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Construction of tightly regulated E-lysis cassette for production of genetically inactivated *Salmonella Enteritidits* ghost

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One of the most widely studied bacterial killing factors is the integral membrane host-toxic protein E expressed by DNA phage PhiX174, which can be utilized for genetic inactivation of Gram-negative bacterium, resulting in lysis of host bacterial cells. Protein E causes lysis in growing Gram-negative cells by blocking cell wall synthesis and this blockage is effected by specific inhibition of MraY, an enzyme involved in the pathway for murein biosynthesis. The application of genetic engineering tools is necessary for proper development of tightly regulated prokaryotic expression systems with stable maintenance of genes whose expression is detrimental to the growth of the host bacteria. The use of a regulatory promoter system that tightly represses expression of gene E during normal culture growth is essential because of its lethality to the bacterial host. Such tight regulation of gene E expression at normal growth temperatures may facilitate optimal and stable growth of host bacterial cells, ultimately resulting in higher production of ghost cell mass. In order to avoid leaky expression of the bacterial host-toxic PhiX174 lysis gene E from the λpR promoter, a convergent promoter construct was made in which gene E was placed between a sense λpR promoter and an anti-sense ParaBAD promoter. In the presence of L-arabinose, leaky transcription of lysis gene E at 28 °C from the sense λpR promoter was repressed by an anti-sense RNA simultaneously expressed from the ParaBADpromoter. The stringent repression of lysis gene E in the absence of induction temperature resulted into higher concentration of bacteria in culture suspension, and consequently higher and stable production of a *Salmonella* Enteritidis (S. Enteritidis) ghost.

Biography

Chetan V Jawale is basically a Veterinarian, currently pursuing PhD in Veterinary Medicine at college of Veterinary Medicine, Chonbuk National University, South Korea. He completed his B.V.Sc. and A. H. (2002-2007) from Bombay Veterinary College, Mumbai, India, and M.V.Sc in Animal biotechnology (2007-2009) from Anand Agricultural University, Anand, India. During 2009-2010 he worked as junior scientist at Xcelris Genomics Center, Ahmedabad, India. His research primarily focuses on the development of the recombinant and genetically inactivated vaccines against the *Salmonella* and E. coli infections in the domestic animals.

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