July 20

Technology of the month – July 2020

Tagging of *Pseudomonas putida* using cumate-inducible GFP

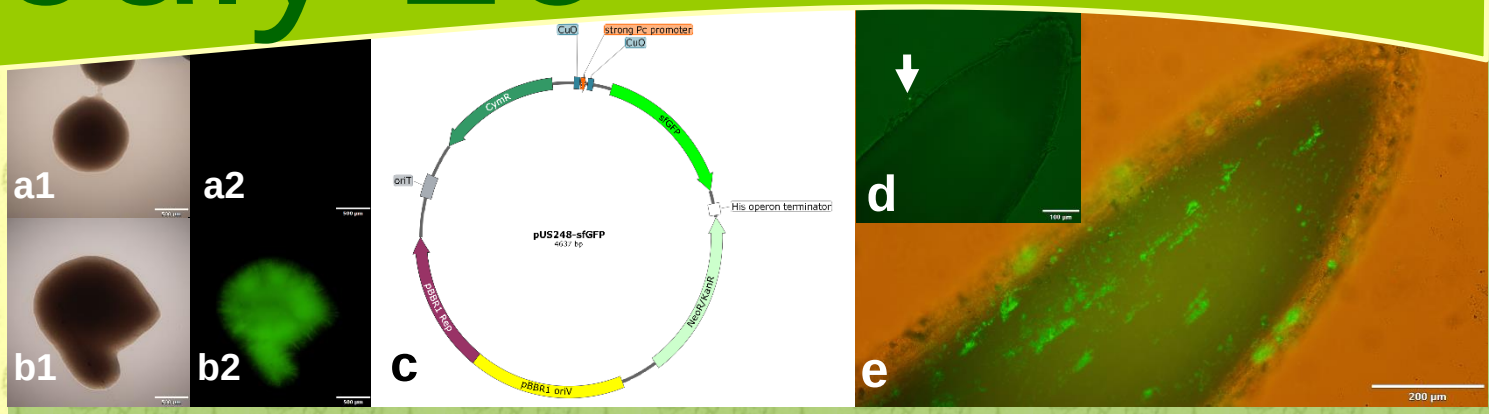

Fig. 1. (a) Colonies of transformed *Pseudomonas putida* grown on PDA plates with kanamycin at 50 µg/mL (Km50), visualized by either bright-field microscopy (a1) or fluorescence microscopy (a2). (b) Same as (a), but with p-cumate added to agar at 1 mM (c) Plasmid map of pUS248-sfGFP. (d) *P. putida* (pUS248-sfGFP) on vetch root tip post 90 mins inoculation (white arrow) (e) *P. putida*(pUS248-sfGFP) on vetch root tip 9 days post-inoculation (dpi), visualized by mixed fluorescence/bright field microscopy. Transformed *P. putida* cultures were kindly provided by Mark Somerville and vetch seeds by Dr Jade Hartley.

Green fluorescent protein (GFP) has been used as a reporter protein in many experiments. Previous work has demonstrated that the cumate-inducible gene expression system from *Pseudomonas putida* F1 works in both Gram negative and Gram positive bacteria (Kaczmarczyk et al (2013), Seo and Schmidt-Dannert (2019)). Here, a plasmid providing cumate-controlled GFP expression was introduced into *Pseudomonas putida* strain KT2440 (a model rhizosphere bacterium) and the system tested for monitoring plant root colonization.

Method: A novel synthetic cumate-inducible system was constructed by combining the strong constitutive promoter Pc from the class 1 integron with the regulatory elements from *Pseudomonas putida* F1 (CymR repressor, and two CuO operator sequences) in a broad host range plasmid backbone (pBBR). The sfGFP reporter gene was cloned under the control of the cumate-inducible promoter to give plasmid pUS248-sfGFP.

The fluorescence of the recombinant *P.putida* culture was tested first on agar, and then *in planta*. For the latter experiments, the bacteria were applied to vetch plants using the inoculation and *in vitro* growth method outlined in Perrine-Walker et al. (2014). After 90 mins inoculation and 9 dpi, 3 to 4 plants were harvested for microscopy observations. Cumate (1 mM) and kanamycin (25 or 50 µg/mL) were added to liquid Jensens' nitrogen free liquid growth medium. For controls, cumate was not added. Plant growth conditions were 22-24ºC at 50% humidity under continuous light. Plant roots observations are representatives of two repeat experiments.

Green fluorescence protein expression was observed using an Olympus BX51 microscope equipped with a WIB and GFP filter under UV fluorescence and DP74 Olympus camera.

Results and possible applications: The system is useful for tagging *Pseudomonas putida* to track colonization of plant surfaces, and due to the broad-host range plasmid replicon, could easily be applied to other Gram-negative bacteria.

Advantages: Inducible promoters can be more useful than constitutive promoters since they minimize stress on the host cell and can be switched on only when needed. Cumate is an ideal inducer, being non-toxic and inexpensive, and it freely diffuses into cells. Characterization of gene expression levels, expression kinetics, and dose-response to cumate inducer concentration can be studied. The tight regulation of the cumate system makes it potentially useful for metabolic engineering and synthetic biology.

Disadvantages: The current plasmid backbone only replicates in Gram negative bacteria and it would need to be changed for application to Gram positive bacteria. Superfolder GFP is patent-protected and would need to be replaced by another marker for use in commercial applications.

Further Reading: Kaczmarczyk et al (2013) Applied and Environmental Microbiology 79:6795-6802
Perrine-Walker et al., (2014) Protoplasma 251:1099–1111
Seo and Schmidt-Dannert (2019) Applied Microbiology and Biotechnology 103:303–313
Volke et al., (2020) Trends in Microbiology 28 (6): 512-513

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