Bioresource Technology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Review

# Improvement of industrially important microbial strains by genome shuffling: Current status and future prospects

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ARTICLE INFO

*Keywords:*
Genome shuffling
Stress tolerance
Strain improvement
Bioconversion
Protoplast fusion

ABSTRACT

The growing demand for biotechnological products against limited metabolic capacity of industrially used microorganisms has led to an increased interest on strain-improvement over the last several decades, which aimed to enhance metabolite yield, substrate uptake and tolerance of the strains. Among a few techniques of strain-improvement, genome shuffling is the most recent and promising approach used for rapid strain-improvement that can yield a new strain by combining whole genomes of multi-parental microorganisms using the principles of protoplast fusion. Genome shuffling has brought a major breakthrough in the strain-improvement concept as it is found to be effective and reliable for expressing complex phenotypes. This review will discuss the technical aspects and applications of genome shuffling for various industrial strains to present its current status and recent progress. In the concluding remarks, a summary will be presented focusing on the major challenges and future outlooks of this technology.

## 1. Introduction

The rapid increase of world population as well as the growing concerns on food insecurity, rapid depletion of fossil fuels and environmental pollution have necessitated realistic research efforts for increasing the yields of different biotechnological products in the limited facility. Biosynthesis of these products are completed mainly by using microorganisms or their enzymes through bioconversion of various substrates (Gao et al., 2017, 2018). However, an increase in the product yield using natural microbial strains are often hindered by their low substrate conversion efficiency, production of by-products, and low tolerance to stresses (De Gérando et al., 2016; Qi et al., 2016). One of the strategies to overcome these challenges is the optimization of fermentation process that may include screening of the best conditions for the parameters related to this basic step in a bioconversion process (Yun et al., 2018). Despite the effectiveness of optimization process, it is practically difficult to find out the best conditions for a wild strain, particularly when both high product yield and high tolerance to the final product are desired at a time. Strain-improvement could be the most vital way to solve these issues, and it has been received much interests over the last several decades (Qi et al., 2017; Zhang et al., 2014).

Classical strain-improvement basically involves screening of high throughput mutants generated by different techniques including random mutation, gene engineering for overexpressing selected genes, intentional mutagenesis and laboratory evolution (Biot-Pelletier & Martin, 2014; Cheigh et al., 2005; Kalra et al., 1973). However, although classical strain-improvement have been studied extensively over the decades for improving different industrially important microorganisms, it is a time consuming and laborious approach. More importantly, obtainment of genetically modified strains in a classical strain-improvement technique require comprehensive information on the genomes of parental microorganisms (Leja et al., 2011). In addition, even though strain-improvement practically means mutation in the genetic patterns of microorganisms, main target is the phenotypic improvement of the strains. Till to date, many approaches have been reported for improving phenotypic expression of strains that include artificial transcription factor engineering, global transcription machinery engineering, ribosome engineering, and genome shuffling (Gong et al., 2009).

Genome shuffling is the most recent and one of the promising technologies for rapid phenotypic improvement that has received much attention for phenotypic improvements of industrially important strains. It allows combinatorial recombination in the genotypes of parent strains related to the desired phenotypes through recursive recombination (Patnaik et al., 2002; Wang et al., 2017b). Genome

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https://doi.org/10.1016/j.biortech.2018.02.118
Received 27 December 2017; Received in revised form 23 February 2018; Accepted 25 February 2018

shuffling has some attributes similar to those of classical strain-improvement as both offer genomic diversification and screening for improved strains. The main difference between these two techniques is that genome shuffling process is sexual and whole populations of improved strains are evolved, as are not observed in the classical approach. In addition, genome shuffling is a faster and more efficient technique for generating required phenotypes compared to classical approach. Moreover, genome shuffling can sporadically induce mutations at different points of the entire genome for complex phenotypes without requiring genome sequencing data or network information of target strains (Biot-Pelletier & Martin, 2014).

Strain-improvement by genome shuffling is usually initiated by subjecting initial microbial population to repeated mutagenesis that results in the selection of desired mutants as parental population. In the subsequent steps, protoplasts are prepared, and recursive protoplast fusion is carried out, which follows the screening and selection of shuffled strains (Gong et al., 2009). The successfully developed strains through genome shuffling have been reported to be used for producing a wide range of biotechnological products, including lactic acid, riboflavin, lipase, bioethanol, antibiotics, bioinsecticide, ayamycin, avilamycin, alkaliphilic lipase, and many other similar products (Luna-Flores et al., 2017; Zhang et al., 2014; Zhao et al., 2016).

The aim of this review is to summarize the recent advances in genome shuffling in the perspectives of technological achievements and applications. The major challenges, prospective solutions and future opportunities of this technology will also be discussed in the concluding remarks.

## 2. Technical aspects of genome shuffling

Although genome shuffling is largely based on protoplast fusion, there is a primary difference between protoplast fusion and genome shuffling. In the former, recombination occurs in the new strains by genome transfer between two parents in each generation, while genome shuffling allows recombination between multiple parents and results a better chance of getting strains with improved genetic traits and capability of enhanced performance. However, it requires to develop a parental library with repeated genetic modifications and selection from a variety of strains prior to conducting recursive protoplast fusion. The achievements of genome shuffling basically rely on the initial screening of strains, effectiveness of recombination techniques, and strength of selection techniques of shuffled strains (Gong et al., 2009).

### 2.1. Parental library construction

Development of an efficient parental library with potent strains would be the first step of genome shuffling, which starts from the preliminary screening and selection of population containing desired genomes. The initial population of microorganisms must have a good genetic diversity and sufficient variation in phenotypes that are very important to get a complex phenotypic expression in the shuffled strains at the final stage. The size and diversity in the parental population depend on the techniques used to achieve the strains.

During the construction of parental library, strains are subjected to a single or several rounds of mutagenesis using either chemicals or physical mutagens. The frequently used chemical mutagens for this purpose include N-methyl-NNN-nitro-N-nitrosoguanidine (NTG) and ethyl methylsulfonate (EMS) (Leja et al., 2011), while UV is widely used as a physical mutagen (Biot-Pelletier & Martin, 2014). Sometimes mutagens can also be used in combination for generating a higher level of diversity in the strains (Yin et al., 2016; Zhang et al., 2014). With the technological achievement, recently advanced techniques and equipment are used more effective mutagenesis, such as ARTP (Zhang et al., 2015). Finally, the best performing strains are identified as the parents for the next step of recursive protoplast fusion. The standards for selection of parental strains depend on the targeted phenotypes that may include productivity and yield of desired products, stress tolerance, and growth traits of the strains. Effective mutation and selection of efficient mutant are very important for the development of successful parental library, which in turn also necessary for the overall genome shuffling process (Leja et al., 2011).

In a typical UV irradiation technique of parental library development, cells are grown in particularly a suitable liquid medium until the obtainment of desired concentration of cells, followed by the exposure of growth culture to UV irradiation (15–20 W) for a few seconds to minutes (Zhang et al., 2015; Wang et al., 2017a). The exposure of liquid culture is more appropriate where the concentration of cells varied from strain to strain based on the growth characteristics, but typically $10^5$–$10^7$ cells/ml (Wang et al., 2017a). The efficiency of mutation and lethality rate of the cells by the exposed dose are determined by the plate culture technique. Almost similar techniques are followed for the chemicals induced mutation except for exposure of the cells to the suitable concentration of the chemicals for several minutes to hours, as for example, 0.5 mg/ml of NTG for 30 min (Yin et al., 2016).

### 2.2. Protoplast fusion

Recursive protoplast fusion is the next step to the parental library development, which is basically the main step of genome shuffling. Prior to fusion, protoplasts are collected from the cells of parental strains, where cells are grown in medium, collected by centrifugation and resuspended in a buffer solution containing either a cell wall degrading enzymes (lysozyme) or enzymes having the similar function like snailase (Leja et al., 2011). This treatment results in the release of protoplasts from the cells that are then aggregated by centrifugation (Otte et al., 2009). Fusion of the obtained protoplasts are subsequently done by various techniques, such as chemical agents or electrical pulses induced cell fusion (Gong et al., 2009), femtosecond laser-induce cell fusion (Gong et al., 2008), using microfluidic chip (Skelley et al., 2009), applying optical tweezers (Mao et al., 2005), and some other techniques. At the end of fusion, the fused protoplasts are centrifuged, washed, re-suspended in buffer, serially diluted and regenerated on the regeneration medium (John et al., 2008; Leja et al., 2011). Strains obtained from the regenerated protoplasts are pooled and subjected to the next rounds of fusion and the process may be repeated for several rounds until achieving the desired strains.

The efficiency of protoplast fusion is affected by the process conditions, which in turn vary from strain to strain. Therefore, optimization of the fusion conditions is required for individual strains prior to conducting protoplast fusion to ensure a high efficiency protoplast fusion and regeneration that helps to obtain the desired shuffled strain (Shi et al., 2014). In addition to method optimization, further technical aspects can be considered for efficient protoplast fusion. For example, prokaryotic and eukaryotic cells have successfully been used to rapidly screen various strains by efficient protoplast preparation and fusion (Cao et al., 2012). In another example, recombinant strains with a wide range of genetic and phenotypic variations are obtained from the repetitive protoplast fusion.

### 2.3. Selection of desired phenotype

The final step of genome shuffling is the screening and selection of the desired phenotypes. It is crucial and very important to have a robust and high throughput screening method in place for genome shuffling to be successful. Unfortunately, compared with the availability and development of strain engineering strategies, selection methods for desired phenotypes are yet to be developed, even though several techniques are being used in this purpose.

The currently used screening techniques may vary in response to the target of strain-improvement (Table 1). For example, when a desired phenotype is required for improving stress tolerance or substrate utilization, growth based high-throughput screening methods are often