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Review

Physical methods for genetic transformation of fungi and yeast

Ana Leonor Rivera [a], Denis Magaña-Ortíz [b], Miguel Gómez-Lim [b], Francisco Fernández [a], Achim M. Loske [a,*]

[a] *Centro de Física Aplicada y Tecnología Avanzada, Universidad Nacional Autónoma de México, A.P. 1-1010, C.P. 76000, Querétaro, QRO, Mexico*

[b] *Unidad de Biotecnología e Ingeniería Genética de Plantas, Centro de Investigación y de Estudios Avanzados del IPN, A.P. 629, C.P. 36500, Irapuato, GTO, Mexico*

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Abstract

The production of transgenic fungi is a routine process. Currently, it is possible to insert genes from other fungi, viruses, bacteria and even animals, albeit with low efficiency, into the genomes of a number of fungal species. Genetic transformation requires the penetration of the transgene through the fungal cell wall, a process that can be facilitated by biological or physical methods. Novel methodologies for the efficient introduction of specific genes and stronger promoters are needed to increase production levels. A possible solution to this problem is the recently discovered shock-wave-mediated transformation. The objective of this article is to review the state of the art of the physical methods used for genetic fungi transformation and to describe some of the basic physics and molecular biology behind them.

Keywords: Genetic transformation; Fungi; Electroporation; Biolistics; Agitation with glass beads; Shock waves

Contents

* Corresponding author.
E-mail addresses: analeonor@fata.unam.mx (A.L. Rivera), dmagana@ira.cinvestav.mx (D. Magaña-Ortíz), mgomez@ira.cinvestav.mx (M. Gómez-Lim), francisco@fata.unam.mx (F. Fernández), loske@fata.unam.mx (A.M. Loske).

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1. Introduction

Fungi are useful organisms in our daily lives. For instance, they break down dead organic material, provide antibiotics [1–3], and can be used to produce various compounds such as insulin, hepatitis B vaccines, the anticoagulant hirudin, and glucagon [4–7]. Fungi are also important model organisms used in experimental and theoretical biology for gene regulation [8,9], the characterization of new genes [10], and to study the molecular and biochemical bases of diseases [11–13]. Moreover, fungal genes have been employed in the production of transgenic plants [14–20]. Fungi provide attractive expression platforms [3], and different processes can be humanized to ensure that the products mimic the human counterparts [21]. They are also employed to produce aminoacids [22,23], and recombinant proteins [5,24–32]. They are an important source of a wide range of endogenous compounds and enzymes employed in the textile [33,34], food [35,36], paper [37], chemical, and other industries [38,39]. Furthermore they are able to produce chemicals used for fermentation [40–44], such as food acidulants [44–46], food preservatives [47], flavor enhancers [48,49], renewable plastics [50], solvents [51,52], biological fungicides [53], pesticides [54], biofuels [43,55,56], and animal feed [57,58]. Commercially relevant enzymes for biodelignification [59], industrial oxidative processes [60], and environmental bioremediation [61,62] are also produced by fungi. On the other hand, fungi also cause plant, animal and human diseases [63–69]. The development of genome sequencing has enabled a better comprehension of the metabolism of fungi [38,70–76].

Completion of the *Saccharomyces cerevisiae* genome project was accomplished in 1996 making it the first genome deciphered from a eukaryotic organism [77]. Currently, 22 fungal genomes have been completely sequenced (see NCBI home page http://www.ncbi.nlm.nih.gov/) and at least a thousand fungal genome-sequencing projects are underway (http://www.genomesonline.org).

The fungal genomes available are from

- *Saccharomyces cerevisiae* [77–79],
- *Kluyveromyces waltii* [80],
- *Kluyveromyces lactis* [81],
- *Yarrowia lipolytica* [81],
- *Candida albicans* [82,83],
- *Candida dubliniensis* [83],
- *Candida glabrata* [81],
- *Debaryomyces hansenii* [81],
- *Saccharomyces pastorianus Weihenstephan* [84],
- *Magnaporthe grisea* [85,86],
- *Schizosaccharomyces pombe* [87],
- *Neurospora crassa* [88],
- *Phanerochaete chrysosporium* [54],
- *Aspergillus fumigatus* [89,90],
- *Aspergillus niger* [91],
- *Pichia stipitis* [92],
- *Vanderwaltozyma polyspora* [93],
- *Gibberella zeae* [94],
- *Podospora anserina* [95],
- *Trichoderma reesei* [96],
- *Nectria haematococca* [97], and
- *Tuber melanosporum* [98].

The first recombinant DNA molecules were produced in the 1970's with the use of biochemical scissors called restriction enzymes [99,100]. In this sense, many approaches have been successfully applied, such as varying the growth conditions in a systematic way, over-expressing activator genes, removing epigenetic silencing, introducing heterologous genes, generating strains with novel properties, and improving bioinformatic programs of random mutagenesis. *Saccharomyces cerevisiae*, a common yeast, was the first eukaryote employed for heterologous gene expression [6,25].