Bacterial cell factories for recombinant protein production; expanding the catalogue

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Escherichia coli has been the pioneering host for recombinant protein production, since the original recombinant DNA procedures were developed using its genetic material and infecting bacteriophages. As a consequence, and because of the accumulated know-how on E. coli genetics and physiology and the increasing number of tools for genetic engineering adapted to this bacterium, E. coli is the preferred host when attempting the production of a new protein. Also, it is still the first choice for protein production at laboratory and industrial scales for an important number of proteins, being fast growth and simple culture procedures critical issues. When searching for an ideal system for protein production, this bacterial species is clearly far from offering, in generic terms, optimal conditions for protein production and downstream. Plasmid loss and antibiotic-based maintenance, undesired chemical inducers of gene expression, plasmid/protein-mediated metabolic burden and stress responses, lack of post-translational modifications (including the inability to form disulphide bonds), none or poor secretion, protein aggregation and proteolytic digestion, endotoxin contamination and complex downstream are among the main obstacles encountered during protein production in E. coli. In the pharmaceutical scenario, proper protein glycosylation is often requested and simplest purification procedures become highly desirable when pursuing cost-effective bioproduction. In this context, the yeast Saccharomyces cerevisiae, diverse mammalian cell lines, insect cells and whole plant and animals (as transgenic systems) are being incorporated to the protein production scenario [1], and many of these products have been already approved for use as protein drugs [2]. Other (less conventional) yeast species and a more limited number of species of filamentous fungi [3], molds [4], moss [5], algae [6] and protozoa [7] are also under development as potential suppliers of recombinant proteins. The engineering of such systems could represent a promising way to the cost effective production of high quality protein versions that biotechnology and biomedical industries are steadily demanding. The potential and versatility of these platforms as protein producers or in general, as cell factories for added value products such as chemicals, amino acids or vitamins has been stressed in recent experimental reports or reviews [8-17]. Despite this, it must be noted that adapting large-scale production processes to the biological complexity of some of these systems might represent, in some cases, an unaffordable task.

From a different angle, bacterial hosts others than E. coli are attracting attention as cell factories due to their metabolic diversity and biosynthetic potential derived from adaptation to extremely diverse environments. The most important bacterial groups explored as cell factories for recombinant proteins and their associated potentialities are summarized in Table 1. The implementation of lactic acid bacteria as a routine cell factory expands their applications from conventional food microbiology [18-21] to protein production and also protein drug display and delivery [22-29], taking advantage of the generically recognized as safe (GRAS) features of this platform. Improved solubility in halophilic and cold-adapted bacteria, enhanced secretion in acid lactic bacteria and in general in endotoxin-free gram-positive species and post-translational modifications in mycobacteria among others are highly appealing properties in protein production, that can be of special value for specific difficult-to-express proteins. While exhibiting most of the above mentioned limitations linked to prokaryotic-based production, exploring bacterial species other than E. coli should be not abandoned but fully supported as it will not only expand the current catalogue of cell factories but also offer novel process opportunities in easily cultivable/scalable systems that might pose, in generic terms, less methodological issues than unconventional protein production systems [30].
Towards a progressively more competitive biological synthesis by microbes [78] and assisted by expanding systems metabolic engineering and synthetic biology tools [79], industrial biotechnology should desirably find within the prokaryotic world, a growing spectrum of alternatives to eukaryotic cell factories, that apart from easy and cost-effective cultivation provide unexpectedly high metabolic versatility and biosafety of their

| Table 1 The most important bacterial groups explored as cell factories for recombinant protein production |
|-------------------------------------------------|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Host                                            | Main features                                          | Reviews*          | Main bacterial Species                                      | Case proteins                        | References      |
| Proteobacteria:                                  |                                                        |                  | Hematopoietic necrosis virus capsid proteins                | [33]                                  |
| Caulobacteria                                   | Easy purification of secreted RsA fusions              | [31,32]          | Caulobacter crescentus                                       | [33]                                  |
| Phototrophic bacteria                           | High production of membrane proteins                   | [35]              | Rodobacter sphaeroides                                       | Membrane proteins                    | [35]            |
| Cold adapted bacteria                           | Improved protein folding                                | [36,37]          | Pseudoalteromonas haloplanktis                               | 3H6 Fab                               | [38]            |
| Cold adapted bacteria                           |                                                        |                  |                                                           | Human nerve growth factor             | [39]            |
| Phototrophic bacteria                           |                                                        |                  |                                                           | β-Lactamase, peptidases, glucosidase  | [40]            |
| Pseudomonads                                     | Efficient secretion                                     | [41]              | Pseudomonas fluorescens                                     | Human granulocyte colony-stimulating factor | [42]          |
| Pseudomonas                                      |                                                        |                  | Pseudomonas putida                                           | Single chain Fv fragments             | [43]            |
| Pseudomonas                                      |                                                        |                  | Pseudomonas aeruginosa                                       | Penicillin G acylase                  | [44]            |
| Halophilic bacteria                              | Solubility favored                                     | [45]              | Halomonas elongata                                           | β-Lactamase                           | [45]            |
| Halophilic bacteria                              |                                                        |                  |                                                           | Chromohalobacter salexigenes          | [46]            |
| Actinobacteria:                                  |                                                        |                  |                                                           |                                        |                 |
| Streptomyces                                      | Efficient secretion                                     | [47,48]          | Streptomyces lividans                                       | M. tuberculosis antigens              | [49]            |
| Streptomyces                                      |                                                        |                  | Streptomyces griseus                                         | Trypsin                               | [50]            |
| Nocardia                                         | Efficient secretion                                     | [48]              | Nocardia lactamundanus                                       | Lysine-6-amino transferase           | [51]            |
| Mycobacteria                                     |                                                        |                  |                                                           | Hsp65-hIL-2 fusion protein           | [53]            |
| Coryneform bacteria                              | High-level production and secretion; GRAS              | [48,55]          | Corynebacterium glutamicum                                   | Mycobacterial proteins                | [54]            |
| Coryneform bacteria                              |                                                        |                  | Corynebacterium ammoniagenes                                 | Protein-glutaminase                   | [56]            |
| Coryneform bacteria                              |                                                        |                  | Brevibacterium lactofermentum                                | Pro-transglutaminase                  | [57]            |
| Firmicutes                                       | High-level production and secretion                     | [59-64]          | Bacillus subtilis                                            | Cellulases                           | [58]            |
| Bacilli                                          |                                                        |                  |                                                           |                                        |                 |
| Bacilli                                          |                                                        |                  | Bacillus brevis                                              | Disulfide isomerase                   | [66,67]         |
| Bacilli                                          |                                                        |                  | Bacillus megatenium                                          | Antibodies                            | [68]            |
| Bacilli                                          |                                                        |                  | Bacillus licheniformis                                       | Subtilisin                            | [69]            |
| Bacilli                                          |                                                        |                  | Bacillus amyloliqufaciens                                   | Amylases                             | [70]            |
| Lactic acid bacteria                             | Secretion; GRAS                                       | [22-24,71]       | Lactococcus lactis                                           | Fibronectin-binding protein A, internalin A, GroEL | [72,73]         |
| Lactic acid bacteria                             |                                                        |                  | Lactobacillus planatarum                                     | β-Galactosidase                       | [74]            |
| Lactic acid bacteria                             |                                                        |                  | Lactobacillus casei                                          | VP2-VP3 fusion protein of infectious pancreatic necrosis virus | [75] |
| Lactic acid bacteria                             |                                                        |                  | Lactobacillus reuteri                                        | Pediocin PA-1                         | [76]            |
| Lactic acid bacteria                             |                                                        |                  | Lactobacillus gasseri                                        | CC chemokines                         | [77]            |

*Generic reviews about the biological platform or about specific tools for protein production.
protein-based products. In some cases and at a large extent, it is solving some of the main issues posed by E. coli as traditional producer or recombinant proteins.

**Competing interests**

The authors declare that they have no competing interests.

**Acknowledgments**

We are indebted to MINECO (BFU2010-17450), AGAUR (2009SGR-0108) and CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN, Spain) for funding our research on protein-based therapeutics and the Protein Production Platform (PPP). CIBER-BBN is an initiative funded by the VI National RD&I Plan 2008–2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions and financed by the Instituto de Salud Carlos III with assistance from the European Regional Development Fund. AV received an ICREA ACADEMIA award.

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Received: 29 October 2013 Accepted: 30 October 2013

Published: 18 November 2013

**References**

1. Sorensen HP: Towards universal systems for recombinant gene expression. Microb Cell Fact 2010, 9:27.

2. Ferrer-Miralles N, Domingo-Espin J, Corchero JL, Vazquez E, Villaverde A: Strategies for high-level recombinant protein production in yeasts: when are these systems better than bacterial production systems? Appl Microbiol Biotechnol 2011, 89:39–94.

3. Corchero JL, Basset B, Reski R: Moss bioreactors producing improved biopharmaceuticals. Curr Opin Biotechnol 2007, 18:393–398.

4. Decker EL, Reski R: Strategies for high-level recombinant protein expression in transgenic microalgae: a review. Biotechnol Adv 2010, 28:910–918.

5. LEXYS Biosafety Status. 2013. http://www.jenabioscience.com/cms/en/1/browse/1879_biosafety.html. 2013 Ref Type: Electronic Citation.

6. Decker EL, Reski R: Moss bioreactors producing improved biopharmaceuticals. Curr Opin Biotechnol 2007, 18:393–398.

7. Peterbauer C, Maischberger T, Haltrich D: Optimization for recombinant protein production in the Antarctic bacterium Pseudoalteromonas haloplanktis TAC125. Microb Cell Fact 2011, 10:115.

8. Villaverde A: Secretory delivery of recombinant proteins in Lactococcus lactis as a live vector: heterologous protein production and secretion. J Mol Microbiol Biotechnol 2008, 14:46–58.

9. Duilio A, Tutino ML, Marino G: Relevant aspects of protein expression in Caulobacter crescentus: proteins and beyond. Microb Cell Fact 2011, 10:48.

10. Shu S, Kong J, Sun Z, Han L, Kong W, Yang P: Intranasal immunisation with recombinant E. coli membrane protein production for structural genomics initiatives. Biochem Biotechnol 2008, 95:97–108.

11. Rippa V, Papa R, Giuliani M, Pezzella C, Parrilli E, Tutino ML, et al: Lactococcus lactis, an efficient cell factory for recombinant protein production and secretion. J Mol Microbiol Biotechnol 2008, 2013. http://www.jenabioscience.com/cms/en/1/browse/1879_biosafety.html. 2013 Ref Type: Electronic Citation.

12. Teusink B, Bachmann H, Molenaar D: Systems biology of lactic acid bacteria: a critical review. Microb Cell Fact 2011, 10:151.

13. Garcia-Frutos E: Lactic Acid Bacteria: a promising alternative for recombinant protein production. Microb Cell Fact 2012, 11:57.

14. Peterbauer C, Maischberger T, Haltrich D: Food-grade gene expression in lactic acid bacteria. Microb Cell Fact 2011, 6:147–161.

15. De Vos WM: Systems solutions by lactic acid bacteria: from paradigms to practice. Microb Cell Fact 2011, 10:152.

16. Specht E, Miyake-Stoner S, Mayfield S: Micro-algae come of age as a platform for recombinant protein production. Biotechnol Lett 2010, 32:1373–1383.

17. Rhee SJ, Lee JE, Lee CH: Importance of lactic acid bacteria in Asian fermented foods. Microb Cell Fact 2011, 10:155.

18. De Vos WM: Systems solutions by lactic acid bacteria: from paradigms to practice. Microb Cell Fact 2011, 10:152.

19. Arens K, Moroni A, Zannini E: Medical nutrition therapy: use of sourdough lactic acid bacteria as a cell factory for delivering functional biomolecules and food ingredients in gluten free bread. Microb Cell Fact 2011, 10:151.
psychrophilic *Pseudoalteromonas haloplanktis*. *J Biotechnol* 2006, 127(1-2):141–150.

40. Miyake R, Kawamoto J, Wei YL, Kitagawa M, Kato I, Kunita H, et al.: Construction of a low-temperature protein expression system using a cold-adapted bacterium, *Shewanella* sp. strain AC10, as the host. *Appl Environ Microbiol* 2007, 73:4849–4856.

41. Retallack DM, Jin H, Chew L: Reliable protein production in a *Pseudomonas fluorescens* expression system. *Protein Expr Purif* 2011, 81:157–165.

42. Jin H, Cantin GT, Maki S, Chew LC, Resnick SM, Nigai J, et al.: Soluble periplasmic production of human granulocyte colony-stimulating factor (G-CSF) in *Pseudomonas fluorescens*. *Protein Expr Purif* 2011, 78:65–77.

43. Dammeyer T, Steinwand M, Kruger SC, Dubel S, Hust M, Timmis KN: Efficient production of soluble recombinant single chain Fv fragments by a *Pseudomonas putida* strain KT2440 cell factory. *Microb Cell Fact* 2011, 10:11.

44. Krzeslak J, Braun P, Wühloux R, Cool RH, Quax WJ, Cool RH, et al.: *Penicillium* sp. strain AC10, as the host. *Appl Microbiol Biotechnol* 2013, 97:9597–9608.

45. Ayala JC, Pimienta E, Rodriguez C, Anne J, Vallin C, Milanes MT, et al.: Gene expression systems in *corynebacteria*. *Microb Cell Factories* 2013, 12:113.

46. Nagayoshi C, Tokunaga H, Hayashi A, Harazono H, Hamasaki K, Ando A, et al.: Efficient expression of halaarohaenic nucleoside diphosphate kinase via strong porin promoter in moderately halophilic bacterium. *Protein Pept Lett* 2006, 13:611–615.

47. Anne J, Maldonado B, Van Li, Van ML, Bernal D: Recombinant protein production and streptomycetes. *J Biotechnology* 2012, 158:159–167.

48. Liu L, Yang H, Shih HD, Li J, Cao P, Chen J: Porin-based biphasic expression vectors for heterologous protein production in *Escherichia coli*. *Biochim Biophys Acta* 2011, 1813:675–687.

49. Chary VK, de la Fuente JL, Leitao AL, Lisas P, Martin JF: Overexpression of the lat gene in *Nocardia lactamurans* from strong heterologous promoters results in very high levels of lysine-6-amino transferase and up to two-fold increase in cephamycin C production. *Appl Microbiol Biotechnol* 2000, 54:282–288.

50.Connell ND: Expression systems for use in actinomycetes and related organisms. *Curr Opin Biotechnol* 2001, 12:446–449.

51. Guo QQ, Wei Y, Yu B: Recombinant *Mycobacterium smegmatis* expressing Hsp65-Hil-2 fusion protein and its influence on lymphocyte function in mice. *Asian Pac J Trop Med* 2012, 5:347–351.

52. Noens EE, Williams C, Anandhakrishnan M, Poulsen C, Ehebauer MT, et al.: Isolation of a *Mycobacterium smegmatis* double promoter for protein expression in *Mycobacterium* subsp. bulgaricus DSM 20081: expression in *Mycobacterium subsp. bulgaricus* and *Mycobacterium smegmatis*. *Appl Environ Microbiol* 2010, 76:87–97.

53. Miyoshi A, Bermudez-Humaran LG, Langella P, Chatel JM, et al.: Purification and characterization of a halotolerant serine proteinase from thermotolerant *Bacillus licheniformis* RKK-04 isolated from Thai fish sauce. *Appl Microbiol Biotechnol* 2010, 86:1867–1875.

54. Pohl S, Harwood CH: Heterologous protein secretion by bacillus species from the cradle to the grave. *Adv Appl Microbiol* 2010, 73:1–25.

55. Liu L, Yang H, Shin HD, Li J, Cao P, Chen J: Porin-based biphasic expression vectors for heterologous protein production in *Escherichia coli*. *Biochim Biophys Acta* 2011, 1813:675–687.

56. Biedendieck R, Bunk B, Furch T, Franco-Lara E, Jahn M, Jahn D: Efficient production of soluble recombinant single chain Fv fragments by a *Pseudomonas putida* strain KT2440 cell factory. *Microb Cell Fact* 2011, 10:11.

57. Kizielak J, Braun P, Wühloux R, Cool RH, Quax WW: Heterologous production of *Escherichia coli* penicillin G acylase in *Pseudomonas aeruginosa*. *J Biotechnol* 2009, 142:250–259.

58. Retallack DM, Jin H, Chew L: Reliable protein production in a *Pseudomonas fluorescens* expression system. *Protein Expr Purif* 2011, 81:157–165.

59. Toyokawa Y, Takahara H, Reungsang A, Fukuta M, Hachimine Y, Tachibana S, et al.: Production of *Mycobacterium smegmatis* groEL1DeltaC expression strain. *Microb Cell Fact* 2011, 10:11.

60. David F, Steinwand M, Hust M, Bohle K, Ross A, Dubel S, et al.: Antibody production in *Bacillus megaterium*: strategies and physiological implications of scaling from micro titer plates to industrial bioreactors. *Bioresource* 2011, 161:1516–1531.

61. Toyokawa Y, Takahara H, Reungsang A, Fukuta M, Hachimine Y, Tachibana S, et al.: Purification and characterization of a halotolerant serine proteinase from thermotolerant *Bacillus licheniformis* RKK-04 isolated from Thai fish sauce. *Appl Microbiol Biotechnol* 2010, 86:1867–1875.

62. Deb P, Talukdar SA, Mohsina K, Sarker PK, Sayem SA: Production and partial characterization of extracellular amylose enzyme from *P. sp. strain Ac10*. *Springerplus* 2013, 2:154.

63. Le LY, Azevedo V, Oliveira SC, Freitas DA, Miyoshi A, Bermudez-Humaran LG, et al.: Protein secretion in *Lactococcus lactis*: an efficient way to increase the overall heterologous protein production. *Microb Cell Fact* 2005, 4:2.

64. Innocentin S, Guimaraes V, Miyoshi A, Azevedo V, Langella P, Chatel JM, et al.: Heterologous expression of *Brucella abortus* GroEL heat-shock protein in *Lactococcus lactis*. *Microb Cell Fact* 2006, 5:14.

65. Miyoshi A, Bermudez-Humaran LG, Ribeiro LA, Le LY, Oliveira SC, Langella P, et al.: Heterologous expression of Brucella abortus GroEL heat-shock protein in *Lactococcus lactis*. *Microb Cell Fact* 2006, 5:14.

66. Wang J, Yang H, Shin HD, Li J, Cao P, Chen J: Porin-based biphasic expression vectors for heterologous protein production in *Escherichia coli*. *Biochim Biophys Acta* 2011, 1813:675–687.

67. Kajino T, Okito C, Muramatsu M, Obata S, Udaka S, Yamada Y, et al.: A protein disulfide isomerase gene fusion expression system that increases the extracellular productivity of *Bacillus brevis*. *Appl Environ Microbiol* 2000, 66:583–586.

68. Kajino T, Kato K, Miyazaki C, Asami O, Hira I, Yamada Y, et al.: Isolation of a protease-deficient mutant of *Bacillus brevis* and efficient secretion of a fungal protein disulfide isomerase by the mutant. *J Biosci Bioeng* 1999, 87:37–42.

69. Fang H, Steinwand M, Hust M, Bohle K, Ross A, Dubel S, et al.: Antibody production in *Bacillus megaterium*: strategies and physiological implications of scaling from micro titer plates to industrial bioreactors. *Bioresource* 2011, 161:1516–1531.

70. David F, Steinwand M, Hust M, Bohle K, Ross A, Dubel S, et al.: Antibody production in *Bacillus megaterium*: strategies and physiological implications of scaling from micro titer plates to industrial bioreactors. *Bioresource* 2011, 161:1516–1531.

71. Toyokawa Y, Takahara H, Reungsang A, Fukuta M, Hachimine Y, Tachibana S, et al.: Purification and characterization of a halotolerant serine proteinase from thermotolerant *Bacillus licheniformis* RKK-04 isolated from Thai fish sauce. *Appl Microbiol Biotechnol* 2010, 86:1867–1875.

72. Deb P, Talukdar SA, Mohsina K, Sarker PK, Sayem SA: Production and partial characterization of extracellular amylose enzyme from *P. sp. strain Ac10*. *Springerplus* 2013, 2:154.

73. Le LY, Azevedo V, Oliveira SC, Freitas DA, Miyoshi A, Bermudez-Humaran LG, et al.: Protein secretion in *Lactococcus lactis*: an efficient way to increase the overall heterologous protein production. *Microb Cell Fact* 2005, 4:2.

74. Innocentin S, Guimaraes V, Miyoshi A, Azevedo V, Langella P, Chatel JM, et al.: Heterologous expression of *Brucella abortus* GroEL heat-shock protein in *Lactococcus lactis*. *Microb Cell Fact* 2006, 5:14.