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Research paper

# Effects of cryoprotectants on the viability and activity of freeze dried recombinant yeasts as novel oral drug delivery systems assessed by an artificial digestive system

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**Abstract**

The aim of this study was to investigate, in a gastric-small intestinal system TIM-1, the effect of cryoprotectants on the survival of freeze-dried *Saccharomyces cerevisiae* expressing the heterologous P450 73A1 and their ability to convert *trans*-cinnamic acid into *p*-coumaric acid. Yeasts were lyophilized in suspensions of trehalose, maltose, lactose, or a milk proteins/trehalose mix. Freeze-dried or native yeasts and *trans*-cinnamic acid were introduced simultaneously into TIM-1 at the beginning of digestion. Yeast survival rate was evaluated by cell counting in the ileal effluents. P450 73A1 activity was followed by HPLC assay of *p*-coumaric acid. Freeze-dried yeasts showed high tolerance to digestive conditions. Nevertheless, their survival rate was lower than that of non-dried cells (around 80% whatever the protective agent vs. 96%). The ability of recombinant freeze-dried *S. cerevisiae* to perform a bioconversion reaction in the digestive tract was shown with all the protectants. The highest *trans*-cinnamic acid conversion rate (24 vs. 41% for native yeasts) was obtained with the milk proteins/trehalose mix. These results show that freeze-drying might be considered for the pharmaceutical formulation of new drug delivery systems based on orally administered recombinant yeasts and that TIM-1 could be a helpful tool for the pre-screening of oral dosage forms.


*Keywords:* Oral formulations; Pre-screening; Freeze-drying; Cryoprotectant; Recombinant *Saccharomyces cerevisiae*; Artificial digestive system

## 1. Introduction

The development of innovative drug delivery systems using recombinant micro-organisms as live vehicles for active compounds in the human digestive environment has been recently considered [1–3]. This new kind of vector offers several advantages over classical dosage forms. First, the micro-organisms, by protecting the active compounds, can allow the administration of drugs sensitive to digestive conditions when given in classical pharmaceutical formulations. Second, the regulation of gene expression (e.g. using an inducible promoter) makes it possible to target specific sites throughout the digestive tract and to control drug release. Thus, similar therapeutic effects can potentially be obtained at lower doses [3]. Recombinant micro-organisms can either carry out a bioconversion reaction or produce compounds of interest directly in the digestive environment. Potential medical applications include the correction of gastric or intestinal enzyme deficiencies [4] or organ failure [5], biodetoxication [6] or direct production in the digestive tract of proteins of interest, such as biological mediators [3] or antigens [7].

Recombinant bacteria, mainly lactic acid bacteria, have been the main potential host candidates [2–7]. However, in view of its eukaryotic structure, its Generally Recognized As Safe (GRAS) status and its easy genetic engineering, baker's yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) emerges as a convenient host for the development of this new kind of drug delivery system [1]. We have chosen

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a recombinant *S. cerevisiae* expressing the plant (*Helianthus tuberosus*) P450 73A1 [8] as a model strain to evaluate the scientific feasibility of this new approach, because of the non-toxicity and easy quantification of both substrate and product. P450 73A1 catalyses the 4-hydroxylation of *trans*-cinnamic acid into *p*-coumaric acid (cinnamate 4-hydroxylase -CA4H- activity), reproducing the first oxidative step in the plant phenylpropanoid pathway. A recent study [9] has shown a high survival rate of *S. cerevisiae* expressing the P450 73A1 and its ability to catalyse the bioconversion of *trans*-cinnamic acid into *p*-coumaric acid in a simulated digestive environment, the gastric-small intestinal system TIM-1 [10].

At present time, TIM-1 is the in vitro model that allows the closest simulation of in vivo dynamic physiological processes occurring within the lumen of the stomach and the three segments of the small intestine of human. This dynamic, computer-controlled system has been designed to accept parameters and data obtained from in vivo studies on human volunteers. The main parameters of digestion, such as pH, temperature, peristaltic mixing and transport, gastric, biliary and pancreatic secretions, passive absorption of small molecules (e.g. nutrients, drugs) and water are reproduced as accurately as possible. This gastric-small intestinal system offers reproducibility, easy manipulation and sample collection at any level of the digestive tract and at any time during digestion. It has been validated by several studies [9–13], including the evaluation of micro-organisms survival [9,12,13] and availability for absorption of acetaminophen [13]. In particular, TIM-1 might be a helpful tool in pharma-related studies by supplying information on the intraluminal fate of a drug compound (i.e. the drug release from a dosage form, its stability or viability—in case of living cells—) and its subsequent availability for absorption [13].

Once the scientific feasibility of our new drug delivery system was established, we focused on the development of pharmaceutical formulations allowing the oral administration of the genetically modified *S. cerevisiae*. Freeze-drying is a technique of dehydration commonly used for the formulation of drugs containing non-recombinant *Saccharomyces* spp. as active compounds [14–16]. The aim of this study was to assess the influence of cryoprotectants on the survival and CA4H activity of freeze-dried recombinant model *S. cerevisiae* in simulated human digestive conditions, using TIM1-system.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Chemicals

*Trans*-cinnamic acid, *p*-coumaric acid and maltose were supplied by Sigma-Aldrich (Saint–Quentin Fallavier, France). Trehalose was purchased from Acros (Noisy-le-Grand, France), mannitol and maltodextrin 1.8 kDa from Roquette (Lestrem, France), milk proteins from Armor-proteine (Saint-Brice en Cogles, France) and lactose (fast flow) from Seppic (Paris, France). All the products for yeast culture media were from Difco (Le Pont de Claix, France) or Acros. All the chemicals used for HPLC analysis were analytical grade and were purchased from Acros.

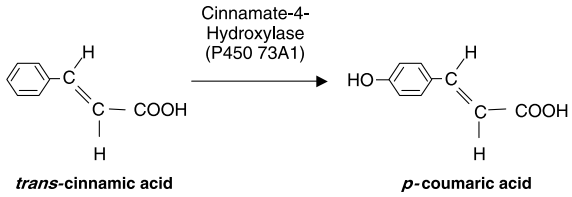


Fig. 1. CA4H activity of *S. cerevisiae* WRP45073A1.

#### 2.1.2. Yeast strain

The *S. cerevisiae* strain (kindly provided by Dr Denis Pompon, CNRS, Gif-sur-Yvette, France) was derived from the haploid strain W303-1B (MATα; *ade*2-1; *his*3-11,-15; *leu*2-3,-112; *ura*3-1; *can*$^{R}$; *cyr*$^{+}$) and was genetically engineered to overexpress *Helianthus tuberosus* CA4H when grown in the presence of galactose [8]. The resulting *S. cerevisiae* strain, called WRP45073A1, transforms *trans*-cinnamic acid into *p*-coumaric acid (Fig. 1).

#### 2.1.3. Gastric-small intestinal system (TIM-1)

The gastric-small intestinal system TIM-1 (TNO, Zeist, The Netherlands) consists of four successive compartments simulating the stomach, duodenum, jejunum and ileum [10]. This system has already been described elsewhere [10,13]. Briefly, each compartment is composed of glass units with flexible inside walls. The system is kept at body temperature by pumping water into the space between the glass jacket and the flexible wall. Peristaltic mixing is simulated by alternate compression and relaxation of the flexible walls following changes in the water pressure. Mathematical modeling of gastric and ileal deliveries with power exponential equations ($f = 1 - 2^{-(t/t_{1/2})\beta}$ where $f$ represents the fraction of meal delivered, $t$ the time of delivery, $t_{1/2}$ the half-time of delivery and $\beta$ is a coefficient describing the shape of the curve) is used for the computer control of chyme transit, as described by Elashoff et al. [17]. In our study, TIM-1 was programmed to reproduce gastrointestinal conditions of the adult after intake of a glass of water, according to in vivo data [10,17–19]. The half-time of gastric emptying was 30 min and the $\beta$ coefficient of the power exponential equation was 1. The half-time of ileal delivery of the chyme was 160 min and the $\beta$ coefficient was 1.6. Chyme transit is regulated by opening or closing the peristaltic valves that connect the compartments. The volume and pH are computer-monitored and continuously controlled in each compartment. In the stomach, the pH followed a pre-set curve: pH 4.5, 4.2, 2.1 and 1.7, at 5, 20,