO:0048523 | Negative regulation of cellular process | 15 | 207 | 0.42 | 0.0331 |
| GO:0051172 | Negative regulation of nitrogen compound metabolic process | 13 | 166 | 0.45 | 0.0382 |
| GO:0009607 | Response to biotic stimulus | 6 | 38 | 0.76 | 0.0423 |
| GO:2000113 | Negative regulation of cellular macromolecule biosynthetic | | | | |
| process | 12 | 148 | 0.47 | 0.0433 | |

## Appendix J. Enriched molecular function (MF) of upregulated CP-DEGs.

| Term ID | Term description | Observed gene count | Background gene count | Strength | FDR |
|---|---|---|---|---|---|
| GO:0015419 | ATPase-coupled sulfate transmembrane transporter activity | 5 | 5 | 1.56 | 0.0037 |

**Appendix K. Enriched biological process (BP) GO terms of downregulated regulated CP-DEGs.**

| Term ID | Term description | Observed gene count | Background gene count | Strength | FDR |
|---|---|---|---|---|---|
| GO:0042956 | Maltodextrin transport | 5 | 5 | 1.1 | 0.0352 |
| GO:0070814 | Hydrogen sulfide biosynthetic process | 6 | 6 | 1.1 | 0.0107 |
| GO:0000103 | Sulfate assimilation | 7 | 8 | 1.04 | 0.006 |
| GO:0006536 | Glutamate metabolic process | 10 | 18 | 0.85 | 0.003 |
| GO:0009065 | Glutamine family amino acid catabolic process | 9 | 18 | 0.8 | 0.0096 |
| GO:0009069 | Serine family amino acid metabolic process | 14 | 43 | 0.61 | 0.006 |
| GO:1901606 | Alpha-amino acid catabolic process | 18 | 62 | 0.56 | 0.0027 |
| GO:0043648 | Dicarboxylic acid metabolic process | 26 | 90 | 0.56 | 8.96E-05 |
| GO:0009064 | Glutamine family amino acid metabolic process | 16 | 57 | 0.55 | 0.007 |
| GO:0044272 | Sulfur compound biosynthetic process | 16 | 66 | 0.49 | 0.0226 |
| GO:1901607 | Alpha-amino acid biosynthetic process | 27 | 122 | 0.45 | 0.0017 |
| GO:1901605 | Alpha-amino acid metabolic process | 44 | 199 | 0.45 | 1.85E-05 |
| GO:0008652 | Cellular amino acid biosynthetic process | 30 | 138 | 0.44 | 0.0009 |
| GO:0006790 | Sulfur compound metabolic process | 26 | 122 | 0.43 | 0.0033 |
| GO:0006520 | Cellular amino acid metabolic process | 50 | 254 | 0.4 | 2.30E-05 |
| GO:0046394 | Carboxylic acid biosynthetic process | 36 | 203 | 0.35 | 0.0033 |
| GO:0043436 | Oxoacid metabolic process | 78 | 525 | 0.27 | 8.28E-05 |
| GO:0044283 | Small molecule biosynthetic process | 46 | 320 | 0.26 | 0.0144 |
| GO:0019752 | Carboxylic acid metabolic process | 71 | 501 | 0.25 | 0.0008 |
| GO:1901566 | Organonitroge n compound biosynthetic process | 63 | 509 | 0.19 | 0.0355 |
| GO:0044281 | Small molecule metabolic process | 108 | 870 | 0.19 | 0.0004 |
| GO:1901564 | Organonitroge n compound metabolic process | 104 | 966 | 0.13 | 0.0462 |

| | | | | | |
|---|---|---|---|---|---|
| GO:0009987 | Cellular process | 269 | 2926 | 0.06 | 0.0009 |

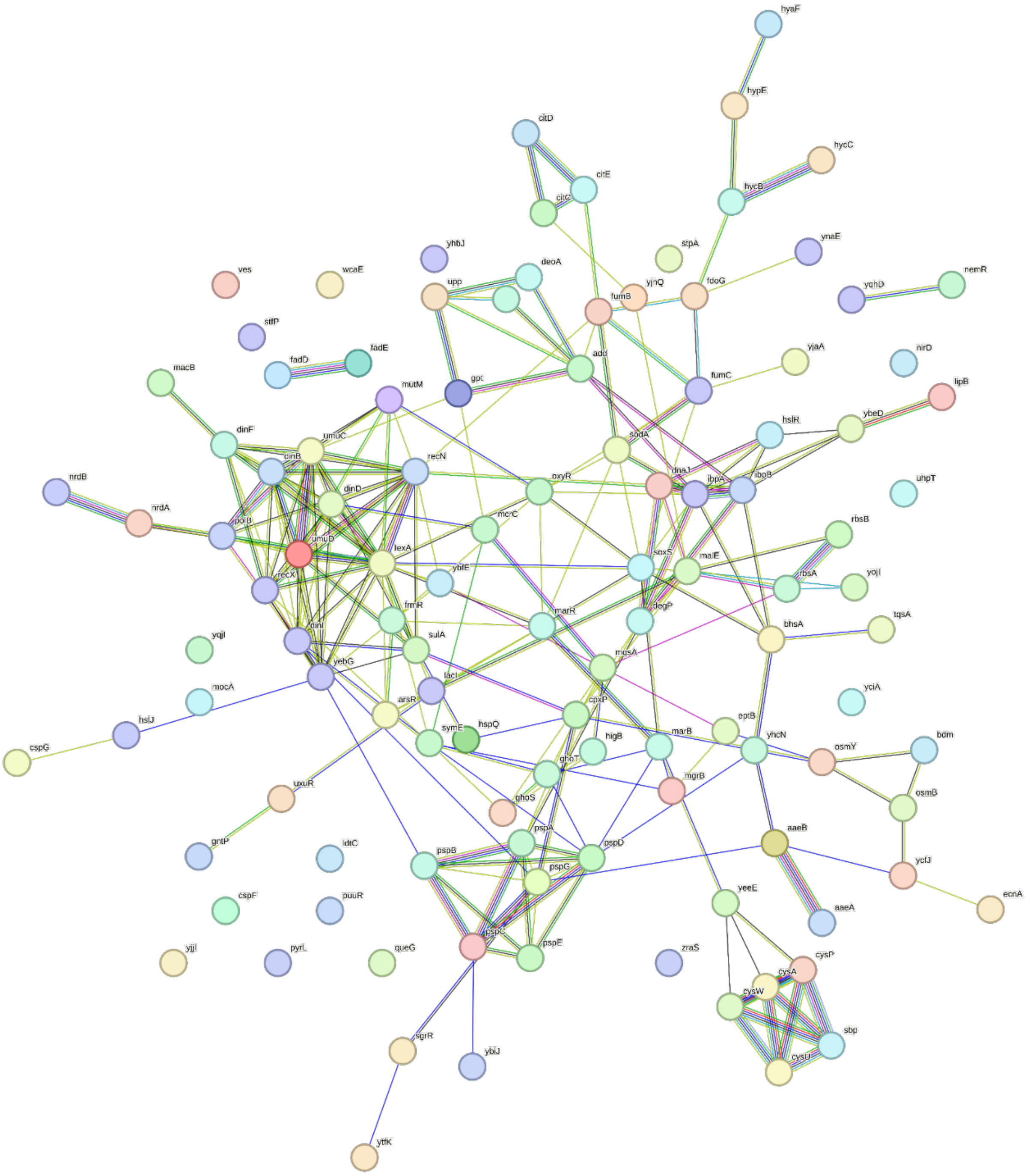
### Appendix L. Enriched molecular function (MF) of downregulated CP-DEGs.

| Term ID | Term description | Observed gene count | Background gene count | Strength | FDR |
|---|---|---|---|---|---|
| GO:0030170 | Pyridoxal phosphate binding | 17 | 57 | 0.58 | 0.0115 |
| GO:0043167 | Ion binding | 145 | 1335 | 0.14 | 0.0115 |
| GO:0043168 | Anion binding | 90 | 734 | 0.19 | 0.0115 |
| GO:0003824 | Catalytic activity | 198 | 2032 | 0.09 | 0.0123 |
| GO:0036094 | Small molecule binding | 96 | 817 | 0.17 | 0.0123 |
| GO:0019842 | Vitamin binding | 19 | 82 | 0.47 | 0.0189 |

### Appendix M. KEGG analysis of downregulated CP-DEGs.

| Term ID | Term description | Observed gene count | Background gene count | Strength | FDR |
|---|---|---|---|---|---|
| eco00561 | Glycerolipid metabolism | 7 | 14 | 0.8 | 0.0095 |
| eco00910 | Nitrogen metabolism | 8 | 24 | 0.62 | 0.0243 |
| eco00260 | Glycine, serine and threonine metabolism | 10 | 37 | 0.53 | 0.0243 |
| eco00630 | Glyoxylate and dicarboxylate metabolism | 10 | 42 | 0.48 | 0.0404 |
| eco00240 | Pyrimidine metabolism | 11 | 51 | 0.43 | 0.0457 |
| eco00920 | Sulfur metabolism | 14 | 31 | 0.76 | 0.0001 |
| eco00250 | Alanine, aspartate and glutamate metabolism | 14 | 33 | 0.73 | 0.0001 |
| eco02024 | Quorum sensing | 15 | 64 | 0.47 | 0.0095 |
| eco01230 | Biosynthesis of amino acids | 20 | 117 | 0.33 | 0.0243 |
| eco02010 | ABC transporters | 35 | 179 | 0.39 | 0.0001 |
| eco01110 | Biosynthesis of secondary metabolites | 52 | 339 | 0.29 | 0.0002 |
| eco01100 | Metabolic pathways | 120 | 890 | 0.23 | 1.88E-07 |

## Appendix N. Protein-protein Interaction Network of upregulated CP-DEGs generated by STRING.



## Appendix O: Protein-protein Interaction Network of downregulated CP-DEGs generated by STRING