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Cell engineering, fine tuning in codon distribution and antibiotic-free selection for recombinant protein production in *E. coli*: A perfect harmony

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During the past decades, the domain of recombinant protein expression has drawn inspiration from molecular engineering techniques associated to fundamental aspects of microbiology and cellular biology. One of the major drivers was the insatiable need for large amounts of biotherapeutic molecules and recombinant vaccines. Molecular biology techniques have, in parallel, gained in sophistication and ease of use, leveraging the creativity associated to the definition of novel expression systems. In addition to industrial needs in terms of productivity and cost effectiveness, safety considerations became increasingly demanding. The consequence was the definition or strengthening of new and logical quality and safety constraints. As a matter of example, the use of alternatives to antibiotic-based selection is promised to introduce new regulatory standards. The use of antibiotic-based selection, frequently associated with genetic manipulation of microorganism is currently undergoing a profound metamorphosis with the implementation and diversification of alternative selection means. We propose a general strategy, based on novel systems validated at both laboratory and pre-industrial scale. Such genetic solutions combine natural plasmid stabilisation and antibiotic-free selection based on post-segregational killing. These novel host/vector systems are completely devoid of antibiotic resistance gene and bring the additional advantage of improving recombinant protein expression and/or plasmid recovery. A dramatic increase in genetic stability and robustness, over high stress fermentation conditions is also often demonstrated. The intrinsic performance of the expression system can be further improved through precise fine tuning of the codon usage, modulating speed and local rhythm of translation. An expected increase in productivity, solubility and global functionality of expressed protein will be discussed.

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Study of the genes involved in the resistance of nosocomial *Pseudomonas aeruginosa* to fluoroquinolone

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The major mechanism of *Pseudomonas aeruginosa* resistance to fluoroquinolones is the alteration of target enzymes, type II and IV topoisomerases due to mutations in the quinolone resistance-determining-regions (QRDR) of the *gyrA* and *parC* genes coding A subunits of these enzymes. 37 isolates from patients with burn wounds and 20 isolates from blood, urine and sputum specimen were selected to evaluate mutations involved in antibiotic resistance and were subsequently verified for their resistance to ciprofloxacin. QRDRs regions of *gyrA* and *parC* were amplified by Polymerase Chain Reaction (PCR) and were subsequently sequenced. 90% of isolates with MIC≥8 µg/ml to ciprofloxacin had a mutation in *gyrA* gene in which threonine at position 83 changed to isoleucine. 87.5% of isolates had mutation in *parC*, Serine 87 changed. 75% had Ser87Leu and 12.5% possessed Serin87Trp. Various silent mutations were also detected such as Val103Val, Ala118Ala, Ala136Ala and His132His in *gyrA* and Ala115Ala in *parC*. The data indicates that the common mutation in *gyrA* is Thr83Ile and in *parC* is Ser87Leu/Trp. No individual *parC* mutation was observed while mutations in *gyrA* and *parC* occurred simultaneously and appears to be the main reason of high level resistance to fluoroquinolones in patients with burn wounds and urine infection. The vast majority of *P. aeruginosa* isolates had mutation in *parC* which can play a crucial role in increased resistance of these isolates. This is the first report of *parC* mutations from resistant *P. aeruginosa* isolates from Iran, Tehran.

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