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Morphology engineering of bacteria for bio-production

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ABSTRACT

The concept of "morphology engineering" is proposed here. There are many genes involved in maintaining the bacterial shapes. The manipulations of these genes allow us to change the bacterial shapes from rods to fibers or to small spheres or large spheres. The advantages of morphology engineered bacteria for bio-production including accelerated growth, high cell density, simplification of downstream separation, enlarged space for more inclusion body accumulation and reduction on the cost of bio-production, have recently started to be exploited. So far only a few shape related genes have been manipulated for bioprocess benefits, many more genes are to be exploited for various cell morphologies. The limits of bacterial lengths and diameters may depend on how we manipulate relevant genes. Over time, these limits can be broken to enhance bioprocess competitiveness including improvements on the effectiveness of up- and downstream bioprocessing. Morphology engineering is just starting to show its promises.

Contents

1. Introduction

Industrial biotechnology usually involves microbial fermentation in large fermentor vessels, followed by separation of microbial biomass from fermentation media and then the processing of either biomass or supernatants. Since the microbial cells are very small, especially prokaryote cells, it is usually difficult to separate tiny cells from fermentation media (Wang et al., 2014a). Expensive and energy-intensive continuous centrifugation or micro-filtration or time-consuming gravity precipitation is required to achieve the separation. It is therefore desirable to find an alternative economic way to achieve the separation (Tan et al., 2014; Wang et al., 2014a, 2014b).

It is now possible to conduct bacterial morphology engineering in which the shapes of bacteria can be manipulated from bars to spheres (Jiang et al., 2015; Kruse et al., 2003) or bars to fibers (Chen et al., 2012). Such shape changes have proven not only beneficial for

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downstream separation but also positive for improvement on inclusion body production (Jiang et al., 2015; Tan et al., 2014; Wang et al., 2014a).

In this paper, we will take microbial polyhydroxyalkanoates (PHA), a family of biodegradable biopolymers useful as environmentally friendly and sustainable plastics as an example to illustrate how morphology engineering can benefit bioprocessing especially downstream bioprocessing (Gao et al., 2011; Wang et al., 2014b).

To enable PHA to be as economically competitive as conventional plastics, efforts should be made on effectively lowering the PHA production cost. Over the last decade, many efforts have been made to reduce PHA production cost including process optimization, the use of low-value substrates such as glycerol, cellulose and starch (Chen and Patel, 2012; Meng et al., 2015), mixed microbial cultures, and the development of efficient culture strategies (Ahn et al., 2001; Javers et al., 2012; Squio et al., 2003; Tan et al., 2011; Yue et al., 2014). Very importantly, bacterial cells should be grown to high cell density within a short period of time on lower cost substrates during the upstream processing to achieve the goal (Choi and Lee, 1997; Keshavarz and Roy, 2010; Kim et al., 1994; Lee and Lee, 1996; Li et al., 2009, 2010).

One of the most costly factors for PHA production is the downstream processing of bacterial cells and their PHA intracellular contents. Most bacteria have small sizes ranging from 0.5–2 μm, the small sizes not only increase the difficulty to separate the bacterial biomass from culture broth, but also limit the amount of PHA granules to be stored in each cell. It is important to investigate what can be done to engineer the bacterial morphology to allow convenient separation and large space for inclusion body storages. Table 1 summarizes the representative determinants for the maintenance of rod-shaped bacteria.

Most bacteria have cell walls that maintain cell shapes and protect the cells against osmotic lysis. Peptidoglycan is the main stress-bearing component of the bacterial cell walls providing mechanical strength to resist osmotic and mechanical challenges (Vollmer et al., 2008). In nearly all bacteria, the cell shape is determined by the architecture of the peptidoglycan cell wall, a macromolecule consisting of glycan chains cross-linked by short peptides (Wang et al., 2012). Membrane-bound and membrane-associated proteins linking the peptidoglycan and the cytoskeleton, can transmit shape information across the cytoplasmic membrane for maintaining the cell shape. This group of shape determinants includes penicillin-binding proteins (PBPs) and other proteins required for shape maintenance, including MreC, MreD, RodZ and RodA (Table 1) (Cabeen and Jacobs-Wagner, 2005). Penicillin-binding proteins (PBPs) are membrane-associated proteins involved in polymerizing and modifying peptidoglycans, the main component of bacterial cell walls. According to their molecular weights, sequences, enzymatic and cellular functions, PBPs are categorized into five high-molecular-weight PBPs (PBPs 1a, 1b, 1c, 2, 3) and seven low-molecular-weight PBPs (PBPs 4, 5, 6, 7, AmpC and AmpH) (Spratt, 1975).

The cytoskeletal protein MreB, exerts spatial and temporal control over peptidoglycan synthesis, plays a critical role in maintaining the rod shape of many species and is often required for viability of bacteria such as *Escherichia coli* and *Bacillus subtilis* during elongation (Gitai, 2005; Wang et al., 2012). The contribution of the MreB cytoskeleton to cellular stiffness is comparable with that of the cell wall (Wang et al., 2010), it plays a stabilization role to maintain cell width (Cabeen and Jacobs-Wagner, 2010).

RodA protein, which belongs to a family of polytopic membrane proteins, is required for normal cell shape formation, and is not strictly essential for cell viability (Shiomi et al., 2013). RodZ is a conserved bitopic membrane protein that interacts with MreB, and both factors are required to maintain the rod shape of *E. coli*. RodZ is also required for the proper assembly of the MreB cytoskeleton (van den Ent et al., 2010). Bitopic membrane protein MreC and integral membrane protein MreD, both transcribed asoperons and functioning in the same morphogenetic pathway, were also reported to be important for cell shape control (Fig. 1) (van den Ent et al., 2010).

FtsZ, a tubulin-like protein, is an essential cell-division protein that is universally required for cell division in nearly all bacteria (Bi and Lutkenhaus, 1991; Erickson et al., 2010). FtsZ forms a ring structure, namely, the Z-ring, at the cell-division site, which constricts the cell membrane during septation (Bi and Lutkenhaus, 1991). The Z ring contains only approximately 30–40% of cellular FtsZ at a given time (Stricker et al., 2002). The other fraction of FtsZ moves rapidly in helical patterns around the cytoplasm (Thanedar and Margolin, 2004). The helical localization of FtsZ encircles the cells along the inside of the cell membrane, perhaps serving as a scaffold to recruit and position a cascade of enzymes involved in peptidoglycan synthesis with helical localization patterns (Møller-Jensen and Löwe, 2005). In a process referred to as preseptal cell elongation in *E. coli*, Z rings direct peptidoglycan synthesis and contribute to cell growth along the long cell axis (Cabeen and Jacobs-Wagner, 2010).

PHA are inclusion bodies accumulated in various prokaryotic microorganisms, larger intracellular space is preferred for PHA polymer production (Jiang et al., 2015; Tan et al., 2014; Wang et al., 2014a). Based on recent successful examples of morphology engineering, we review methods to change bacterial morphology and its applications for economical inclusion body production.

2. Manipulation on bacterial morphology

The rigid cell wall is one of the many factors limiting accumulation of inclusion bodies (intracellular granules) in bacterial cells. Since a weak cell wall may allow easy expansion of cell size for more storage of inclusion bodies such as PHA granules, cell wall (peptidoglycan) synthesis genes can be deleted to weaken the cell walls. Penicillin binding

Table 1
Proteins that affect bacterial morphologies.

Name	Function(s)	Intracellular localization	Reference
FtsZ	Cell-division determinant; formation of Z-ring	Dynamic Z-ring and cytoplasmic helices	Bi and Lutkenhaus (1991), Thanedar and Margolin (2004)
FtsA	Stabilization of the Z-ring; recruitment of proteins to the division site	Z-ring associated	Geissler et al., 2003
SulA	Stress-induced inhibitor of FtsZ polymer assembly	Z-ring associated	Cordell et al., 2003; Justice et al., 2000
MinC	An inhibitor of FtsZ polymerization	Z-ring associated	Gueiros-Filho and Losick, 2002
MinD	Recruits MinC to the membrane	Z-ring associated	Gueiros-Filho and Losick, 2002
MreB/Mbl	Cytoskeletal structure, ATPase, GTPase; cell shape determination in non-spherical bacteria	A cytoplasmic, membrane-attached helix or patches	Heichlinger et al., 2011; Kruse et al. (2005)
MreC,	MreB-associated proteins	Inner membrane-associated proteins	Kruse et al. (2005); Wachi et al., 1989
MreD	MreB-associated proteins	Inner membrane-associated proteins	(Kruse et al. (2005); Wachi et al., 1989
RodZ	MreB-associated proteins; required for cell shape maintenance	Inner membrane-associated proteins	Bendezú et al. (2009)
PBP2	Essential for cell elongation; murein DD-transpeptidase	Dependent on MreB filament for localization	de Pedro et al. (2001)
PBP3	Essential for cell division; part of the divisome	Anchored in the inner membrane	Denome et al. (1999)
RodA	Pivotal to the cell wall elongation; lipid II flippase	Polytopic membrane proteins	Shiomi et al. (2013)