
Kinetic of Transit of a *Saccharomyces Cerevisiae* Probiotic Strain along Gastrointestinal Tract of Cannulated Healthy Pigs

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Abstract: Probiotic yeasts display several dose-dependent actions in gut but little is known about their survival and transit across gastro-intestinal tract. Therefore we have analyzed the kinetic of transit of one probiotic *S. cerevisiae* strain.

Cannulated and sequentially-sacrificed pigs received diets containing 0, 10⁶, 10⁷ or 10⁸ CFU/g of feed of a *S. cerevisiae* probiotic (Actisaf, Lesaffre, France). Yeasts were counted at 0h, 3h, 6h, 9h, 12h, 24h, then every 24h until 120h. Data were analyzed using the GLM procedure for repeated measures. The 2-cannula system was validated by comparison with slaughtered animals and used for the rest of the study.

In ileum, Log₁₀ CFU/g increased from t0 to t6 with 4.86±0.05 for 10⁶ (P<0.05), 5.48±0.23 for 10⁷ (P<0.05) and 6.52±0.11 for 10⁸ (P<0.1). Then significant decrease and stabilization occurred to 3.16±0.19 from t24 to t120 for 10⁶, 2.33±0.25 from t72 to t120 for 10⁷ (P<0.05 with t6) and 6.16±0.11 until t120 for 10⁸ (P>0.1 with t6). Similar pattern with lower CFUs was observed in colon.

Present study demonstrated survival of the probiotic yeast towards GIT conditions. Increasing the doses resulted in increased maximum CFU counts and duration of their maintenance in the different GIT sections without changing transit time.

Keywords: *Saccharomyces cerevisiae*, probiotic, weaned pigs, gut transit, cannula

1. INTRODUCTION

Probiotics, defined by WHO as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”, have been used for decades in Human to improve gut microflora stability (Fooks and Gibson, 2002) and to avoid dysbiosis during stresses or pathogenic challenges such as *C. difficile* (Friedman, 2012). Recently, various probiotic bacteria strains belonging to *Bacillus cereus* var. *toyoi* (Taras et al., 2005), *Enterococcus faecium* (Zeyner and Boldt, 2006), *Lactobacillus sobrius* (Konstantinov et al., 2008), *Pediococcus acidilactici* (Di Giancamillo et al., 2008) and yeast strains belonging to *Saccharomyces cerevisiae* (Jurgens et al., 1997; Li et al., 2006) and *Saccharomyces cerevisiae* var. *boulardii* (Collier et al., 2011) have been applied successfully to improve health and zootechnical performances in animals such as pigs (Kenny et al., 2011). Focusing on yeast probiotics in pigs, recent works on an industrial strain of *S. cerevisiae* (CNCM I-4407) have confirmed an ability to improve transfer of immunoglobulins from sows to their piglets (Jang et al., 2013; Zanello et al., 2013) to modulate microflora *in vitro* (Pinloche et al., 2012) or *in vivo* (van Heugten et al., 2003) and to have protective effects against *E. coli*-induced inflammation *in vitro* (Zanello et al., 2011a; Zanello et al., 2011b) and *in vivo* (Trckova et al., 2014; Trevisi et al., 2015). These effects are generally proportional to the number of live cells (Colony Forming Units, CFU) used *in vitro* or fed to the animals. However, despite numerous studies on the effects of probiotics in humans or animals, little is known on the precise survival and kinetic of transit of yeast probiotic along gastrointestinal tract of mammals. Only few works have been conducted on probiotic strains such as *Bifidobacterium lactis* in humans (Waller et al., 2011), *Saccharomyces cerevisiae* var. *boulardii* in humans, mice (Blehaut et al., 1989) and lambs (Durand-Chaucheyras et al., 1998) or *Lactobacillus casei* (Ohashi et al., 2004) and *Enterococcus faecium* (Macha et al., 2004) in swine. Currently, no work reported the survival and transit time of a *Saccharomyces cerevisiae* probiotic strain in small intestine and colon of pigs. Nevertheless, demonstration of the ability of any probiotic to remain alive up to the more distal parts of the digestive tract of its host is absolutely required to transpose the *in vitro* results to an *in vivo* prediction of the potential efficacy of a probiotic microorganism.

The present work aimed to describe the kinetic of transit and survival rates of a probiotic strain *S. cerevisiae* administered continuously in the feed of weaned pigs at different CFU levels along the gastrointestinal tract (GIT). We have developed, validated and used a 2-cannulas model on weaned pigs allowing simultaneous enumeration of CFUs in small intestine, in colon and in feces of the animals along time of administration. We have hypothesized that the dose-dependending results reported *in vitro* and in animals across literature for this *S. cerevisiae* strain will be supported by the demonstration of significant differences of CFU levels in the different sections of the gut, differences in transit time and/or differences in time required to stabilize the CFU counts after starting product administration.

2. MATERIAL AND METHODS

2.1. Animal Feeding and Housing

The experiment was conducted at the VetAgro Sup veterinary school in Lyon, France. Experiments followed the directive n°59/VAL/0411 (28/06/2011) and n°1160 (4/10/2011) of ethical commission for animal welfare and was performed by an accredited staff.

A total of 43 healthy castrated males between 10 and 12 kg body weight from a classical farm with excellent epidemiological status was used. Pigs were housed in group (non-cannulated animals, n=34) or individually (cannulated animals, n=9) and had *ad libitum* access to drinking nipples. Space, temperature, light cycle, aeration and cleaning were in agreement with European recommendations for pig housing.

Animals were fed a high quality classical barley/wheat/soybean post-weaning diet provided by Euronutrition SAS (accreditation alpha FR-72321400) not containing probiotic (feed was controlled for the absence of yeast) or any other feed additive or medicine.

Additionally to the non-supplemented (Control) diet, 3 complementary diets were prepared and only differed from the control diet by the incorporation of the probiotic yeast *S. cerevisiae* CNCM I-4407 (Actisaf, Phileo Lesaffre Animal Care, France) to reach theoretical 1×10^6 (diet S0), 1×10^7 (diet S1) and 1×10^8 (diet S2) CFU/g of feed. Real probiotic concentrations were determined in the different feeds before starting the experiment and reached 1.6×10^6 for S0, 6.6×10^7 for S1 and 1.1×10^8 for S2, close to the expected values and reaching the objective of a 1-log scale difference of CFU rates between the diets.

Feed was administered twice a day (between 8:00 to 9:00 and 18:30 to 20:00) under liquid form at equal feed/water volume. Before any experiment, control diet was given to the pigs for 3 days and between experiments, control diet was given for at least 2 days.

When required by the protocol (validation of the transit kinetic in non-cannulated pigs) or at the end of the experiment (cannulated pigs), animals were humanely killed by intracardiac Pentobarbital injection after anesthesia using Zoletil before sampling the contents of the digestive tract at the different sites of interest and peripheral organs to check the absence of yeast translocation from GIT.

2.2. Cannulation of the Animals

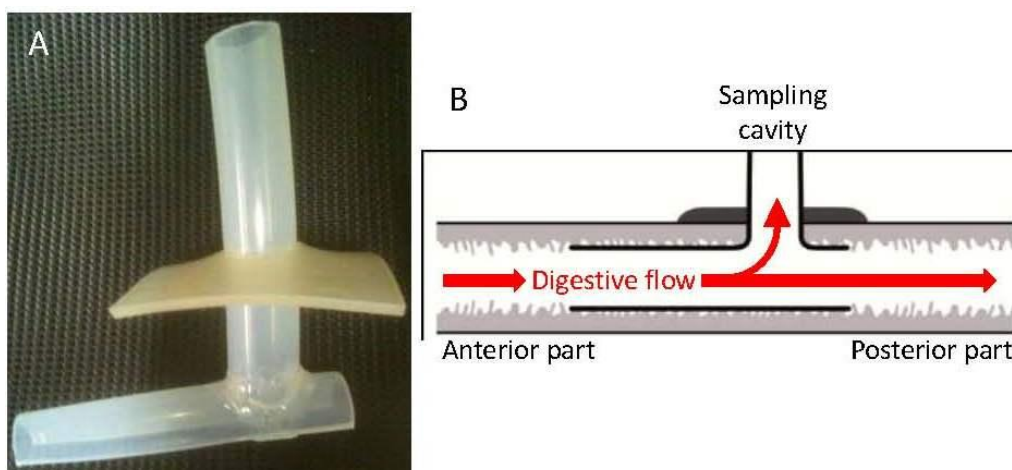


Figure 1. Cannula picture (A) and schematic view of the cannula when placed in ileum or in colon (B).

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Nine animals were cannulated using 2 cannulas/pig, the first cannula was placed in the ileum, the second in the colon. Picture and a schematic view of the cannula on site are provided in Figure 1. Preliminary sedation was performed using 0.6ml of atropine associated with 1ml of azaperon (Stresnil®). Anesthesia was performed 30 min later using 1.5ml of Zoletil 100® (Tiletamine and Zolazepam). Pre- and post-surgery analgesic procedures using 0.2ml of morphine were respected, associated with a fentanyl pain patch for the post-surgery procedure. Cannula in ileum was fitted before colon cannula using classical surgery procedures.

2.3. Yeast Counting

For each sample, 10g of intestinal content were placed in sterile water at 37°C for a total volume of 100ml then incubated at 37°C for 30min. After homogenization, 10-fold serial dilutions were performed in sterile water and incubated for 30min at 37°C. One ml of each dilution was incorporated in YM media containing 0.7% of Oxytetracyclin to prevent growth of bacteria or molds and then laid down on Petri plates following usual laboratory procedures.

Countings were realized after 48h of culture of the samples at 30°C and based on morphological identification of *S. cerevisiae* CNCM I-4407 under binocular magnifier. At the first counts realized for the experiment, molecular identification of the postulated *S. cerevisiae* CNCM I-4407 colonies was performed using specific PCR primers for the strain. Hundred percent of the colonies suspected to be *S. cerevisiae* CNCM I-4407 were identified as the relevant strain. Consequently, following enumerations were only based on the counting on YM plates.

2.4. Statistical Analysis

Data were statistically computed using SPSS 11.5 for Windows. Presence of outliers was tested using Dixon's test. Comparison of the values between doses was made using the GLM procedure for repeated measures.

Differences were considered as a tendency for $P < 0.1$; significant for $P < 0.05$, very significant for $P < 0.01$ and highly significant for $P < 0.001$. Data presented are expressed as mean \pm SEM on a Log₁₀-basis after removal of outliers according to Dixon's test.

3. RESULTS

Exp 1 - Timepoint establishment using fecal CFU counts and final yeast counts in GIT sections:

At their arrival in the experimental facilities, pigs were controlled for wild yeast presence in the feces. Colony Forming Unit counts (Log₁₀ CFU) in the feces were similar between animals with 4.61 \pm 0.38 ($P > 0.05$). After 3 days of control diet feeding, Log₁₀ CFU counts significantly decreased to 3.61 \pm 0.21 ($P < 0.05$), with no significant differences between animals.

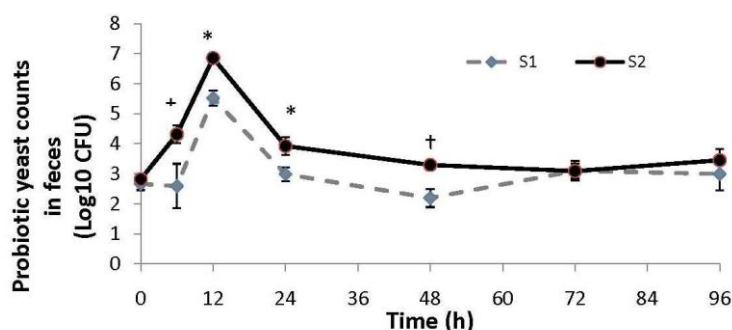


Figure 2. Kinetic of probiotic yeast transit in the feces of weaned pigs. Animals were given *S. cerevisiae* CNCM I-4407 probiotic live yeast in the feed at 10^7 CFU/g of feed (S1) or 10^8 CFU/g of feed (S2) since t_0 for 4 consecutive days. Data presented are means \pm SEM of Log₁₀ CFU. GLM statistical analysis for repeated measures indicated a significant difference between the 2 doses ($P < 0.001$) without interaction. For each time point, additional comparison using T-test was made (\dagger for $P < 0.1$ and $*$ for $P < 0.05$).

Results of CFU counts in the feces of the non-cannulated pigs after administration of S1 for 4 days then S2 diets for 4 days are provided in Figure 2. A significant difference between the 2 doses without interaction with time was observed. Except at t_0 and after t_{72} , tendency at t_6 and t_{48} ($P < 0.1$) and significant differences at t_{12} and t_{24} ($P < 0.05$) were observed with 1 Log₁₀ CFU difference between the 2 supplementation levels. Whatever the dose, maximum Log₁₀ CFU counts were reached at t_{12} , significantly higher than the other time points within each dose ($P < 0.05$). For the S1 dose CFU counts

started to increase from t6. In pigs receiving control diet, wild yeast counts remain low and constant along the time of experiment with 2.47±0.07 Log10 CFU.

After 5 days of administration of the highest yeast dose (S2), animals were sacrificed to enumerate yeast counts in the different section of the GIT. An average of 7.24± 0.15 Log10 CFU in stomach, jejunum and ileum was observed in these sites without significant differences among them. In colon then in feces, a progressive and significant decrease of CFUs was observed (P<0.05, Figure 3). In control animals, wild yeast counts were respectively 1.69 ±/ 0.40^{ab} in stomach, < 1.00^a in jejunum, < 1.00^a in ileum, 2.30 ±/ 0.42^b in colon and 2.42 ±/ 0.42^c in feces (values sharing different superscripts significantly differ at P<0.05).

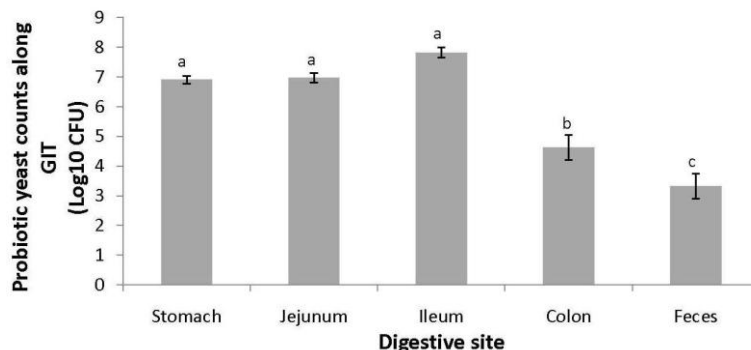


Figure3. Probiotic yeast counts in different sections of the gastrointestinal tract of weaned pigs. Animals were given *S. cerevisiae* CNCM I-4407 probiotic in the feed at 10⁸ CFU/g of feed (S2) for 5 days. After sacrifice, probiotic yeasts were enumerated in stomach, jejunum, ileum, colon and feces. Data presented are means ± SEM of Log10 CFU. GLM statistical analysis confirmed a significant difference of CFU levels across digestive sites (P<0.001). Values sharing different superscripts significantly differ (P<0.05).

Exp 2 – Comparison of transit of live yeast in conventional sequentially-slaughtered and 2-cannulas-equipped pigs

Pigs were controlled at their arrival in the experiment building for wild yeast counts. No significant differences in CFUs were observed between animals and CFUs in the feces were in average 2.53 ±/ 0.15 Log10 CFU.

To establish the kinetic of transit of the probiotic live yeast along GIT of non-cannulated pigs, sequential sacrifice of pigs receiving the dose S1 was performed. Results are presented in Figure 4. In jejunum, higher CFU counts were observed at t3 and t12, significantly higher than t0, t9 and t24 (P<0.05). In ileum, maximum CFU count was observed at t3, and tended to be higher than at t0 (P<0.1). Then intermediary CFU counts were observed until t24. In colon, CFU counts started to increase numerically at t6 and reached a maximum at t9, these 2 time points being significantly different from t24 (P<0.05). Colony forming units at t0 and t3 also tended to be different from t24 (P<0.1).

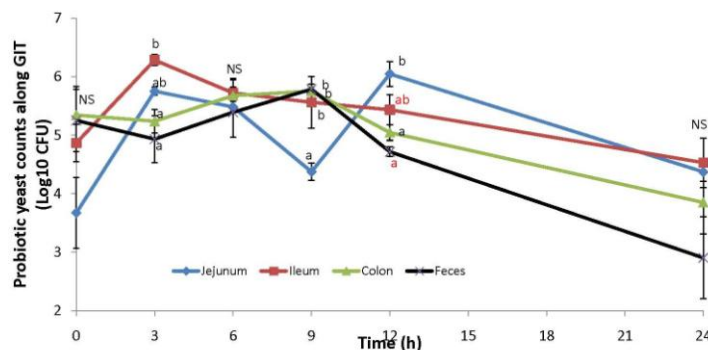


Figure4. Kinetic of probiotic yeast strain in different sections of the gastro intestinal tract. Animals were given *S. cerevisiae* CNCM I-4407 probiotic live yeast in the feed at 10⁷ CFU/g of feed (S1). Sequential sacrifice was performed and yeasts were enumerated in jejunum, ileum and colon on 4 animals/time point. Data presented are means ± SEM of Log10 CFU. GLM statistical analysis for repeated measures indicated a significant difference across time for every site (P<0.001) and interaction with the site (P<0.05). Within each time point, values sharing similar color of the superscript tend to differ (P<0.1) and values sharing different superscripts significantly differ (P<0.05).

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A comparison of the probiotic live kinetic of transit across GIT between sacrificed animals and cannulated animals sampled at the same time points has been conducted to test whether or not the cannula system would alter the parameters of gut transit of the animals. Results for the dose S1 in ileum is provided in Figure 5A. Comparison of the kinetic indicated a maximum CFU count at t3 in non-cannulated animals with 6.28 +/- 0.10 Log₁₀ CFU, tending to be different from t0 with 4.87 +/- 0.32 Log₁₀ CFU ($P < 0.1$) and significantly different from t24 with 4.53 +/- 0.42 Log₁₀ CFU ($P < 0.05$). In cannulated animals maximum CFU counts were observed at t6 with 6.33 +/- 0.05 Log₁₀ CFU, significantly different from t3 with 4.83 +/- 0.56 Log₁₀ CFU ($P < 0.05$), t6 and t3 being also significantly different from t0 with an average of 3.36 +/- 0.46 Log₁₀ CFU ($P < 0.05$). When considering a correction of a 1 time-point delay in the appearance of yeast in the cannulated animals, similar Log₁₀ CFU counts were measured (Figure 5B). In cannulated animals receiving control diet, wild yeast CFU counts remained low and non-significantly different along time, with an average 1.43 +/- 0.20 Log₁₀ CFU during the experimental period.

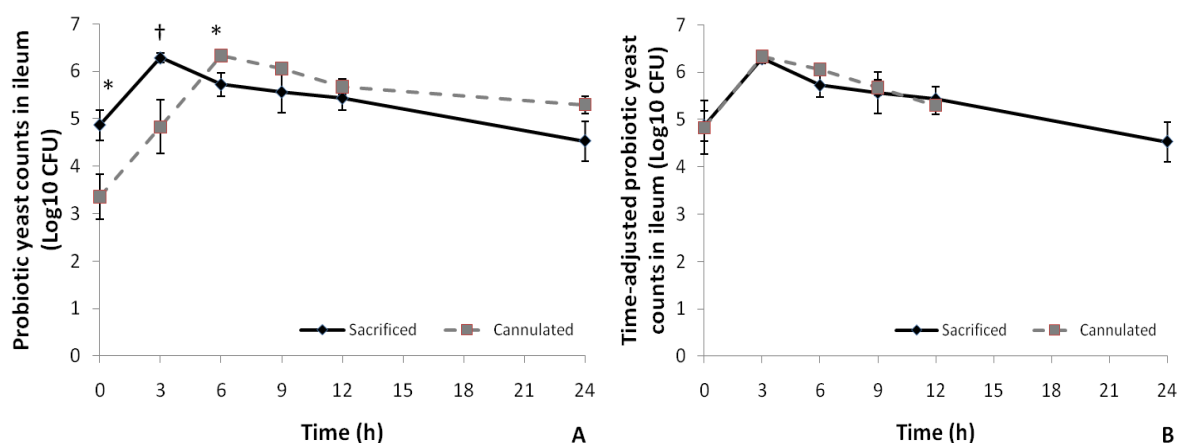


Figure 5. Comparison of kinetic of transit in the ileum of sacrificed non-cannulated pigs and sampled cannulated pigs receiving a probiotic live *S. cerevisiae* CNCM I-4407 probiotic live yeast in the feed at 10^7 CFU/g of feed (S1). Data presented are means +/- SEM of Log₁₀ CFU. GLM statistical analysis for repeated measures indicated a significant interaction between status of the pigs (non-cannulated pigs vs. cannulated pigs) and time ($P < 0.01$) when the 2 profiles were compared (A). Values tended to be different at t3 ($P < 0.1$) and were significantly different at t0 and t6 ($P < 0.05$). However, no significant differences or interaction remained in the kinetic of transit of the live yeast between the non-cannulated and cannulated animals when values for cannulated animals were shifted one time point earlier to correct for progressive digesta accumulation into the cannula (B).

Similar pattern of the kinetic was noticed in colon, with a maximum CFU count observed in non-cannulated animals at t9 with 5.75 +/- 0.12 Log₁₀ CFU, non-significantly different from values observed at t6 with 5.67 +/- 0.27 Log₁₀ CFU and t12 with 5.05 +/- 0.13 Log₁₀ CFU, but tending or being significantly different from values observed at t0, t3 and t24 with an average of 4.81 +/- 0.31 Log₁₀ CFU. In cannulated animals, maximum CFU count was observed at t12 with 6.04 +/- 0.08 Log₁₀ CFU, very close to CFU counts at t9 with 5.87 +/- 0.26 Log₁₀ CFU and t6 with 5.57 +/- 0.12 Log₁₀ CFU, all significantly different from CFU counts at t0 with 3.69 +/- 0.31 Log₁₀ CFU and t3 with 3.25 +/- 0.78 ($P < 0.05$). At t24, counts were intermediary with 4.83 +/- 0.18 Log₁₀ CFU and tended to be different from CFU counts at t0 and t3 ($P < 0.1$). Similarly to ileum, GLM statistical analysis for repeated measures indicated a significant interaction between the non-cannulated vs. cannulated pigs and time ($P < 0.01$) when the 2 profiles were compared. Shifting CFU counts of the cannulated animals one time-point earlier lead to non-significant differences between the 2 profiles of transit in the colon. In cannulated animals receiving control diet, wild yeast CFU counts remained low and non-significantly different, with an average 1.09 +/- 0.09 Log₁₀ CFU during the experimental period.

Exp 3 – Transit of various doses of probiotic yeast in cannulated pigs

Double-cannulated pigs were then used for analysis of CFU counts of the probiotic yeast in ileum and colon and received successively control diet for 3 days, S0 diet for 5 days, control diet for 2 days, S1 diet for 5 days, control diet for 2 days and S2 diet for 5 days.

In ileum for S0 diet, increased Log₁₀ CFU counts were observed at t3 (3.59 +/- 0.39) then t6 (4.86 +/- 0.05), both significantly different from t0. Then intermediary values were observed at t9 (4.00 +/- 0.09) and t12 (3.95 +/- 0.22), and finally a sharp decrease was observed at t24, with non-significantly (except compared with t0, P<0.05 vs. all other timepoints) different stable values until t120 (in average 3.16 +/- 0.19).

In ileum for S1 diet, increase of yeast counts was observed at t3 (5.04 +/- 0.43) then at t6 (5.48 +/- 0.23) with a tendency to be different from t0 (2.86 +/- 1.02 P<0.1). Counts remained therefore stable at t9 (5.20 +/- 0.03), t12 (5.42 +/- 0.30), t24 (5.70 +/- 0.21) and t48 (5.13 +/- 0.59) and non-significantly different among them. Then a significant decrease (P<0.05) and stabilization occurred from t72 with an average 2.33 +/- 0.25 Log₁₀ CFU.

In ileum for S2 diet, significant increase of yeast counts was observed (P<0.05) between t0 (1.62 +/- 0.47) and t3 (6.59 +/- 0.05). Then stable Log₁₀ CFU were observed all along the experimental period (in average 6.16 +/- 0.11 Log₁₀ CFU, P>0.05).

Overall complete comparison of the kinetic profile in ileum for the 3 doses is provided in Figure 6 and includes comparison between doses for each time point.

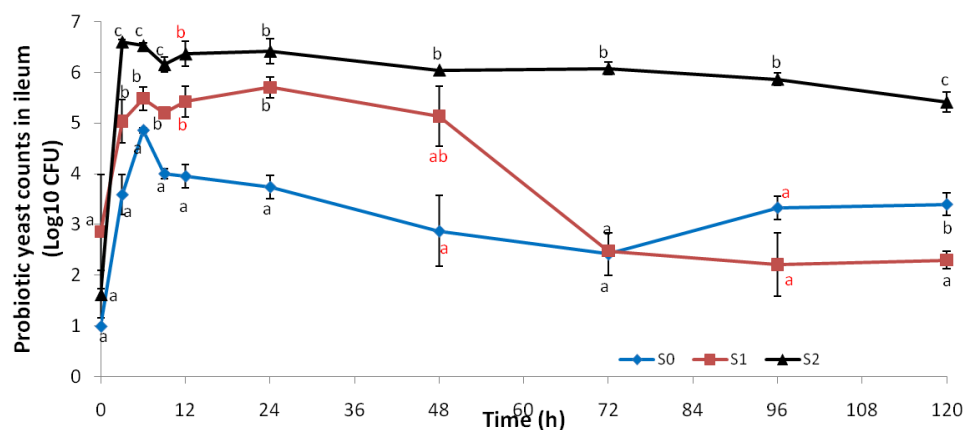


Figure 6. Determination of Log₁₀ CFU counts in the ileum of cannulated weaned pigs fed continuously different doses of a *S. cerevisiae* CNCM I-4407 probiotic live yeast in the feed at 10⁶ CFU (S0), 10⁷ CFU (S1) or 10⁸ CFU (S2) per gram of feed. GLM analysis for repeated measure confirmed a significant effect of probiotic yeast dose (P<0.001), significant effect of time for each dose (P<0.001) and no significant interaction between doses and time (P>0.05). Post-hoc analysis was realized to determine the differences between doses for each time point and differences mentioned by the superscript must be read only time point by time point, for clarity reasons (statements about statistical differences within dose across time is summarized in the text). For each time point, values sharing superscripts with similar colors tend to differ (P<0.1) and values sharing different superscripts significantly differ (P<0.05).

In colon for S0, Log₁₀ CFU progressively increased from t0 to t3 (2.60 +/- 0.00) then become significantly higher than t0 at t6 (3.68 +/- 0.39), then significantly higher than t0 and t3 (P<0.05) at t9 (4.27 +/- 0.03) and t12 (4.31 +/- 0.04). A decrease occurred there after from t24 (3.79 +/- 0.17) up to stable intermediary values from t48 (average 2.68 +/- 0.16, not significantly different from any other time point for this dose but tending to be different from t9 and t12, P<0.1).

In colon for S1, Log₁₀ CFU were <1.00 at t0 and t3, increased progressively from t6 (3.31 +/- 2.31), tending to be different from t0 and t3 (P<0.1), then yeast counts stabilized from t9 up to t48 (average 4.58 +/- 0.21, t9, t12, t24 and t48, not being significantly different between them or with t6). Then a strong decrease occurred from t72, t96 and t120 with an average 1.38 +/- 0.16, similar to t0 and t3, tending to be different from t6 (P<0.1) and significantly lower from t9 to t48 (P<0.05).

In colon for S2, Log₁₀ CFU increased progressively from t0 (1.00 +/- 0.00) to t3 (3.00 +/- 0.00) to t6 (4.88 +/- 0.37) then to t9 (5.52 +/- 0.29) and to t12 (5.47 +/- 0.68), t9 and t12 being significantly different from t0. Then a slight decrease of yeast counts occurred between t24 and t72 leading to intermediary values (in average 4.44 +/- 0.27, not significantly different from any other time point for the dose). A second drop occurred thereafter, leading to low CFU counts at t96 (3.15 +/- 0.39, significantly different from t9 and t12, P<0.05) and at t120 (3.28 +/- 0.41).

Overall complete comparison of the kinetic profile in colon for the 3 doses is provided in Figure 7 and includes comparison between doses for each time point.

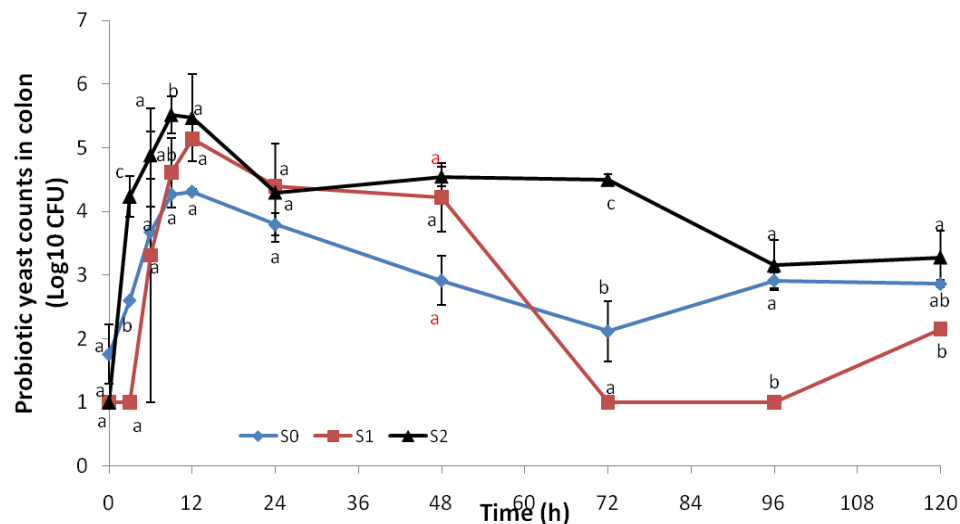


Figure 7. Determination of Log₁₀ CFU counts in the colon of cannulated weaned pigs fed continuously different doses of a *S. cerevisiae* CNCM I-4407 probiotic live yeast in the feed at 10⁶ CFU (S0), 10⁷ CFU (S1) or 10⁸ CFU (S2) per gram of feed. GLM analysis for repeated measure confirmed a significant effect of probiotic yeast dose ($P < 0.001$), significant effect of time for each dose ($P < 0.001$) and a significant interaction between doses and time ($P < 0.05$). Post-hoc analysis was realized to determine the differences between doses for each time point and differences mentioned by the superscript must be read only time point by time point, for clarity reasons (statements about statistical differences within dose across time is summarized in the text). For each time point, values with superscripts sharing similar colors tend to differ ($P < 0.1$) and values sharing different superscripts significantly differ ($P < 0.05$).

4. DISCUSSION

Studying the characteristics of transit of probiotics is important to justify the possibility to exert some (or all) of the numerous actions described *in vitro* or *in vivo* in the targeted intestinal site(s), generally small intestine and/or colon. In addition, justification of modulating the probiotic dose must be justified by some differences of CFU levels *in vivo*. In the present experiment, we have analyzed the kinetic of transit of the probiotic yeast *Saccharomyces cerevisiae* CNCM I-4407 in pigs after validation of an *in vivo* 2-cannulas system.

Probiotic yeast CFU appeared very fast in the different GIT section after first administration, i.e. 3h in the ileum, between 9 and 12h in the colon and 12 h in the feces. Despite no transit markers were used in the present study, these times are fully compatible with a transit of the yeast with the liquid phase (Kararli, 1995) and in agreement with a previous work describing the correlation between the transit of a probiotic *Lactobacillus casei* strain Shirota and a marker of liquid phase transit in in caecum-cannulated pigs (Ohashi et al., 2004). It seems that despite the biggest size of a yeast compared to a bacterium (~10 times in size, ~100 times in volume), the 2 types of probiotic organisms transit with the liquid phase.

The strain used in the study was able to survive to the gastro-intestinal conditions, i.e. low pH in the stomach and exposition to various digestive enzymes in the small intestine. However, an important decrease of CFU counts was observed after passing the ileo-caecal junction. It can be considered that, after achieving steady-state of CFU in the different section of the GIT and with reference to the concentration of the probiotic yeast in the feed, successive 2 log CFU were lost between ileum and colon, then an additional 1 log between colon and feces. Even if this progressive disappearance of the yeast along the GIT seems to be important, similar losses were previously described during the study of the transit of a *S. cerevisiae* var. *boulardii* in rat (Blehaut et al., 1989), with less than 1% of the initial yeast load recovered in the feces. Similar statement was done with the probiotic bacteria *Lactobacillus casei*, showing a reduction of the initial probiotic load of 50% in ileum and 70% in the feces (Oozeer et al., 2006). Among the possible explanations, increasing bacteria counts from proximal to distal part of the whole may progressively increase the pressure for any external microorganism to occupy, even transitorily, the more and more limited available space in the digestive ecosystem. Additionally, passing through industrial processes may have favored heterogeneity of the yeast population metabolic status, increasing sensitivity of some cells to lysis.

Last but not least, CFU determination only considers cells able to divide whereas part of the cells may remain alive but not being anymore able to divide consequently underestimating the real number of yeast cells able to exert probiotic effects. On this aspect, further investigations are needed to determinate the ability of non-dividing cells to exert or not probiotic effects.

Despite appearance of the probiotic yeast in the feces occurred at a relevant time after probiotic administration, CFU enumerated in the feces were not considered further in the present study, after observing that yeast counts in the feces largely underestimate the real number of CFU reaching the colon (significant -1 Log₁₀ CFU difference with feces, P<0.05) and ileum (significant -4 Log₁₀ CFU difference with feces, P<0.05), and cannot reflect the real amount of probiotic yeast crossing the GIT.

Actually, comparison of CFU counts between sacrificed animals and pigs fitted with the 2-cannulas in the major sites of action of probiotic products, i.e. distal small intestine and colon, confirmed the relevancy of the 2-cannula system to determine exact, reproducible and realistic CFU counts of the probiotic strain reaching each section of the GIT, allowing repeated measures in the same animals. The only limitation of the model raised in the present study lay in the progressive accumulation of content in the exit of the cannula, leading to a reproducible delay of the transit time of the probiotic yeast of 3h in ileum and between 3 and 6h for colon. On the basis of the usual uses of probiotics (long duration of administration to the animals), we can consider this delay in the model as negligible, except if short term administration where, for instance, single dose(s) of probiotic could be provided. In these situations, the reproducible delay must be taken into account but will not alter the total CFU counted or the action of the strain. Such an experimental model provides possibilities to compare kinetic of transit and viability of various probiotics such as different strains or strains passing through different technological preparations. One of the key discoveries of the present work lay on the reliable dose-effect that was observed between the different doses, with a reproducible 1 log difference in the CFU counts whatever the intestinal site when 1 log-differentiated feeds were given to the pigs. Additionally, decrease of CFU counts observed in all sites were all the more delayed than the provided dose was high. For example in ileum, the decrease was observed from t24 for S0, t48 for S1 and t144 for S2. The same statement can be done for colon, with a decrease starting from t48 for S0, t72 for S1 and from t96 for S2. These results are in agreement with the description in mouse by (Blehaut et al., 1989) of the disappearance up to 3 LogCFU of a probiotic yeast after 24h.

At the end of the experiment, all animals were sacrificed to sample liver, spleen, mesenteric lymphoid nodes and ileo-caecal junction. Microscopical examination concluded on the absence of lesion on the different organs and no yeasts were found in these peripheral organs confirming that in healthy situations no translocation of the probiotic occurs. In digestive tissues, yeast were only visible on the luminal part of the samples, confirming the absence of massive penetration of the microorganism in the GIT of animals

5. CONCLUSION

In the present study, we have investigated kinetic of transit of one yeast probiotic already proven to have various mechanisms of action in pigs with a dose-effect pattern. We have postulated that these reported effects would be due to increasing CFU counts reaching the 2 major digestive sites of action of the probiotic (ileum against bacterial disease or inflammation such as *E. coli*, and colon for microbiota modification and subsequent improved fiber digestibility and for both sites for more general dysbioses). Major results of the study showed that 1) after stabilization of the CFU in the different sections of the gut, CFU progressively decrease along GIT sections, 2) CFU in the feces were largely underestimating the CFU reaching the colon, 3) 2-cannula system in weaned pigs is a reliable and reproducible animal model to study kinetic of transit of various probiotics in pigs, 4) increasing the provided doses to the animals resulted in increased CFU counts reaching the different part of the gut with a reproducible dose-effect pattern and 5) that higher doses of yeast led to longer persistence of the CFU in the different GIT section.

ACKNOWLEDGMENTS

We sincerely thank VetAgro Sup staff for their collaboration for both surgery and lab analyses.

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