



Biomedicine & Prevention

An Open Access Transdisciplinary Journal

Dual-Use Molecules from Yeast

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Introduction

This year the OPCW, the implementing body for the Chemical Weapons Convention, celebrates the 20th anniversary of entry into forces. In 2014, this organization examined the impact of new technologies in the field of chemical and biological weapons, in particular the "Convergence" of Chemistry and Biology. An OPCW report of the Scientific Advisory Group highlighted the importance of monitoring developments in science and technology: "New production processes, combined with developments in drug discovery and delivery, could be exploited in the development of new toxic chemicals that could be used as weapons."¹ Indeed, since 2008, Synthetic Biology is monitored also by other international organizations, such as the Nonproliferation Export Control Regimes Australia Group.^{2,3}

Until now, the interest in Synthetic Biology research as a proliferation risk,^{4,5,6} was essentially based on the possibility to create from scratch lethal viruses; indeed, whole bacterial genomes can be now synthesized in laboratories and assembled in yeast cells and non-essential genes can be reshuffled and/or eliminated from the genome. A synthetic yeast genome itself is under construction,^{7,8,9,10} (this field of Synthetic Biology is characterized by a *bottom-up* approach, Fig. 1A). This achievement is considered a "Convergence" of Chemistry towards Biology because it is possible to construct a biological molecule, a genome, starting with chemicals in laboratory.¹¹ The second type of Synthetic Biology approach (*top-down*), has not been considered a threat because it is widely used to create new metabolic pathways in bacteria or yeasts, to produce new compounds such as biofuels, biologically active molecules (often of pharmaceutical value) and chemicals.^{12,13} Along this way, the synthesis and the insertion of functional biological components into natural genomes is achieved creating metabolic pathways, often combining genes from different organisms. Unfortunately, recently, even this *top-down* approach raised concerns for the possibility to produce toxic chemicals from microorganisms.¹⁴ This type of experiments represents the "Convergence" of Biology towards Chemistry, because chemicals are produced by biological means.^{15,16} Yeasts are considered excellent cell factories for industrial applications, with the possibility to create engineered cells for efficient production of a desired compound (metabolic engineering). In particular, the yeast *Saccharomyces cerevisiae* plays an essential role in this field,¹⁷ in addition to that of food industry (production of bread, beer, wine and chocolate). With yeasts, it is possible to produce pure amount of chemicals of high quality at low costs

and with a standardised process and continuous production all over the year, independent from seasonal harvest.¹⁸ Of great interest for the pharmaceutical industry, the biological synthesis could expand the chemical diversity of natural products and their structural complexity;^{19,20} indeed, model organisms are suitable for advancing new approaches to drug discovery. The most successful production in yeast is the synthesis of the antimalarial artemisin precursor,^{21,22} Fig. 1B, but *S. cerevisiae* has been used as a biomanufacturing platform for a large number of molecules.^{23,24} In addition to biofuels (bioethanol and farnesene),²⁵ high value metabolites were produced in yeast:²⁶ penicillin,²⁷ vindoline,²⁸ noscapine,²⁹ anthranilates,³⁰ raspberry ketone,³¹ resveratrol,^{32,24} vanillin,³³ rose oil and perfumes.³⁴

Illicit Chemicals from Yeast Cells

For the first time, in 2015, illicit molecules (brain acting molecules) were produced applying Synthetic Biology *top-down* approach to yeast cells. Concerns were raised when the production of codeine and morphine was achieved in *S. cerevisiae*.^{35,36} Different groups successfully inserted genes from bacteria, opium poppy and sugar beet into yeast, reconstructing the pathway for morphine or codeine production.^{37,38,39} The entire biosynthetic pathway of thebaine (precursor of morphine) and hydrocodone has been achieved by inserting genes from plants, bacteria and mammals.⁴⁰ The expression of the entire opiate pathway in a single yeast cell has now become a reality but could be subject to misuse because anyone with access to morphine-producing yeast strains, and with basic fermentation skills, could easily produce morphine in large quantity, starting from glucose.⁴¹ Opioids are of great pharmaceutical value because are the primary analgesic alkaloids used for pain relief; the chemical synthesis of these molecules is not commercially competitive, when compared with the extraction from the *Papaver somniferum* plant; so, with Synthetic Biology tools, it soon will be possible to produce cheaper, less addictive, safer and more effective analgesics. Another type of brain active molecules, which are used to inhibit inflammation and pain, are the cannabinoids, extracted from *Cannabis sativa*.⁴² Cannabinoids act on human endocannabinoid system, which is involved in a wide array of bodily processes, including appetite, memory, pain and mood. *Cannabis sativa* plant makes over 100 different types of cannabinoids, but the most important are tetrahydrocannabinol (THC), which is responsible of psychoactive effects, and cannabidiol (CBD), which has solely therapeutic effects. These molecules bind to different human cannabinoid re-

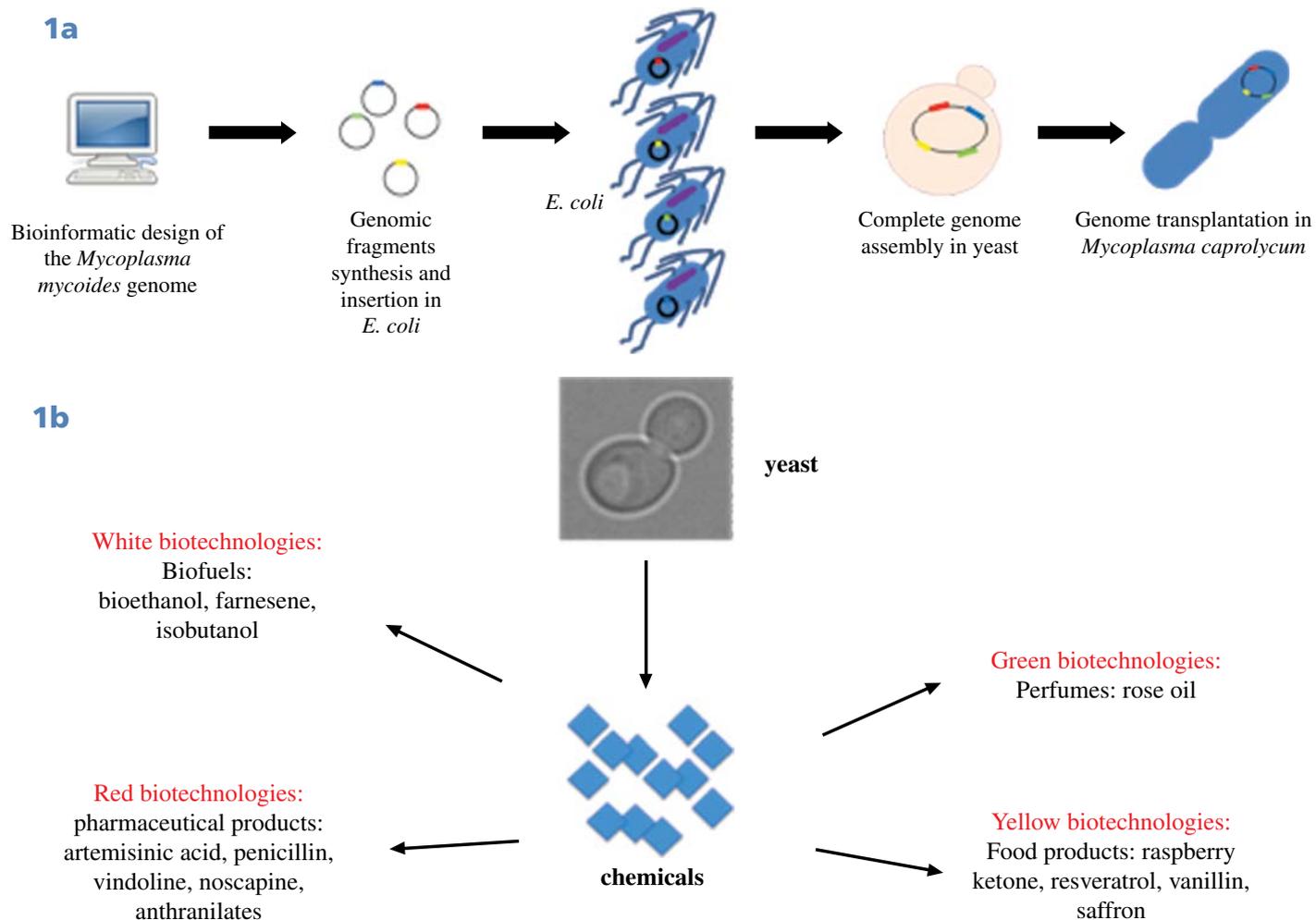


Figure 1. Yeast in synthetic biology. **a:** *Bottom-up* approach. Yeast cells were used to assemble the first synthetic bacterial genome. The *de novo* synthesis of the *Mycoplasma mycoides* genome utilized synthetic DNA oligonucleotides assembled first *in vitro* and then in *Saccharomyces cerevisiae*. The whole construct was transplanted to *Mycoplasma capricolum*, yielding a viable organism. **b:** *Top-down* approach. Yeast as a cell factory for chemical production in biotechnology.

ceptors: THC binds to the cannabinoid CB1 receptor in the brain triggering psychotropic and psychedelic effects, while the cannabidiol binding to the CB2 receptor has an anti-inflammatory effect. The obstacle to the wide use of cannabinoids in medicine is that the extraction of cannabidiol contains a small percentage of THC. The pharmaceutical industry is seeking a synthetic form of cannabis that inhibits inflammation and pain, but without the psychedelic or psychotropic effect. The advantage of using yeast to produce cannabinoids is that strains can be engineered to produce only solely therapeutic cannabinoids, like CBD, and not those that are psychedelic or psychotropic, like THC. In other words, yeast could be used to make pure products used for nutraceutical or pharmaceutical purposes: until now, using yeast cells, Germany's Technical University of Dortmund, achieved the production of Δ^9 -tetrahydrocannabinol and cannabidiol; Canada's Hyasynth Bio produced cannabigerol; England's Anandia Laboratories make cannabinoids and US BioTork LLC, cannabidiol.

Conclusions on *Dual-Use* Implication

The pharmaceutical interest on opioids and cannabinoids will result in a further future development of metabolic engineering pathways to improve the yields and produce other molecules acting in the brain. These discoveries could lead to the massive production of illicit drugs in fermenters, with cheap raw materials and in an easy way, in any place in the world. Unfortunately,

yeast cells, thanks to the harmless nature of this organism, can be easily transported without biosafety restrictions.⁴³ In the future, the *bottom-up* will meet the *top-down* technology and it will be possible to introduce into yeast synthetic genome the biological modules containing the entire biosynthetic opiates or cannabinoids pathways and select yeast cells overproducing morphine or cannabidiol. For the above reason, this type of experimentation should rise attention of the scientific community on the so called *dual-use* technology,⁴⁴ being this the first case of production of controlled narcotics by synthetic biology. In the light of the fast development of biotechnology, the *outreach* to academia should be as broad as possible, to capture the attention also of researchers involved in basic science. In the short to medium term, Synthetic Biology is unlikely to pose new risks or threats, but the long-term possibility to insert foreign synthetic genes into a natural organism, with the aim of producing biological weapons or drugs could turn even a harmless organism, such as the *Saccharomyces cerevisiae* into a dangerous threat. Toxins have already been expressed in microorganisms: bacterial toxins from *Escherichia coli*, *Vibrio cholerae*, *Bacillus thuringiensis*, *Clostridium botulinum* and *Clostridium tetani* were successfully expressed in the yeast *Pichia pastoris*.⁴⁵ The non-enzymatic heavy chain of the botulinum toxin B was expressed in yeast with the aim of expressing a secretory toxin antigen, and the subunit A (the catalytic subunit) of the ricin toxin has been expressed in *S.*

cerevisiae, to study how ricin kills the cells for the development of a protective ricin antidote.^{46,47} Finally, engineered anthrax toxin in bacteria was successfully used to selectively deliver toxic molecules in cancer cells, resulting in successful tumor therapy in animal models.^{48,49,50,51} The examples reported indicate that we are facing a real genomic and biotechnological revolution in life

sciences and that researchers who manipulates GRAS organisms should also be aware of bio-threats in the future. Simple actions would be sufficient to prevent the unconscious spread of intangible technologies or strains and plasmids among potential bioterrorists.⁴³

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