Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Identification of a resonance Raman marker for cytochrome to monitor stress responses in *Escherichia coli*

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Fig. S1 Comparison of Raman spectra of *E. coli* recorded with two excitation wavelengths (633 nm and 785 nm) at three time points of growth (3h: Lag phase, 6h: Log phase, 24 h: Death phase). Each spectrum shown is an average of three independent experiments. The standard deviation from these three experiments are shown as a shaded region in each spectrum. For each independent experiment at least 60 spectra were collected to account for spatial inhomogeneity

Fig. S2 Growth dependent Raman spectra of *E. coli* recorded with 633 nm laser excitations in two different temperatures. Each spectrum shown is average of three independent experiments. The standard deviation from these three experiments are shown as a shaded region in each spectrum. For each independent experiment at least 60 spectra were collected to account for spatial inhomogeneity

Fig. S3 Ratiometric markers under different stress conditions induced by chemical compounds. Statistical analysis was performed using one-way ANOVA where *** represents $p < 0.001$

Fig. S4 Confirmation of *cydB* and *ccmE* deletion by PCR amplification: Primers flanking 100 bp upstream and downstream of *cydB* and *ccmE* were used. *E. coli* WT (MG 1655) strain showed the presence of 400 bp and 1200 bp band which was absent in the *E. coli* mutant strains $\Delta cydB$ and $\Delta ccmE$ respectively. The band at ~1500 bp was indicative of the presence of kanamycin resistance cassette

Fig. S5 Representative growth dependent Raman spectra of *E. coli* mutant strains. ($\Delta cydB$ and $\Delta ccmE$) Each spectrum shown is average of three independent experiments. The standard deviation from these three experiments are shown as a shaded region in each spectrum. For each independent experiment at least 60 spectra were collected to account for spatial inhomogeneity

(a) Multivariate analysis for *E. coli* grown in different temperatures

(b) Multivariate analysis for three different strains of *E. coli*

Fig. S6 Multivariate analysis indicates that the newly discovered peak can assist in differentiating bacteria grown under different temperatures or different bacterial strains (a) PCA scores plot for *E. coli* grown in different temperatures (Left) and corresponding loading (Right). (b) PCA scores plot for three different *E. coli* strains (Left) and corresponding Loadings (Right). Around 60 spectra were considered for each group for the analysis. Log phase (6 h) spectra were considered for analysis for each condition. Each spectrum was truncated (600 to 1700 cm⁻¹), baseline subtracted, normalized and smoothed prior to multivariate analysis

Fig. S7 Pairwise PCA indicates the major differentiating factor between two mutant strains and bacteria grown in two different temperatures of the newly found peak. **(a)** PCA scores plot for *E. coli* grown in different temperatures (Left) and corresponding loading (Right). **(b)** PCA scores plot for two different *E. coli* strains (Left) and corresponding Loadings (Right). Around 60 spectra were considered for each group for the analysis. Log phase (6 h) spectra were considered for analysis for each condition. Each spectrum was truncated (600 to 1700 cm⁻¹), baseline subtracted, normalized and smoothed prior to multivariate analysis

Table S1 Information of the genes which are deleted in the mutant strains

Gene	Protein	Function
<i>cydB</i>	Cytochrome bd-I ubiquinol oxidase subunit II	Expressed when cells are grown in low aeration, a component of aerobic respiratory chain. Generates proton motive force transferring 1 proton/electron
<i>ccmE</i>	Cytochrome C-type biogenesis protein ccmE	A heme chaperone which is needed for the biogenesis of c-type cytochromes. Binds heme delivered by ccmC and facilitated by ccmF and ccmH

The information about the mutants has been taken from <https://bio.tools/ecogene>.