Insights in Genetics and Genomics

Review Report Open Access

Article Title: Growth on Azo Compounds and Decolorization Capacity of Some Eubacteria not Conferred by Plasmidic DNA

Reviewer: Tamás Fehér, Hungarian Academy of Sciences, Hungary

Submission Date: January 19, 2017

Review Status: Revision Required

How to Cite this Report: Tamás Fehér. Review Report for: Growth on Azo Compounds and Decolorization Capacity of Some Eubacteria not Conferred by Plasmidic DNA [Version 1, Awaiting Peer Review]. Insights Genet Genomics. (2016) 1: 2.1

Review Report

The authors isolated three bacterial strains from environmental samples contaminated by azo-dyes, identified the species, and verified their capability of azo-dye breakdown.
The importance of the topic is quite high, for azo-dyes are common contaminants found in the environment surrounding textile, paper or chemical production facilities. The capability of the isolated strains to decolor azo-dyes is well demonstrated during growth in rich medium.

Major Comments:

1. The authors must be more explicit when stating which strain is capable of growth on azo dyes as the sole carbon source. To me, it seems From Figure 2 that only SNRC1 is capable of utilizing an azo-dye as a carbon source (Figure 2A). The growth starts after a 60h lag phase. The strains S2RC1 and S2RM2, seen in Figures 2B and C, respectively, cease growth at cells numbers of 5 E7 - 6 E7. This is the typical picture of growth incapability, for the small initial increase of the biomass is permitted only by the trace contaminants within the minimal medium. Note, that this cell number (cca. 5 E7) corresponds to the cell numbers seen in the lag phases of Figure 1 and Figure 2A. There is therefore no point of defining a “shortened lag phase” in Figures 2B and C. Furthermore, the growth curves obtained with strains claimed to be cured of plasmids are missing. These would be critical to investigate the roles of the plasmids.
2. The gel photo (Figure 3) does not fully convince the reader that the three strains carry plasmids. Those high-MW bands could well be genomic fragments in the range of 20-40 kbp. A simple restriction digestion with robust and inexpensive endonucleases (e.g. EcoRI, BamHI, HindIII, PstI, or their combinations) could quickly verify that the DNA originates from plasmids if one or more discrete bands are obtained with measurable lengths (<11 kbp).
3. Once the presence of plasmids is verified, then their loss should be reattempted. After treatment with EtBr, however, plating and analysis of individual clones would be essential to justify cell lines totally free of plasmids. The loss of plasmids has to be verified with the same experiment as described in point

2. above, and the result must be shown. Batch treatment of cells with EtBr, and plasmid prepping (as described in the manuscript) does not exclude the possibility of a fraction of cells still carrying the plasmids, and this fraction later overgrowing the cured population.

4. When repeating the growth and azo-dye decoloration assays with the verified plasmid-free lines, the results must be shown.

Minor comments:

1. A thorough revision is necessary to correct all mistakes concerning English grammar
2. In the Methods section concerning the PCR, the concentration of primers should be 20 uM, not mM.

Overall, the manuscript describes an important and interesting study. I recommend a more stringent evaluation of the growth and plasmid preparation experiments and displaying the results completely, in order to fully exploit the value of this work.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.