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

Version: 1 Date: 26 June 2008

Reviewer: Tom Coenye

Reviewer's report:

Minor Essential Revisions

1. It is unclear how the authors determined that the patients investigated were chronically infected with ET12 strains identical to strain J2315 (page 7, line 132). This information needs to be included in the manuscript or an appropriate reference (e.g. to an earlier epidemiological study) needs to be provided.

2. Confusion may arise regarding the methodology used for normalisation of the qPCR data. I would strongly recommend to include fold-expression data based on only one type of normalisation. Both types of normalisation obviously show the same trend and mentioning both makes the manuscript (especially Table 1) unnecessarily complicated. I personally would prefer to include data based on the delta delta Ct method, if the E values are sufficiently close to 2.

3. On page 14, lines 306-307 the authors mention “the twofold filter”. However, this twofold filter is not mentioned in the section on microarray analysis. Is there a direct correlation between genes identified by the twofold filter and genes identified with the statistical significance filter of p < 0.05? This should be clarified.

4. I was a bit surprised to see both semi-quantitative PCR and qPCR being used in the same study. Why was the expression of BCAL1165 not investigated with qPCR as well? My understanding is that semi-quantitative PCR is more a “quick & dirty” approach. This seems to be confirmed by the data in Fig 1: there appears to be a (very) faint signal in lane 2 after 35 cycles, while there appears to be no signal at all after 40 cycles. If there is a specific reason why this gene was only investigated with this method this should be mentioned. If not I would strongly recommend to include qPCR data for this gene as well.

5. Other comments
- Can the authors clarify whether patients 5 and 6 (both infected with P. aeruginosa) were coinfected with B. cenocepacia? If so, this should be clearly mentioned in the text (page 9, lines 16-170).
- Define the abbreviation BSM when it is first used (page 9, line 186).
- Include the power output (in watt) during sonication (page 10, lines 203-204).
Discretionary Revisions

6. The authors could include patient history data (including antibiotic use) in a separate Table.

**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