Reviewer's report

Title: Gene expression changes linked to antimicrobial resistance, oxidative stress, iron depletion and retained motility are observed when Burkholderia cenocepacia grows in cystic fibrosis sputum

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Reviewer: Mark Thomas

Reviewer's report:

Generating a picture of the global changes in Burkholderia cepacia complex (Bcc) gene expression that occur during growth in CF sputum would be a key step in the process leading to the identification of genes that are crucial for Bcc survival and virulence in the CF lung. To this end, Drevinek and colleagues describe the assessment and validation of a newly designed B. cenocepacia microarray. The authors have chosen at the outset to assess the performance of their new microarray on cells growing in CF sputum. I would have been tempted to start by altering a more controllable parameter such as temperature or iron availability. Nevertheless, the authors have done a good job, and a number of genes have been flagged as potential players in CF infection that will warrant further study.

Minor revisions:

1. My 'take' on the the semi-quantitative RT-PCR result (Figure 1) is that it shows expression of the constitutively expressed house-keeping gene, BCAL1861, is downregulated in sputum medium relative to BSM. How do the authors explain this?

2. Does the fact that detection of the BCAL0270 transcript occurs after five fewer cycles than the BCAL1861 transcript actually mean that it is upregulated in sputum medium? For example, if the control house-keeping transcript was of low abundance, then transcripts originating from the test gene still may be detectable before that of the house-keeping gene, even if the test gene has not derepressed. Surely it is the difference between the number of cycles of amplification required to detect the test transcript under either condition that is important? This could be compared with the ratio of cycles for the house-keeping gene transcript amplified under both sets of conditions.

3. Why are the BCAL1861 data not included in Table 1, as it is stated in Table 2 that this was included in the real time analysis?

4. As little as 5 microM iron is fully repressing for some iron-regulated genes in B.cenocepacia. That would suggest that the concentration of iron that the authors measure in BSM and diluted sputum (~35 microM) corresponds to repressing conditions, and may explain why they do not observe derepression of iron-regulated genes. Also note that there may be iron carry over from the LB, as
the cells were not washed. It would have been useful to have determined the iron concentration in their neat (i.e. undiluted sputum), since the iron they measure in the diluted sputum may have largely originated from the diluent (BSM). At least we would know whether the results obtained for genes involved in iron homeostasis reflect the true situation in the CF lung.

It should be noted that although the iron concentration in sputum samples may correspond to a value that is known to support growth in laboratory media, its 'availability' may be restricted. For example, any free iron present in sputum is likely to be mopped up by lactoferrin, which binds iron very tightly (notwithstanding the fact that the freezing procedure that the sputum has been subjected to may inactivate this protein). Also, there is evidence that CF mucus has iron sequestering properties (Wang et al., 1996).

NB FeIII is "readily utilised" if the bacteria produce siderophores.

Regarding the establishment of the reconstituted sputum medium I have a few questions/comments. I think it is important that the method for preparing the medium and setting up the cultures is clear.

5. Line 173, I am assuming that the frozen sputum sample was not lyophilised, since this is not stated?

6. Line 175, it is stated that the sputum concentration was 12.5% (w/v) in the initial homogenate. To what volume of this was 1 ml of overnight cells added? What is the fold dilution of the o/n bacteria in the sputum medium? The statement on line 188 does not make sense. Is the final concentration of sputum now 10%, as stated on line 468 (meaning that it was a 1 in 5 dilution, i.e. 1 ml cells plus 4 ml 12.5% sputum medium)?

7. The initial OD of the diluted culture must have been quite high. How many doublings occurred in the new medium before gene expression was analysed? For example, if the starting OD was 0.2 and cells were harvested 0.6 OD units later (OD 0.8) that is two doublings. I assume that is enough time to allow adaptation to the new medium and turnover of any long-lived mRNAs.

Other minor comments:

Was P. aeruginosa present in any of the sputum samples infected with B.cenocepacia?

Line 174 "...in a minimal salts medium (BSM) containing..."

Line 339-340, delete "and were hypothetical in nature," (redundant)

Line 348 "semi-quantitative reverse transcriptase PCR" (?). Ditto line 353.

Lines 355-358, I presume the authors are referring to growth in sputum medium? Please clarify

Line 556, is growth in CF sputum going to be as fast as it is in pure sputum?
Incidentally, how did the growth rates in the two media compare?

Line 565, BCAL3521

Line 599, what do the authors mean by 'interesting'? This is a bit vague.

Line 609, BCAL0269-0270

Please shorten the Discussion

Discretionary revisions:

Line 328, I presume there are multiple copies of rRNA operons in B. cenocepacia (as there are in many bacteria). This would make the observation that only two rRNA genes are upregulated in sputum medium odd (the different rRNA operons are co-regulated in E.coli). The authors could comment on this observation.

Table 1: I would like to have seen some down-regulated genes included in the data set in Table 1 as well. Also, to validate the iron-limited and oxidative stress conditions, it would have been useful to have included in the analysis genes that are known to be regulated under these conditions (orb, kat etc). What does the house-keeping gene do under these conditions?

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests