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RESEARCH PAPER

Use of Artificial Digestive Systems to Investigate the Biopharmaceutical Factors Influencing the Survival of Probiotic Yeast During Gastrointestinal Transit in Humans

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ABSTRACT

Purpose To evaluate the influence of the main biopharmaceutical factors on the viability of a new probiotic yeast strain, using dynamic *in vitro* systems simulating human gastric/small intestinal (TIM) and large intestinal (ARCOL) environments.

Methods The viability of *Saccharomyces cerevisiae* CNCM I-3856 throughout the artificial digestive tract was determined by microbial counting. We investigated the effects of galenic formulation, food intake, dose, mode and frequency of administration on yeast survival rate.

Results In both fasted and fed states, yeast viability in the upper digestive tract was significantly higher when the probiotic was administered in hydroxypropylmethylcellulose (HPMC) capsules compared to tablets. Food intake led to a delay in yeast release and a two-fold increase in strain survival. Whatever the dose, yeasts were particularly sensitive to the large intestinal environment. High concentrations of probiotic could only be maintained in the colon when it was inoculated twice a day over a 5-h-period.

Conclusions TIM and ARCOL are complementary *in vitro* tools relevant for screening purposes, supplying valuable information on the effects of galenic form, food intake and dose regimen on the viability of probiotics throughout the human digestive tract.

KEY WORDS biopharmaceutical factors · gastrointestinal models · probiotics · viability

INTRODUCTION

The Food and Agriculture Organization and World Health Organization define probiotics as "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host" (1). Probiotics exert their beneficial effects through various mechanisms, including inhibition of pathogen growth by competition for nutritional sources and adhesion sites, secretion of antimicrobial substances, or modulation of the host immune response (2). Species commonly used include lactic acid bacteria such as *Lactobacillus* sp., *Bifidobacterium* sp. or *Streptococcus thermophilus*, and the non-pathogenic yeast *Saccharomyces cerevisiae* var *boulardii* (*S. boulardii*). *S. boulardii* is clinically effective for the prevention and treatment of antibiotic-associated diarrhea, and recurrent *Clostridium difficile* intestinal infections and has also shown promise for the treatment of irritable bowel syndrome or Crohn's disease (3).

S. boulardii was, to date, the only yeast commercialized for human use and consequently the only preparation

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recognized as probiotic. However, other *Saccharomyces* spp. have shown interesting properties (4,5) and are likely to find uses as biotherapeutic agents. In particular, *Saccharomyces cerevisiae* CNCM I-3856 is a new probiotic yeast strain recently marketed as Lynside Pro GI + (Lesaffre Human Care, Milwaukee, USA). This strain has shown anti-inflammatory activities in experimental colitis in mice (5) and strong visceral analgesic effects in a rat model of colonic hypersensitivity (Rousseaux *et al.*, Digestive Diseases Week, Chicago, May 2009). It has also shown very interesting antagonistic properties against a pathogenic strain of *E. coli* O157:H7 by inhibiting its growth renewal in simulated human gastrointestinal conditions (6).

By definition (1), probiotics need to be alive to exert their beneficial effects in the human digestive tract. Consequently, probiotics that are given orally have to survive gastrointestinal transit and therefore transiently colonize the gut. Their survival rate may depend on how the probiotics are administered, *i.e.* on factors such as food matrix (7–9), galenic form (10–13) and dosage regimen (14,15). *In vivo* studies are too complex and expensive to be used in screening experiments aimed at evaluating the influence of these biopharmaceutical factors on probiotic viability during transit through the human digestive tract, creating a real need for relevant *in vitro* tests ahead of *in vivo* experiments.

Numerous studies on probiotic survival in the upper digestive tract have been conducted in oversimplified mono-compartmental static *in vitro* systems which involve incubation at low pH or in a medium supplemented with different types of bile (16,17). These models do not adequately represent the sequential stresses to which ingested microorganisms are exposed during *in vivo* transit, which are key parameters in their survival and/or activity. In order to more closely mirror human gastric and small intestinal conditions, several dynamic *in vitro* models have been developed (18–20). Among them, the TNO (Netherlands Organization for Applied Scientific Research) gastro-Intestinal tract Model (TIM) allows the closest simulation of *in vivo* dynamic physiological processes occurring in the human stomach and small intestine lumen (20). The TIM model uses data collected on human volunteers to reproduce the key parameters of gastrointestinal digestion: body temperature, kinetics of gastric and intestinal pH, peristaltic mixing and transport, gastric, biliary and pancreatic secretions, and passive absorption of small molecules and water. It has been validated for various pharmaceutical or microbiological applications (21,22) and has proven useful for studying the survival of probiotics, such as lactic acid bacteria or yeasts (6,10,21,23,24).

Another important feature of the gastrointestinal transit of probiotics is their ability to persist in the gut and their interaction with intestinal microbiota. Few *in vitro* studies have investigated the behavior of probiotic bacteria in human large intestinal conditions (9,25–27), and none with probiotic yeast. Here, we use a new *in vitro* model of the human gut, ARCOL (for "ARtificial COLon"), adapted from the model described by Gérard-Champod *et al.* (28). ARCOL is a one-stage fermentation system that integrates the main parameters of *in vivo* fermentation in the human large intestine, such as pH, temperature, anaerobiosis (which is maintained, like *in vivo*, by the only activity of the microbiota – no addition of N_2 or CO_2 as usually done in other colonic models -), supply of simulated ileal effluents, presence of a complex, high-density, metabolically-active microbiota of human origin (fecal microbiota as a commonly used model of colonic microbiota), and passive absorption of water and microbial metabolites.

The study reported here aimed to use the two complementary *in vitro* models TIM and ARCOL to investigate the influence of the main biopharmaceutical factors, *i.e.* food matrix, galenic form, dose and mode and frequency of administration, on the survival of *S. cerevisiae* CNCM I-3856 during gastrointestinal transit in humans.

MATERIALS AND METHODS

Materials

S. cerevisiae CNCM I-3856 (Lynside Pro GI+) was obtained from Lesaffre Human Care (Milwaukee, USA) in its active dried powder form. Dicalcium phosphate dihydrate was purchased from Budenheim (Budenheim, Germany), magnesium carbonate was from Particle Dynamics (St. Louis, MO, USA), and magnesium stearate was from Peter Greven (Venlo, The Netherlands). Hydroxypropylmethylcellulose (HPMC) capsules were provided by Suheung capsule (Seoul, Korea). Lipase was supplied by Amano Pharmaceuticals (Nagoya, Japan), potato starch by Roquette (Lestrem, France), microcrystalline cellulose by JRS Pharma (Vivapur®, Rosenberg, Germany), and guar and arabic gums by Cooper (Melun, France). Sabouraud Dextrose Agar was obtained from BD-Difco (Pont-de-Claix, France). All other chemicals or media used for artificial digestions and fermentations were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France).

Preparation and Characterization of Oral Dosage Forms

Tablet Formulation

Tablets were prepared by direct compression using a lab-scale rotary tablet press (Rimek, Karnavati Engineering, Ahmedabad, India) connected to a computerized compression force analyzer, under constant environmental conditions (35% RH, 20–22°C). Tablets were compressed using 10-mm

round, normal-concave tooling. Each tablet contained 31% (w/w) yeast (corresponding to 155 mg of yeast), 31% magnesium carbonate, 37% dicalcium phosphate dehydrate, and 1% magnesium stearate as lubricant. The total weight of one tablet was 500 mg. Resistance to crushing, friability and tablet disintegration were tested in accordance with European Pharmacopoeia (5th edition).

In order to evaluate the influence of compression force on yeast viability, a tablet was suspended in 200 ml of a solution containing 8.5 g/l NaCl and 1 g/l Tween 80. The suspension was magnetically stirred for 30 min (37°C, 80 rpm), then blended for 90 s using a paddle blender (Interscience, Saint-Nom-la-Bretèche, France). Viable yeasts were enumerated using the plate culture method described below. Experiments were carried out in triplicate.

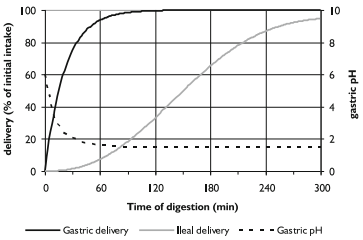
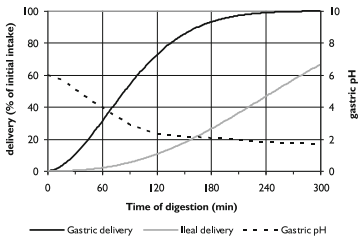
Capsule Formulation

Size-1 HPMC capsules were filled with yeast powder (without excipient) using a lab-scale Feton apparatus (Brussel, Belgium). Content weight was 400 mg. The viable yeast content in one capsule was determined as described above for a tablet ($n=3$).

Digestions in the TIM Gastric and Small Intestinal System

In vitro digestions were performed in the TIM dynamic gastric and small intestinal system (TNO, Zeist, The Netherlands), in which four serial compartments simulate the stomach and the three segments of the small intestine, *i.e.* duodenum, jejunum and ileum. This model has already been described elsewhere (20,24). Here, the TIM system was

Table I Parameters of the TIM System When Simulating Digestive Conditions of a Healthy Adult After Intake of a Glass of Water (A, "fasted state") or a Solid Meal (B, "fed state")[a]

Parameters of *in vitro* digestion	Glass of water (A)	Solid meal (B)
		
Gastric compartment		
pH	2 at T0, then from 6 (5 min) to 1.5 (90 to 300 min)	2 at T0, then from 6 (5 min) to 1.7 (300 min)
Volume	200 mL (at initial time)	300 mL (at initial time)
Secretions	130 U/min of pepsin 5 U/min of lipase HCl 0.3 M if necessary	520 U/min of pepsin 20 U/min of lipase HCl 2 M if necessary
Time of half emptying	$t_{1/2}$ = 15 min	$t_{1/2}$ = 85 min
β coefficient	β=1	β=1.8
Duodenal compartment		
pH	maintained at 6.4	maintained at 6
Volume	30 mL	30 mL
Secretions	20 mg/min of bile salts during the first 30 min of digestion then 10 mg/min 20 mg/min of pancreatic juice 4 USP $NaHCO_3$ 0.5 M if necessary	20 mg/min of bile salts during the first 30 min of digestion then 10 mg/min 80 mg/min of pancreatic juice 4 USP $NaHCO_3$ 1 M if necessary
Jejunal compartment		
pH	maintained at 6.9	maintained at 6.8
Volume	115 mL	125 mL
Secretions	$NaHCO_3$ 0.5 M if necessary	$NaHCO_3$ 1 M if necessary
Ileal compartment		
pH	maintained at 7.2	maintained at 7.2
Volume	115 mL	125 mL
Secretions	$NaHCO_3$ 0.5 M if necessary	$NaHCO_3$ 1 M if necessary
Time of half emptying	$t_{1/2}$ = 150 min	$t_{1/2}$ = 250 min
β coefficient	β=2.4	β=2.5

[a] A power exponential equation ($f = 1\text{-}2^{-(t/t1/2)\beta}$ where f represents the fraction of meal delivered, t the time of delivery, $t_{1/2}$ the half-time of delivery, and β a coefficient describing the shape of the curve) was used for the computer control of gastric and ileal deliveries in the TIM.

programmed with *in vivo* data to reproduce the gastrointestinal conditions of healthy adults after intake of a glass of water or a solid meal (Table 1). The glass of water consisted of 200 ml of mineral water (Volvic®, Volvic, France). The solid meal was made of 19 g mixed diced vegetables, 1.5 g salad dressing, 2 g sunflower oil, 25 g cooked ground beef (15% fat), 8 g instant mashed potato, 17.5 g UHT full-cream milk, 4 g cream cheese, 25 g apple-sauce and 19 g sliced white bread. All the ingredients were purchased from a local store. The volume of the meal was adjusted to 300 ml with mineral water, before homogenization for 20 min with an Ultra Turrax system (T25, IKA®, Werke, Staufen, Germany), set at 24,000 rpm.

Four series of digestions were performed: (a) water with a capsule containing 400 mg of *S. cerevisiae* CNCM I-3856 ($n=3$), (b) a similar capsule with a solid meal ($n=3$), (c) water with a tablet containing 155 mg of *S. cerevisiae* CNCM I-3856 ($n=3$), and (d) a similar tablet with a solid meal ($n=3$). To evaluate the yeast survival rates in the TIM, control digestions were carried out in the same experimental conditions ($n=3$ with liquid meal parameters, $n=3$ with solid meal parameters) with water containing 0.8% (w/v) of a non-absorbable water-soluble marker: blue dextran (20).

Before each experiment, the system was washed with detergent and sterilized by steaming at 105°C for 45 min to avoid microbial contamination. Total duration of each digestion was 300 min. Samples were taken in the test meal prior to its introduction into the stomach compartment of the TIM (initial intake) and regularly collected during digestion in the stomach, duodenum, jejunum, ileum and ileal deliveries. Ileal effluents were collected on ice and pooled at 0–60, 60–120, 120–180, 180–240 and 240–300 min.

Fermentations in the ARCOL Artificial Colon

A 2-l bioreactor (Applikon, Schiedam, The Netherlands) equipped with various ports and probes was used in semi-continuous conditions to simulate the human colonic environment. Fresh feces from a healthy volunteer were used to prepare the inoculum under strict anaerobic conditions in a vinyl anaerobic chamber (Coy, Grass Lake, MI, USA). Stools (~50 g) were mixed with 350 ml of a 200 mM sodium phosphate buffer and filtered through a double layer of gauze. The fecal suspension was rapidly transferred to the bioreactor, flushed with O_2-free N_2 gas, and brought to 450 ml with culture medium. The culture medium, which was sequentially introduced into the bioreactor (15 ml/h), contained various carbohydrate, protein, lipid, mineral and vitamin sources, as previously described by Gérard-Champod *et al.* (28). During fermentation, the fermentation medium was continuously stirred at 400 rpm, pH was kept at a constant 6.0 by adding NaOH 3 M, and temperature was held at 37°C. The atmospheric phase was also continuously homogenized and maintained at an overpressure of 0.1 bar. The fermentative process allowed the maintenance of anaerobic conditions in the bioreactor, with the initial sparging with O_2-free N_2 gas being stopped after inoculation. Fermentation medium was sequentially (15 ml/2 h) withdrawn from the bioreactor. A dialysis system using hollow fiber membranes (cut-off 30,000 Da) maintained the appropriate electrolyte and metabolite concentrations and the operating volume.

Two series of experiments were designed to investigate *S. cerevisiae* CNCM I-3856 survival in the colonic environment under various administration conditions. Each experiment was started 3 days after bioreactor inoculation (stabilization phase). The stabilization phase was checked by microbial (numeration of the main cultivable populations of the microbiota) and metabolic (dosage of gases and short chain fatty acids) analyses followed by comparison with *in vivo* data (data not shown). In a first series of three experiments (one replicate for each condition), 0.4, 4 or 20 g of yeast powder was introduced into the reactor by pulse delivery, once a day for 5 days. In a second series of two experiments (one replicate for each condition), 0.4 g of yeast suspended in a sterile saline solution (NaCl 9 g/l) was continuously introduced over a 5-h period, once or twice a day for 5 days. Samples of fermentative medium were regularly taken to count viable yeasts.

Determination of Viable Yeasts

The viability of the yeasts was evaluated after serial 10-fold dilutions using a plate culture method with Sabouraud Dextrose Agar supplemented with 50 mg/l chloramphenicol and 10 mg/l gentamicin. Petri dishes were incubated at 30°C for 48 h, and the number of viable yeasts was determined by visual counting. Results were expressed as survival percentages (effect of formulation or transit through the TIM) or in $\log_{10}$ colony forming units (cfu) of yeast/ml of fermentative medium (survival in the ARCOL).

Blue Dextran Analysis

The concentrations of blue dextran (used as a transit marker) in the digestive samples from the TIM system were determined colorimetrically using a spectrophotometer (DU® 640 B Spectrophotometer, Beckman Coulter, Villepinte, France) at $\lambda=595$ nm. Results were expressed as percentages of initial intake.

Data Analysis

Values are given as means ± standard deviations. Significant differences between treatments were tested by ANOVA with repeated-measures analysis followed by a post hoc test. All analyses were performed using the SAS 9.1 software (SAS Institute, Inc., Cary, NC, USA). A probability level of $P<0.05$ was considered to be statistically different.

RESULTS

Effect of Formulation on Yeast Viability

The tablets were prepared with a hardness of 79 ± 10 N and friability was conform to European Pharmacopoeia requirements, with a loss of mass of 0.2%. Tablet disintegration time was about 18 min.

The initial number of viable *S. cerevisiae* CNCM I-3856 in the active dry powder used to prepare capsules and tablets was $7.3 \pm 2.9 \times 10^9$ cfu/g ($n=3$), corresponding in theory to 11.3×10^8 cfu per tablet (155 mg of yeast) and 2.9×10^9 cfu per capsule (400 mg of yeast). After compression, the viable yeast content in tablets was $3.7 \pm 0.3 \times 10^8$ cfu ($n=3$). Therefore, the compression significantly decreased viable yeast count, with a 70% loss compared to the initial number of cells ($P<0.05$). In contrast, viability was not altered by filling into capsules, with one capsule containing $2.9 \pm 0.7 \times 10^9$ cfu of yeast ($n=3$).

Yeast Release and Viability in the TIM

The release and survival (cell death or growth) of *S. cerevisiae* CNCM I-3658 during gastrointestinal transit through the TIM were followed by cross-comparing the results obtained for yeast numerations and blue dextran concentrations. The blue dextran is a transit marker which is not degraded or absorbed during digestion. Its concentrations throughout the TIM will fluctuate depending on the volume of each digestive compartment, the rate of dilution by digestive secretions and the chyme flow between two successive compartments.

The cumulative ileal deliveries of viable yeasts are given in Fig. 1. When the probiotic was administered in a capsule with a glass of water (Fig. 1a), yeast recovery followed a similar trend to the transit marker, leading to a survival rate at the end of digestion (Tf) close to 100% (94.8±4.9%, $n=3$). In contrast, when the probiotic was delivered in tablet format (Fig. 1a), yeast recovery was much lower ($P<0.05$) than transit marker recovery, suggesting that yeast cells were killed during transit through the TIM. At the end of digestion (Tf), only 30.1±3.0% ($n=3$) of the initial yeasts were recovered in the digestive medium. When the probiotic was administered in a capsule in a complex food matrix (Fig. 1b), the yeast profile followed the marker curve until 180 min of digestion, then surpassed it, suggesting that cell division occurred during gastrointestinal transit. At Tf, the number of yeasts was almost twice the number initially introduced in the stomach at the beginning of digestion (181.8±4.2%, $n=3$). When delivered in tablet format (Fig. 1b), yeast recovery remained significantly ($P<0.05$) lower than transit marker recovery throughout the digestion period, leading to a 68.8±18.4% ($n=3$) survival rate at the end of the experiment. Overall, whatever the food matrix (water or solid meal),

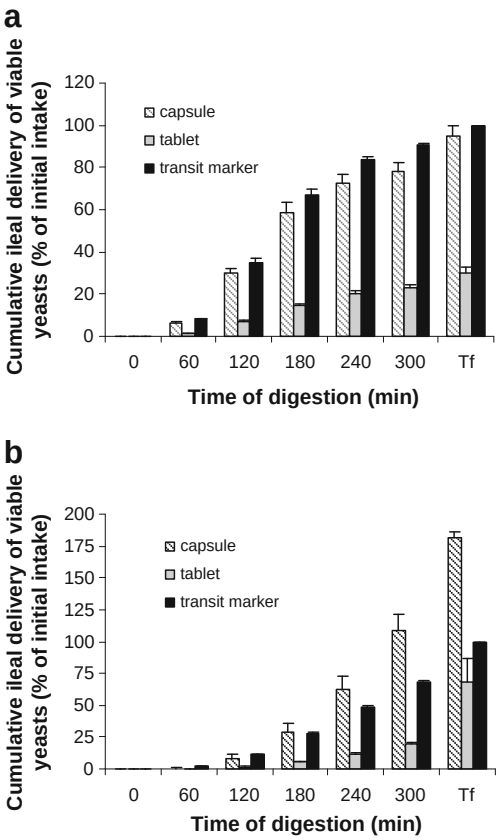


Fig. 1 Effect of galenic form and food matrix on yeast survival rate in the TIM. Yeasts in capsule or in tablet form were introduced into the *in vitro* system in a glass of water (**a**, "fasted state") or in a solid meal (**b**, "fed state"). The cumulative ileal delivery of viable yeasts was plotted and compared to that of the transit marker blue dextran. At the end of digestion, the values obtained in the ileal effluents (0–300 min) and the gastrointestinal residue were added (Tf). Values are expressed as mean percentages ± standard deviations ($n=3$) of the initial intake.

yeast viability throughout digestion was significantly ($P<0.05$) higher when the probiotic was administered in capsule format than in tablet format. Moreover, whatever the galenic form (capsule or tablet), survival percentages at Tf were significantly higher when the yeasts were delivered in a solid meal than in a glass of water ($P<0.05$).

The yeast survival percentages in each digestive compartment of the TIM are presented in Fig. 2 (glass of water, $n=3$) and 3 (solid meal, $n=3$) and compared to the marker plotting. As the transit marker was non-formulated when introduced in the system, it was fully available at the initial time of digestion in the stomach (100% recovery at t0). In the stomach (Fig. 2a), viable yeasts were counted as early as 10 min after the beginning of digestion, indicating that both formulations quickly began disintegrating in the gastric environment when administered in a glass of water. The curve for yeast administered in the capsule was above that of the transit marker before surpassing it, indicating that this galenic form delayed yeast release in the gastric compartment. Conversely, the curve for yeast administered

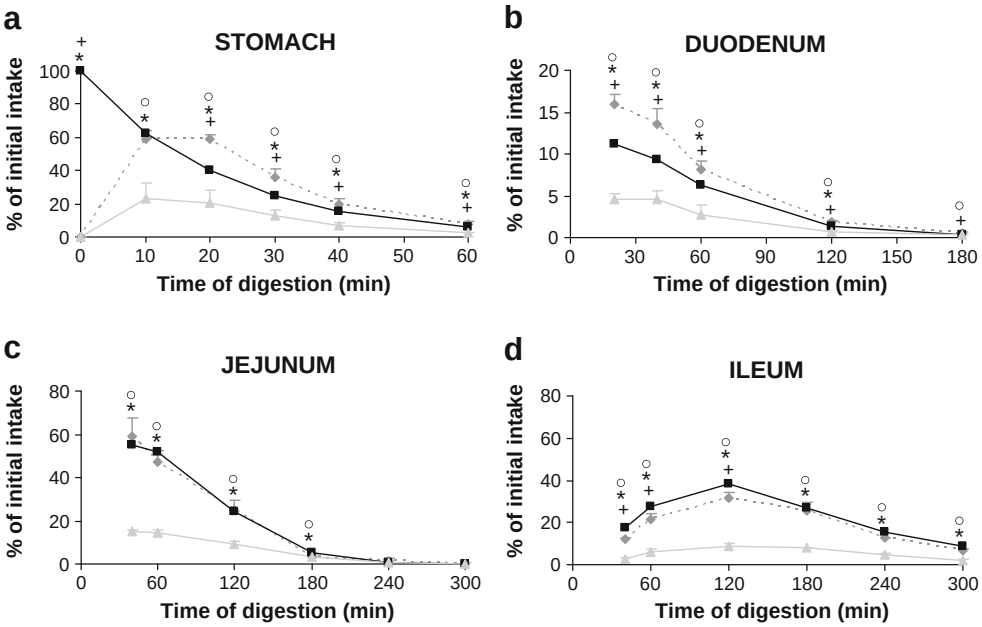


Fig. 2 Yeast viability in the artificial stomach (**a**), duodenum (**b**), jejunum (**c**) and ileum (**d**) of the TIM system, after ingestion with a glass of water ("fasted state"). Recovery profiles of yeast delivered in capsules (in grey, dotted line) or in tablets (in grey, solid line) were compared to the recovery profile of the transit marker bleu dextran (in black). Values are expressed as mean percentages ± standard deviations ($n = 3$) of the initial intake. Yeast in capsules (+) or in tablets (*) showing significantly different patterns to the transit marker ($P < 0.05$). Yeast in capsules showing significantly different patterns to yeast in tablets at $P < 0.05$ (○).

in the tablet remained below that of the marker ($P < 0.05$), suggesting that either yeasts were rapidly killed in the stomach or that the tablet was not fully disintegrated. When the probiotic was delivered in a complex food matrix, similar trends (than in water) were observed in the stomach (Fig. 3a). Nevertheless, the delay in yeast release with the capsule format was more pronounced than previously (with water), and was also reproduced by the tablet format. We found strong variability for yeast at 30 min digestion, suggesting that mixing of the stomach content was more difficult to achieve if the food matrix was present. In the jejunum and ileum compartments of the TIM model (Fig. 2c and d, with water), yeast profiles matched the marker profile when the probiotic was administered in capsules but were below it when the probiotic was administered in tablets ($P < 0.05$). These results confirmed those previously obtained in the ileal effluents of the model (Fig. 1a), *i.e.*, that cell mortality occurred during digestion when a tablet was used, but not when a capsule was used. Regardless of galenic form, when the probiotic was administered within a solid meal (Fig. 3b, c and d), there was a delay in yeast release in the duodenum, jejunum and ileum. For example, in the jejunum (Fig. 3c), survival percentages were maximum at 180 min for capsules and

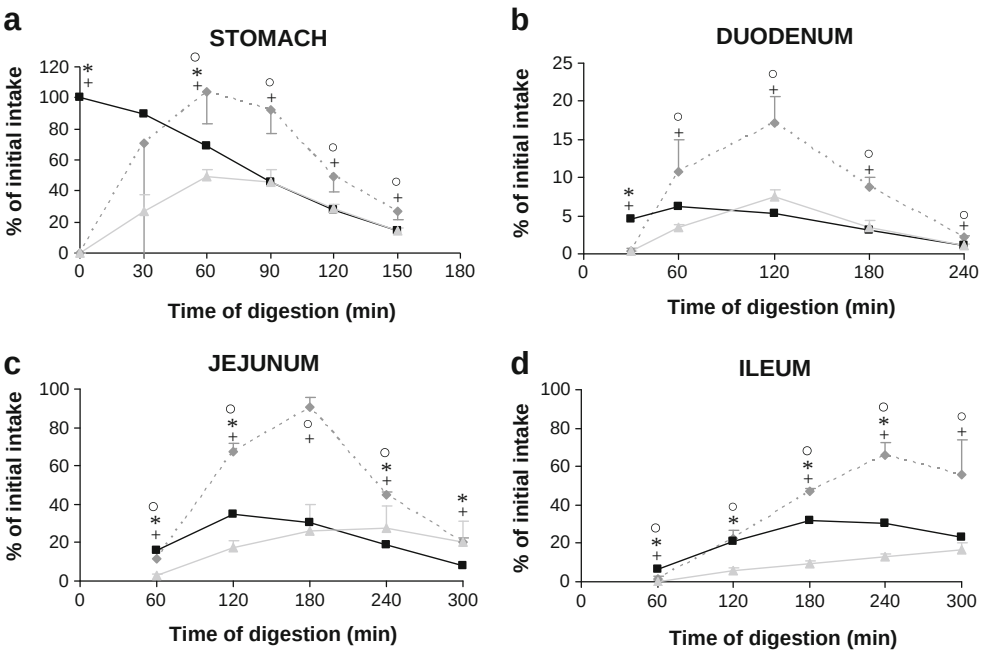


Fig. 3 Yeast viability in the artificial stomach (**a**), duodenum (**b**), jejunum (**c**) and ileum (**d**) of the TIM system, after ingestion with a solid meal ("fed state"). Recovery profiles of yeast delivered in capsules (in grey, dotted line) or in tablets (in grey, solid line) were compared to the recovery profile of the transit marker blue dextran (in black). Values are expressed as mean percentages ± standard deviations ($n = 3$) of the initial intake. Yeast in capsules (+) or in tablets (*) showing significantly different patterns to the transit marker ($P < 0.05$). Yeast in capsules showing significantly different patterns to yeast in tablets at $P < 0.05$ (○).

240 min for tablets *vs* 120 min for the transit marker. The results obtained in each compartment of the TIM with the solid meal also corroborated those observed in the ileal effluents of the system (Fig. 1b). In particular, the recovery profiles for yeast in capsules surpassed the marker recovery profile in all compartments ($P<0.05$), suggesting that cell multiplication occurred during digestion.

Yeast Viability in the ARCOL

Figure 4 shows the concentrations of viable yeasts in the ARCOL following daily pulse administration of three different doses (0.4, 4 or 20 g, $n=1$). Profiles obtained with the three doses followed similar trends. Following each injection, viable yeast concentrations immediately reached peak levels, ranging from 10^6 to 10^9 cfu/ml depending on the dose. Whatever the dose, these levels were not maintained in the fermentative medium, where yeast concentrations decreased more or less rapidly as soon as the administrations were stopped. Few if any living yeasts (10^2–10^3 cfu/ml) were detected in the bioreactor 24 h after each injection.

Figure 5 illustrates the effect of mode and administration frequency on probiotic yeast survival in the ARCOL. When 0.4 g of yeast was continuously injected once a day over a 5-h period (Fig. 5a), high concentrations (from 10^6 to 10^7 cfu/ml) were rapidly reached in the bioreactor and maintained until administration was stopped. Afterwards, the probiotic was quickly cleared from the fermentor, except for the first injection (0–5 h) where concentrations higher than 10^2 cfu/ml were maintained for 19 h. Similar trends were observed when 0.4 g of yeast was injected twice a day over a 5-h period (Fig. 5b). High cellular concentrations (10^6 cfu/ml) were maintained in the fermentative medium between the first two injections. Afterwards, concentrations only remained maximal during the administration period (5 h) but rapidly decreased when the injections were stopped, with yeasts generally disappearing from the colonic medium between two consecutive additions.

DISCUSSION

Survival in the human gastrointestinal tract is a key feature for probiotic strains. It has been shown that several of the beneficial effects of *S. boulardii* are dependent on the viable yeast concentration in the digestive tract (29,30). However, due to the cost and complexity of *in vivo* studies, there is little available on the behavior of probiotic microorganisms in the human digestive environment. Furthermore, the issue is made more complex by the fact that their survival is broadly dependent on how they are administered. In this study, we assessed the influence of the main biopharmaceutical factors on the survival of *S. cerevisiae* CNCM I-3856 in simulated human digestive conditions. This is the first study to explore the behavior of a probiotic yeast strain throughout the full gastrointestinal tract *in vitro* and to establish the influence of dosage form, food matrix, and dose regimen.

The first objective of the present study was to evaluate the effect of galenic formulation on yeast viability during gastrointestinal transit. Two dosage forms were made and compared: tablets formulated by direct compression and HPMC capsules. Both are common dosage forms chosen for their ease of administration, good patient acceptance, and suitability for large-scale production. Several compression forces were tested to achieve tablet formulation (data not shown), but only the highest (79 N) made it possible to obtain tablets conform to the European Pharmacopoeia friability requirements. With such experimental conditions, a 70% loss of viability was observed for *S. cerevisiae* CNCM I-3856. These results corroborate Graff *et al.* (11) who reported an 80% loss of viability for *S. boulardii* when formulated in HPMC tablets with similar compression forces. Direct compression was less effective than encapsulation (which preserves the 100% viability of yeast) in terms of loading of the dosage form with viable yeast cells. This phenomenon was amplified by the lower resistance of yeast to gastric and small intestinal conditions when administered in tablet form compared to capsule form. The ileal effluents of the TIM were treated (blending with Tween 80) to make sure that the loss of

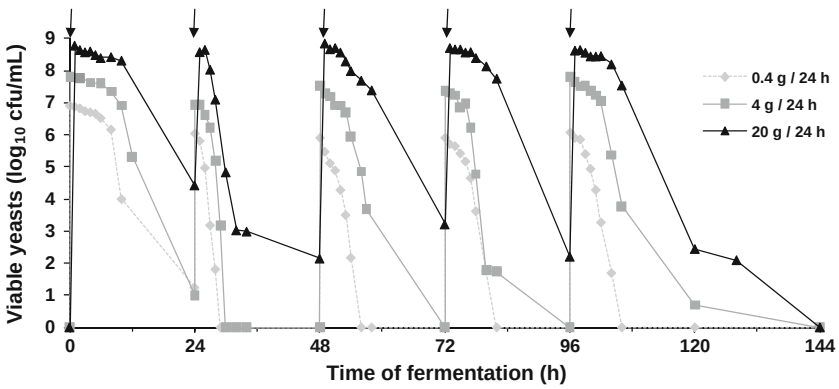


Fig. 4 Dose effect on yeast survival rate in the ARCOL. 0.4, 4 or 20 g of yeast powder was added by pulse to the bioreactor once a day for 5 days. Experiments started (T0) after 3-days stabilization phase. Viable yeasts were regularly counted during fermentation. Values are expressed as $\log_{10}$ cfu/ml of fermentative medium ($n=1$).

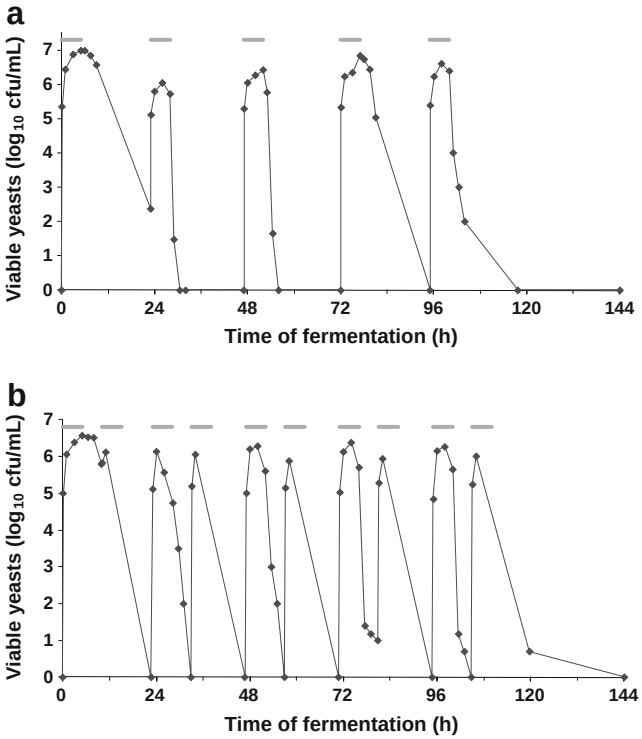


Fig. 5 Effect of administration frequency on yeast survival rate in the ARCOL. 0.4 g of yeast suspended in sterile physiological water was continuously added to the bioreactor over a 5-h period once (**a**) or twice (**b**) a day for 5 days. Experiments started (T0) after a 3-days stabilization phase. Viable yeasts were regularly counted during fermentation. Values are expressed as $\log_{10}$ cfu/ml of fermentative medium ($n = 1$).

viability observed in the *in vitro* system resulted from cell death and not from an incomplete dispersion of compressed yeast. The compression force, by altering cell components of the yeast, should have rendered it more sensitive to the harsh physicochemical conditions of the upper digestive tract, such as acidic pH and digestive secretions. Nevertheless, we showed that there is no effect of transit through the TIM on the growth capability of yeasts (data not shown), which tends to indicate that the probiotic is not too stressed in human gastric and small intestinal conditions (and therefore may still perform its activity).

Another important feature is the influence of food intake on probiotic release and survival in the human gastrointestinal tract. Until now, no *in vitro* or *in vivo* studies comparing the viability of probiotic yeast between fasted or fed state have been available. Here, we performed this comparison by exploiting the potentialities of the TIM system. The model uses *in vivo* data in healthy adults (31–34) to factor in the main digestive parameters influenced by food intake, *i.e.* drop in gastric pH, half-time of gastric and ileal deliveries, time of transit, and luminal concentrations of digestive secretions. To closely mimic the physiological conditions of the fed state, the probiotic yeasts were administered in a typical western meal. Whatever the galenic form, yeast survival rate after gastrointestinal transit was higher when a food matrix was added. This may be explained by the buffering capacity of the meal and/or its high content in ingredients with protective properties, such as the lipid fraction (35). Another explanation could be that food provides the ability of forming niches in which less harsh conditions (less acidic) prevail. A similar protective effect of food has previously been reported for lactic acid bacteria, *e.g.* with milk and *Lactobacillus rhamnosus* GG (7), sausage and *L. plantarum* (8) or chocolate and *L. helveticus* or *Bifidobacterium longum* (9). Moreover, for the first time, we showed that a probiotic yeast was able to multiply (one cell division) during its transit in the upper part of the digestive tract, when administered within a food matrix. This is of particular interest because it means that this strain would be delivered to the intestine in higher amounts than those ingested. The presence of the food matrix also led to a delay in yeast release from both formulations. This may be explained by a lower efficiency in water uptake in the fed state, resulting in a slower swelling of capsules and tablets.

Once the gastric and small intestinal barriers are crossed, the probiotics have to succeed in competing with the resident colonic microbiota. *S. cerevisiae* CNCM I-3856 was added to the ARCOL in non-formulated form, as occurs in the human large intestine. The first dose administered (0.4 g) was chosen to fit the dose brought by a capsule, which, according to the results in the TIM, emerges as the best formulation. Between two consecutive inoculations, yeasts were rapidly eliminated from the fermentative medium (in less than 12 h). A 10-fold increase in the amount of yeast delivered (4 g) only slightly improved its survival in the bioreactor. The only dosage (20 g) which allowed the maintenance of the probiotic between two injections (at levels close to 10^3 cfu/ml) was barely compatible with human use. These results suggest that *S. cerevisiae* CNCM I-3856 was strongly affected by the colonic conditions. This extensive elimination of yeasts may result from the "barrier" effect of the endogenous microbiota, as already observed in mice (36) and in humans (37) for *S. boulardii*. Moreover, yeast cell-wall polysaccharide glucans and mannans can be hydrolyzed by intestinal bacterial enzymes, such as the β 1–3 glucanases produced by *Bacteroides* (38). In a second series of experiments in the ARCOL, the probiotic (0.4 g) was continuously administered over a 5-h period to get nearer to physiological conditions where the ileal effluents are regularly flowing into the large intestine. High yeast concentrations (up to 5×10^6 cfu/ml) were obtained, but only during the administration periods. Consequently, twice a day administration appears as the best way to maintain high levels of probiotics in the human large intestine.

Few studies have evaluated probiotic yeast survival in the human gastrointestinal tract. Furthermore, survival rates

have only been evaluated in feces and not throughout the length of the digestive tract. The available data report fecal recoveries of viable yeasts after single or multiple administrations of dried *S. boulardii* (14,29,37) or *S. cerevisiae* (39). The results obtained in human volunteers corroborate those obtained in the present *in vitro* study. Indeed, much of the oral dose was destroyed, leading to less than a 1% survival rate in feces, and the yeasts were rapidly cleared when the administrations were stopped. A half-life of 6 h was determined *in vivo* for *S. boulardii* (14), which is close to that observed in the ARCOL for *S. cerevisiae* CNCM I-3856. In addition, our results suggest that the major barrier to the survival of ingested live yeasts in humans is not the acidic gastric environment, as previously suggested for *S. boulardii* (40,41), but rather the conditions found in the large intestine.

CONCLUSION

Gastrointestinal models, such as the TIM or the ARCOL, can provide valuable information during the development of a probiotic product. They are particularly relevant for screening purposes, such as for studying the effects of biopharmaceutical factors (such as dosage form, food matrix and dose regimen) on the viability of probiotic strains throughout the human digestive tract. Our results indicate that giving *S. cerevisiae* CNCM I-3856 twice a day, in capsule form and with a food matrix, would increase the percentages of living cells within the human intestine. These results may help to potentiate the therapeutic benefits of this new probiotic yeast, such as its antimicrobial activity against pathogenic bacteria (6).

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