

## Basic Study

**Effects of *Saccharomyces cerevisiae* or *boulardii* yeasts on acute stress induced intestinal dysmotility**

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**Abstract****AIM**

To investigate the capacity of *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Saccharomyces boulardii* (*S. boulardii*) yeasts to reverse or to treat acute stress-related intestinal dysmotility.

**METHODS**

Adult Swiss Webster mice were stressed for 1 h in a wire-mesh restraint to induce symptoms of intestinal dysmotility and were subsequently killed by cervical dislocation. Jejunal and colon tissue were excised and placed within a tissue perfusion bath in which *S. cerevisiae*, *S. boulardii*, or their supernatants were administered into the lumen. Video recordings of contractility and gut diameter changes were converted to spatiotemporal maps and the velocity, frequency, and amplitude of propagating contractile clusters (PCC) were measured. Motility pre- and post-treatment was compared between stressed animals and unstressed controls.

**RESULTS**

*S. boulardii* and *S. cerevisiae* helped to mediate the effects of stress on the small and large intestine. Restraint stress reduced jejunal transit velocity (mm/s)

from  $2.635 \pm 0.316$  to  $1.644 \pm 0.238$ ,  $P < 0.001$  and jejunal transit frequency (Hz) from  $0.032 \pm 0.008$  to  $0.016 \pm 0.005$ ,  $P < 0.001$ . Restraint stress increased colonic transit velocity (mm/s) from  $0.864 \pm 0.183$  to  $1.432 \pm 0.329$ ,  $P < 0.001$  and frequency to a lesser degree. Luminal application of *S. boulardii* helped to restore jejunal and colonic velocity towards the unstressed controls;  $1.833 \pm 0.688$  to  $2.627 \pm 0.664$ ,  $P < 0.001$  and  $1.516 \pm 0.263$  to  $1.036 \pm 0.21$ ,  $P < 0.001$ , respectively. *S. cerevisiae* also had therapeutic effects on the stressed gut, but was most apparent in the jejunum. *S. cerevisiae* increased PCC velocity in the stressed jejunum from  $1.763 \pm 0.397$  to  $2.017 \pm 0.48$ ,  $P = 0.0031$  and PCC frequency from  $0.016 \pm 0.009$  to  $0.027 \pm 0.007$ ,  $P < 0.001$ . *S. cerevisiae* decreased colon PCC velocity from  $1.647 \pm 0.187$  to  $1.038 \pm 0.222$ ,  $P < 0.001$ . Addition of *S. boulardii* or *S. cerevisiae* supernatants also helped to restore motility to unstressed values in similar capacity.

## CONCLUSION

There is a potential therapeutic role for *S. cerevisiae* and *S. boulardii* yeasts and their supernatants in the treatment of acute stress-related gut dysmotility.

**Key words:** Intestine; *Saccharomyces cerevisiae*; *Saccharomyces boulardii*; Restraint stress; Motility

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**Core tip:** The use of *Saccharomyces cerevisiae* and *Saccharomyces boulardii* yeasts as therapeutic agents were tested for their ability to reverse the intestinal discomfort caused by acute stress. Most studies investigate the role of microbes in the prevention of stress, however the yeasts showed promising acute therapeutic effects for the treatment of stress. Additionally, the residual supernatant (Snt) after centrifugation of the yeasts was able to recapitulate much of the effect of the microbes themselves. *Saccharomyces* yeasts or Snt may be potential probiotic therapies in the treatment of acute stress-related intestinal dysmotility.

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## INTRODUCTION

Most studies on beneficial ingested microbes including probiotics have focused primarily on bacteria. However, beneficial roles have been ascribed to certain yeasts, such as the sugar-fermenting *Saccharomyces*<sup>[1]</sup>. *Saccha-*

*romyces boulardii* (*S. boulardii*) and *Saccharomyces cerevisiae* (*S. cerevisiae*) are two closely related strains used either as a probiotic or in the preparation of food and wine. The two strains have been closely examined; revealing that although they are nearly identical at a molecular level, *S. boulardii* shows more physiological resistance to heat and acid stressors<sup>[2]</sup>. It should also be noted that *S. boulardii* does not produce ascospores or use galactose, while *S. cerevisiae* does<sup>[3,4]</sup>.

*S. boulardii* has been systematically studied for its beneficial and probiotic effects<sup>[5]</sup>, but *S. cerevisiae* has limited research supporting a probiotic role<sup>[1]</sup>. *S. boulardii* has been used to help in the prevention of antibiotic or *Clostridium difficile* induced diarrhea, and there is evidence that it may be useful in attenuating acute gastroenteritis and traveller's diarrhea<sup>[5,6]</sup>. Another study showed that treatment with *S. boulardii* helped to shorten the duration of acute diarrhea in children and to normalize the frequency and consistency of stool<sup>[7]</sup>. *S. boulardii* may also help in the treatment of bowel inflammation and infection by reversing mucosal injury<sup>[8]</sup>.

*S. cerevisiae* food and wine strains have a long history in the food and wine industry<sup>[9]</sup> and are generally considered safe for consumption<sup>[1]</sup>. Suggestive for a potential beneficial role for *S. cerevisiae* are reports indicating that this strain may provoke immune stimulation in mice infected with *Staphylococcus aureus*<sup>[10]</sup>. Supplementation with a *S. cerevisiae* I-3856 strain may improve symptoms in constipation-predominant irritable bowel syndrome patients<sup>[11]</sup>. There is also evidence that a *S. cerevisiae* UFMG 905 strain can bind to bacteria and modulate inflammation pathways in a murine model of *Salmonella enterica* serovar *Typhimurium* infection<sup>[12]</sup>. In summary, there appears to be published data supporting roles for *S. boulardii* or *S. cerevisiae* as beneficial or probiotic microbes. The evidence seems to be stronger for *S. boulardii* than *S. cerevisiae*, perhaps because the latter has been less frequently investigated in this regard.

We used an acute *ex vivo*, before and after, motility recording paradigm similar to that used previously to test for the effects of JB-1™ on stress-induced dysmotility<sup>[13]</sup>. *Saccharomyces* or *Saccharomyces* supernatant (Snt) were added to the Krebs buffer perfusing the lumen of isolated, previously stressed or unstressed, mouse intestinal segments. Treatment effects were interpreted by comparing propagating contractile clusters (PCC) of control with treatment recording periods. The *ex vivo* design allowed us to localize any effects to the intestine, thus avoiding multisystem homeostatic feedback between the gut and its extrinsic nervous system. This design also allowed us to separate treatment effects from confounding preventative actions, as would have been the case if the yeasts had been fed to the animal.

The preventative effect of *Saccharomyces* strains in relation to diarrhea suggests a possible action on

disordered gut motility. It has not been experimentally tested whether *S. boulardii* or *S. cerevisiae* are able to treat (effectively reverse) stress-related dysmotility in an experimental model. We have recently shown that restraint stress induces colonic propulsive hypermotility, while disorganizing and reducing motility in the small intestine<sup>[13]</sup>. The effects of stress on motility could be reversed *ex vivo* by introducing a bacterial probiotic (*Lactobacillus rhamnosus* JB-1™) into the lumen<sup>[13]</sup>. The example of JB-1™ in treating stress-induced dysmotility provides a way to compare putative beneficial actions of the *Saccharomyces* strains with a probiotic bacterium whose effects on motility and the enteric nervous system have been previously studied<sup>[14-20]</sup>.

## MATERIALS AND METHODS

### Animals

We used 6-8-wk-old adult male Swiss Webster mice (20-30 g) from Charles River Laboratories (Wilmington, MA, United States). All procedures following acute restraint were *ex vivo*<sup>[20]</sup> and their conduct were approved by the Animal Research Ethics Board of McMaster University (AUP 12-05-17).

### Gut motility

The following experiments and data analysis were performed as described as in West *et al.*<sup>[13]</sup>. Mice were placed in a wire mesh restraint device for 1 h or kept in their cage for 1 h; after which they were killed by cervical dislocation. A 4 cm long segment of jejunum or colon was excised and placed into a tissue bath perfusion chamber<sup>[20]</sup>. The oral and anal ends were cannulated with silicone tubing and the oral end was attached to a stopcock manifold, allowing inflow with oxygenated Krebs buffer or buffer to which yeast or Snt had been added. Krebs buffer was of the following composition (mmol/L): 118 NaCl, 4.8 KCl, 25 NaHCO<sub>3</sub>, 1.0 NaH<sub>2</sub>PO<sub>4</sub>, 1.2 MgSO<sub>4</sub>, 11.1 glucose, and 2.5 CaCl<sub>2</sub> bubbled with carbogen gas (95% O<sub>2</sub> and 5% CO<sub>2</sub>). PCCs were evoked by filling the lumen with Krebs buffer using a pressure differential of 2 hPa (cmH<sub>2</sub>O) for the inflow and 3 hPa for outflow for jejunum, and 2-3 hPa for colon inflow with the outflow raised 1 cm above the inflow.

Contractions of the gut were video-recorded on a JVC camcorder placed 10 cm above the tissue bath. Conversion of videos to spatiotemporal diameter maps (Dmaps) were performed using Image J software, as described in Wu *et al.*<sup>[20]</sup>. Dmaps are a form of heat map in which the oral to anal propagation of the intestine runs down the vertical axis and time runs across the horizontal axis. The intestine's diameter is colour coded using red to represent contraction, and yellow to green to represent varying degrees of relaxation (Figure 1). PCCs are identified in Dmaps as described in West *et al.*<sup>[13]</sup>. The PCCs appear as

broad bands<sup>[20]</sup> that propagate in the oral to anal direction. They are believed to require ENS activity because they are abolished by the Na channel blocker tetrodotoxin<sup>[18-23]</sup>. From these Dmaps, velocity can be measured as the slope of the bands (distance/time), frequency from the intervals between the bands, and amplitude as the difference between gut diameters.

### Luminal stimuli

Lyophilized *S. boulardii* CNCM I-1079 or *S. cerevisiae* LYCC 6029 were obtained from Lallemand Health Solutions (Montreal, QC, CA). The microbes (starter counts) were diluted in 50 mL Krebs buffer and incubated for 45 min at 37 °C. In pilot experiments, 5 × 10<sup>7</sup>, 5 × 10<sup>8</sup> and 5 × 10<sup>9</sup> starter counts of *S. boulardii* reduced PCC velocity in unstressed colon segments by 5%, 25% and 27%. We thus used 5 × 10<sup>8</sup>-lyophilized microbes for dilution in all other experiments as this starter count produced near maximal effect in the pilots. In some experiments we used *S. boulardii* or *S. cerevisiae* Snt after completion of the incubation period. The microbes were diluted and incubated as previously described above. The Snt was separated from the post-incubation yeast microbe solution by centrifugation at 1400 g. using a Beckman Model TJ-6 centrifuge for 30 min. After sediment removal, the mixture was passed through a 0.2 μm pore-size filter and the filtered Snt was applied to the lumen of gut segment to be tested.

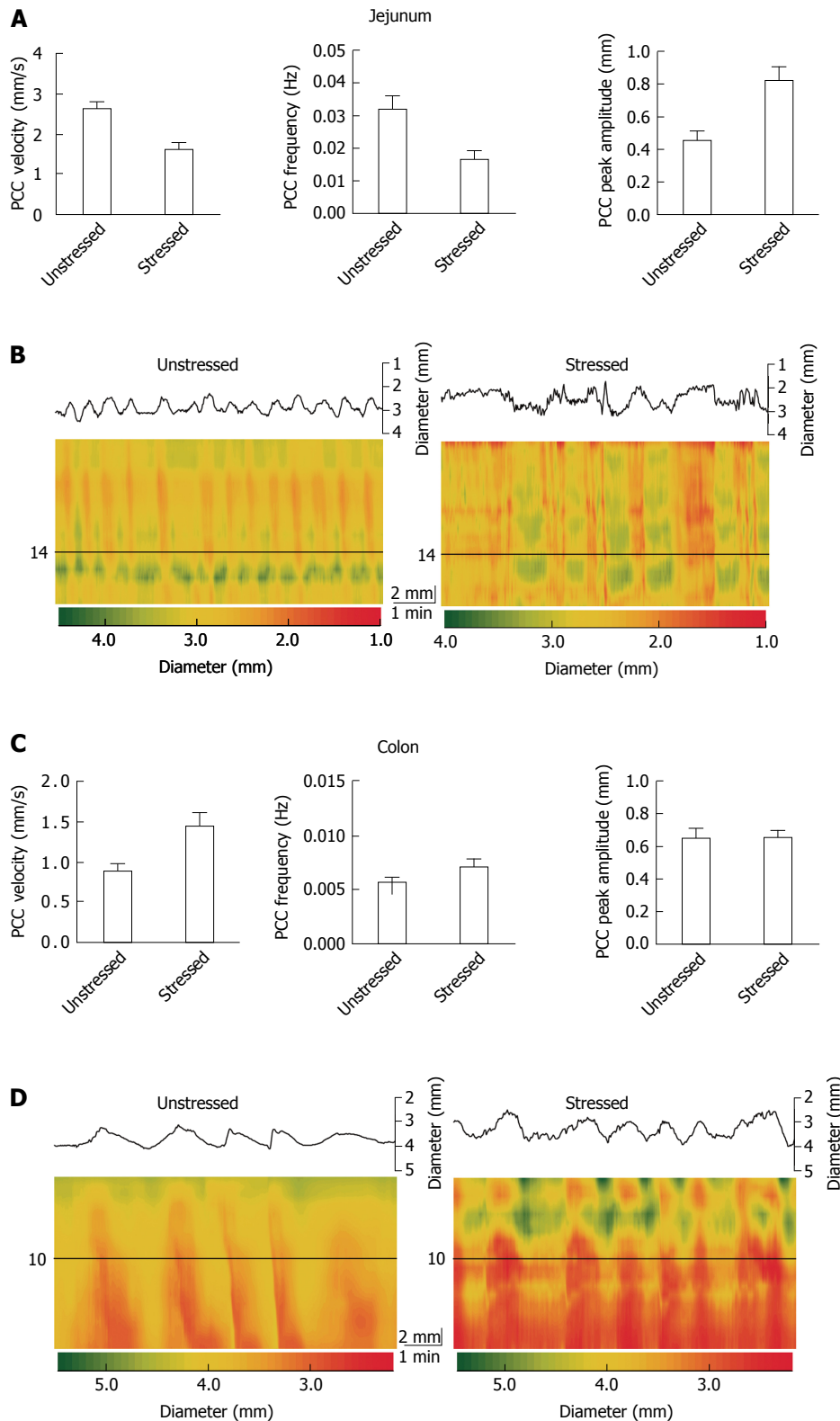
### Statistical analysis

Effects of restraint stress on motility were measured in unpaired experiments by comparing velocity, frequency and peak amplitude for PCCs. The same parameters were compared before and after application of *S. boulardii*, *S. cerevisiae*, or their Snt. Control recordings were made in stressed or unstressed mice perfused with Krebs for a maximum of 20 min. Treatment recordings were made during and after addition of one of the stimuli (yeast or Snt) to the same segment for additional 20 min duration. Descriptive statistics were given as mean ± SD (N, where N denotes the number of mice used). In the results, treatment effect sizes are presented as % mean differences and the probability of superiority (PS) based on distribution of difference scores and standard deviations is presented in brackets<sup>[24]</sup>. Differences were also presented in the tables using unpaired or paired *t* tests under the null hypothesis of no difference.

## RESULTS

### Effects of stress on PCCs

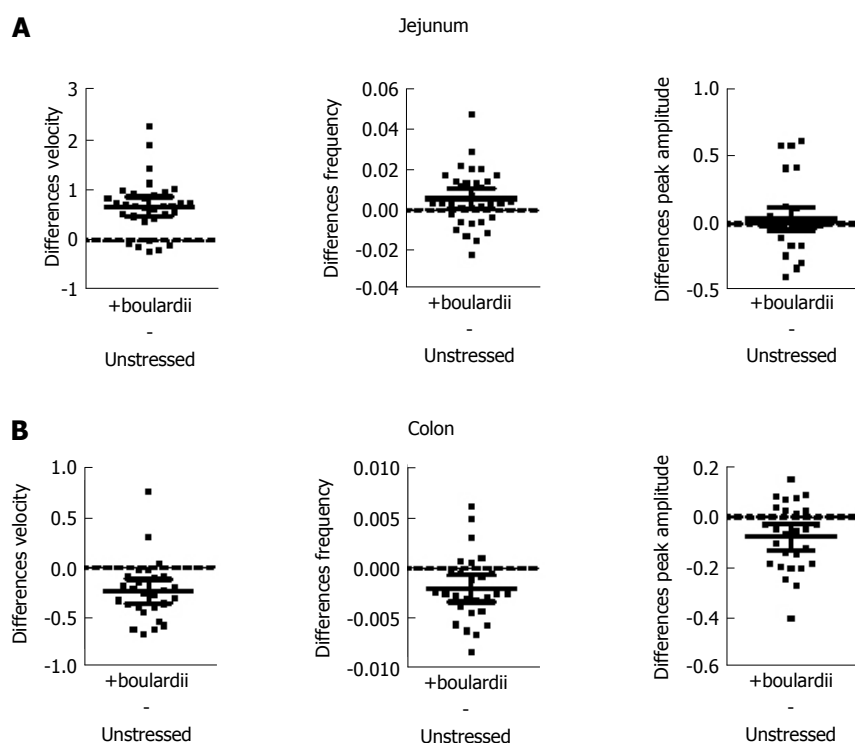
Stress had different effects on the jejunum vs the colon. Stress decreased propulsive motility and decreased the regularity of PCCs in jejunum, but increased motility in colon (Figure 1). Stress decreased jejunal PCC velocity



**Figure 1** Effects of acute restraint stress on gut contractility, *ex vivo*. A and C: Plot PCC velocity, frequency, and peak amplitude in the jejunum and colon respectively. The bar graphs show the mean difference between the control and treatment parameters. Stress decreased PCC velocity ( $P < 0.001$ ) and frequency ( $P < 0.001$ ) and increased peak amplitude in the jejunum ( $P < 0.001$ ). Stress increased PCC velocity ( $P < 0.001$ ) and frequency ( $P = 0.008$ ), but had virtually no effect on peak amplitude in the colon ( $P = 0.902$ ); B and D: Spatiotemporal diameter maps demonstrating contractility of the gut over time. The colour scale of the heat maps denote gut diameter starting from red for the smallest diameter (contraction) to green for the largest diameter (relaxation). The contractions run orally to anally across the vertical axis, and across time along the horizontal axis. Above the heat map is a plot of diameter vs time or a single time-point along the vertical (oral-anal) axis, which is denoted by the horizontal line. The total vertical length of the map is 20 mm and time is 480 s for the jejunum and 560 s for the colon. The scale bar measures 1 min (60 s) horizontally and 2 mm vertically. All subsequent spatiotemporal (Dmaps) followed the same parameters.

**Table 1** Effects of stress on gut propagating contractile clusters

Parameter	Tissue	Unstressed	Stressed	P value (t test)
Velocity (mm/s)	Jejunum	2.635 ± 0.316 (17)	1.644 ± 0.238 (17)	< 0.001
	Colon	0.864 ± 0.183 (17)	1.432 ± 0.329 (17)	< 0.001
Frequency (Hz)	Jejunum	0.032 ± 0.008 (17)	0.016 ± 0.005 (17)	< 0.001
	Colon	0.005 ± 0.001 (17)	0.007 ± 0.001 (17)	0.008
Peak amplitude (mm)	Jejunum	0.437 ± 0.020 (17)	0.790 ± 0.015 (17)	< 0.001
	Colon	0.640 ± 0.118 (17)	0.645 ± 0.101 (17)	0.902



**Figure 2** *Saccharomyces boulardii* increased motility across most parameters in the unstressed jejunum and decreased motility across parameters in the unstressed colon, *ex vivo*. Mean differences in parameters are plotted (unpaired) and standard deviations of the mean are indicated. A: *Saccharomyces boulardii* (*S. boulardii*) increased PCC velocity and frequency, but had little to no effect on peak amplitude, in the unstressed jejunum; B: *S. boulardii* decreased PCC velocity, frequency, and peak amplitude in the unstressed colon.

by 38% (PS = 99%,  $P < 0.001$ ), but increased it by 66% (PS = 98%,  $P < 0.001$ ) for colon (Table 1 and Figure 1). Frequency decreased by 50% (PS = 99%,  $P < 0.001$ ) for jejunum, but increased by 27% (PS = 93%,  $P = 0.008$ ) for colon. Peak amplitude increased by 81% (PS = 99%,  $P < 0.001$ ) for jejunum and by 1% (PS = 51%,  $P = 0.902$ ) for colon.

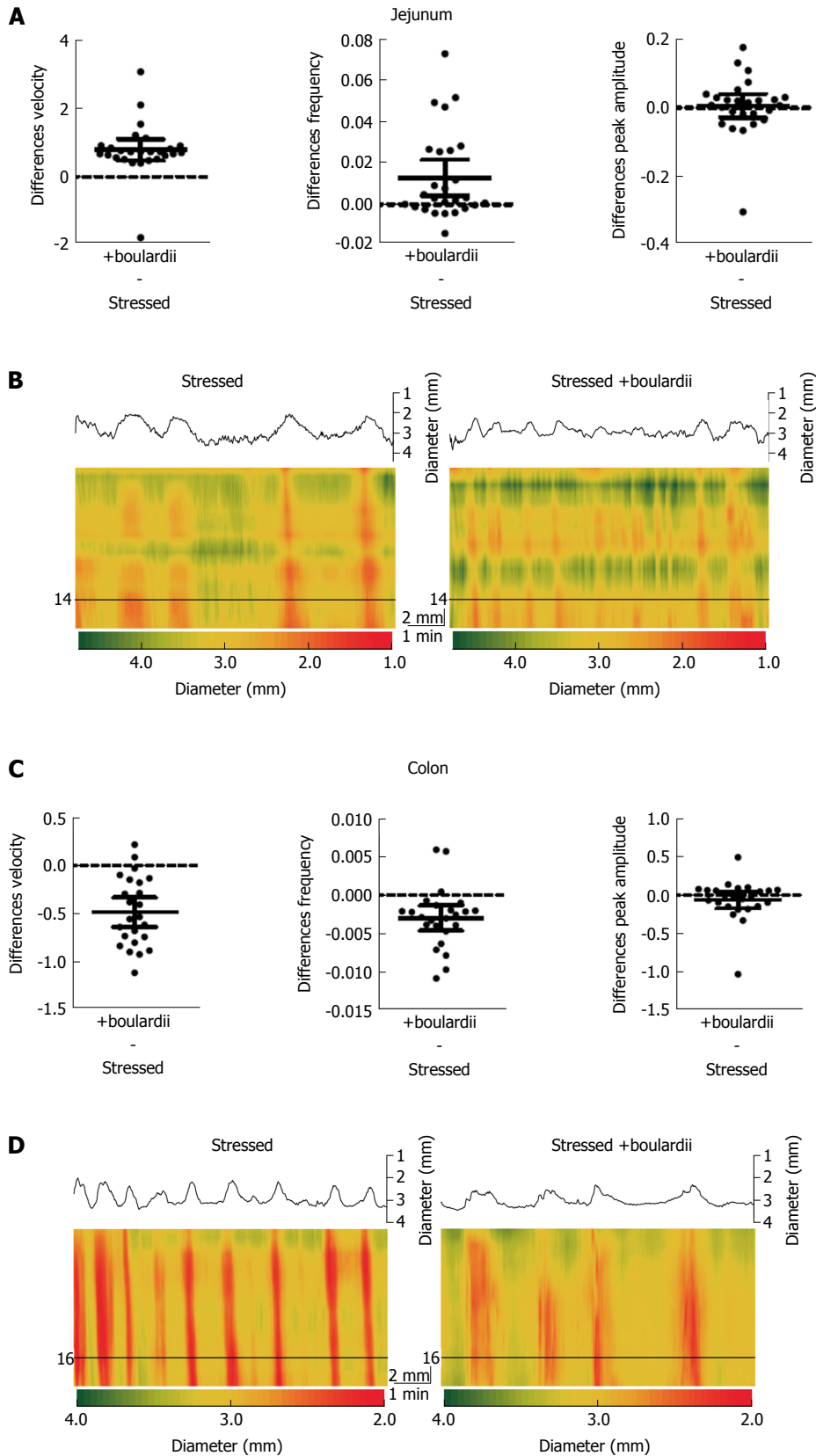
### *S. boulardii* unstressed gut

*S. boulardii* increased PCC velocity in unstressed jejunum by 26% (PS = 94%,  $P < 0.001$ ), but decreased velocity by 27% (PS = 89%,  $P < 0.001$ ) for colon. Frequency was increased by 19% (PS = 74%,  $P = 0.030$ ) for jejunum, but decreased by 22% (PS = 85%,  $P = 0.005$ ) for colon. Peak amplitude increased by 6% (PS = 60%,  $P = 0.290$ ) for jejunum, but decreased by 13% (PS = 85%,  $P = 0.005$ ) for colon (Table 2). Figure 2 also shows paired mean differences (Unstressed + *S. boulardii* Unstressed) with 95% CIs. When the confidence intervals did not

straddle the "difference = 0" line, the value of no paired difference was excluded for these intervals with 95% confidence<sup>[25]</sup>; when it did straddle the "difference = 0" line the value of no paired difference was included within the 95% CIs.

### *S. boulardii* stressed gut

*S. boulardii* counter the effects of stress in both jejunal and colon segments, with the exception of peak amplitude (Figure 3 and Table 3). *S. boulardii* restored the regularity and frequency of contractions in both tissues, as shown in Figure 3. There is a marked increase in frequency of bands in the jejunal Dmap and a decrease in the colon Dmap. Addition of *S. boulardii* increased velocity by 43% (PS = 93%,  $P < 0.001$ ) for stressed jejunum and decreased velocity by 32% (PS = 96%,  $P < 0.001$ ) for colon. PCC frequency was increased by 69% (PS = 85%,  $P = 0.005$ ) for jejunum but decreased by 29% (PS = 90%,  $P = 0.001$ ) for colon. Peak amplitude changed by +1% (PS =



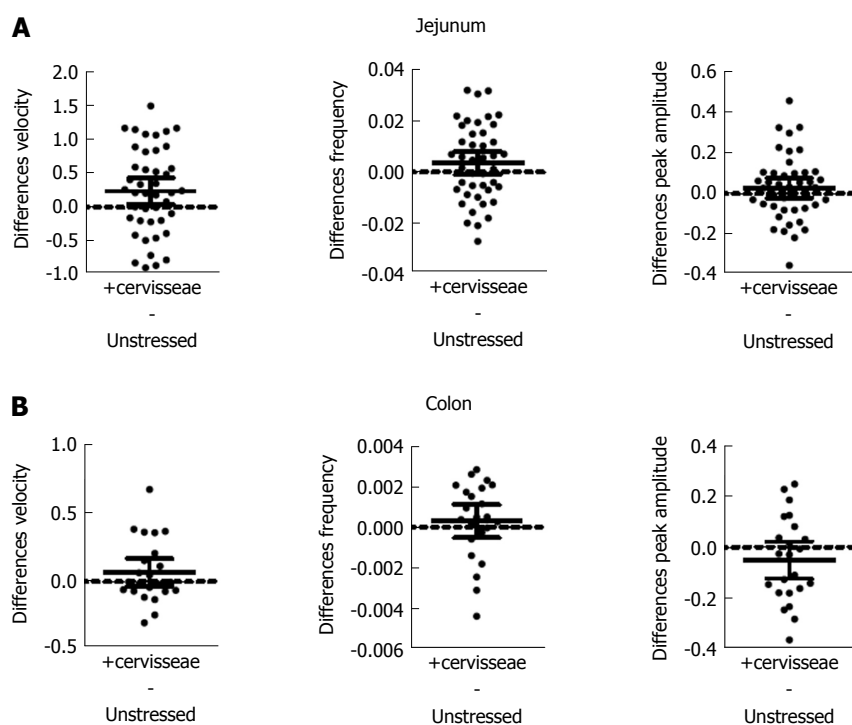
**Figure 3** *Saccharomyces boulardii* reduced the effects of acute stress on the small and large intestine. A and C: Mean paired differences, using 95% CIs across parameters for jejunum and colon [(Stressed + *S. boulardii*) - stressed]. *Saccharomyces boulardii* (*S. boulardii*) increased jejunal velocity and frequency after stress. *S. boulardii* decreased colonic velocity and frequency after stress. *S. boulardii* had little to no effect on peak amplitude in the stressed jejunum and colon. Mean differences straddled 0 for peak amplitude in both jejunum and colon; B and D: Dmaps comparing stressed jejunum and colon before and after addition of *S. boulardii*.

**Table 2** Effects of *Saccharomyces boulardii* on propagating contractile clusters in unstressed gut

Parameter	Tissue	Unstressed	+ <i>boulardii</i>	P value (t test)
Velocity (mm/s)	Jejunum	2.556 ± 0.299 (32)	3.229 ± 0.620 (32)	< 0.001
	Colon	0.865 ± 0.148 (27)	0.633 ± 0.225 (27)	< 0.001
Frequency (Hz)	Jejunum	0.030 ± 0.018 (33)	0.035 ± 0.025 (33)	0.030
	Colon	0.009 ± 0.004 (27)	0.007 ± 0.003 (27)	0.005
Peak amplitude (mm)	Jejunum	0.587 ± 0.241 (33)	0.623 ± 0.291 (33)	0.290
	Colon	0.621 ± 0.190 (27)	0.543 ± 0.179 (27)	0.005

**Table 3** Effects of *Saccharomyces boulardii* on propagating contractile clusters in stressed gut

Parameter	Tissue	Stressed	+ <i>boulardii</i>	P value (t test)
Velocity (mm/s)	Jejunum	1.833 ± 0.688 (26)	2.627 ± 0.664 (26)	< 0.001
	Colon	1.516 ± 0.263 (24)	1.036 ± 0.210 (24)	< 0.001
Frequency (Hz)	Jejunum	0.019 ± 0.013 (26)	0.032 ± 0.024 (26)	0.005
	Colon	0.010 ± 0.003 (24)	0.007 ± 0.003 (24)	0.001
Peak amplitude (mm)	Jejunum	0.692 ± 0.215 (26)	0.698 ± 0.212 (26)	0.705
	Colon	0.719 ± 0.249 (24)	0.657 ± 0.152 (24)	0.259



**Figure 4** *Saccharomyces cerevisiae* slightly increased parameters in both the unstressed small and large intestine (excluding colon peak amplitude). A: 95% CIs for paired mean differences were near 0 for jejunal peak amplitude. Jejunal PCC velocity and frequency slightly increased; B: *Saccharomyces cerevisiae* (*S. cerevisiae*) had less potent effects in the colon, slightly increasing PCC velocity and frequency, but decreasing peak amplitude. PCC: Propagating contractile clusters.

56%,  $P = 0.705$ ) for jejunum and decreased by 9% (PS = 68%,  $P = 0.259$ ) for colon. Mean paired differences (Stressed + *S. boulardii* Stressed) are given in Figure 3B and D with their 95% CIs. Washout with Krebs buffer for 20 min during the control period did not moderate the effects of *S. boulardii* on the stressed segments.

#### *S. cerevisiae* unstressed gut

*S. cerevisiae* had the most noticeable effect on the jejunum (Figure 4 and Table 4). Velocity increased

9% (PS = 71%,  $P = 0.017$ ) for jejunum and 9% (PS = 73%,  $P = 0.161$ ) for colon. Frequency increased by 13% (PS = 64%,  $P = 0.003$ ) for jejunum and by 6% (PS = 63%,  $P = 0.462$ ) for colon, while peak amplitude increased by 7% (PS = 61%,  $P = 0.242$ ) for jejunum, but decreased by 9% (PS = 73%,  $P = 0.164$ ) for colon.

#### *S. cerevisiae* stressed gut

*S. cerevisiae* reduced most of the effects of stress, except for those of colon PCC frequency and amplitude

**Table 4** Effects of *Saccharomyces cerevisiae* on propagating contractile clusters in unstressed gut

Parameter	Tissue	Unstressed	+ <i>cerevisiae</i>	P value ( $t$ test)
Velocity (mm/s)	Jejunum	2.779 ± 0.499 (43)	3.017 ± 0.457 (43)	0.017
	Colon	0.788 ± 0.212 (23)	0.858 ± 0.257 (23)	0.161
Frequency (Hz)	Jejunum	0.027 ± 0.012 (43)	0.031 ± 0.008 (43)	0.003
	Colon	0.0051 ± 0.001 (23)	0.0054 ± 0.001 (23)	0.462
Peak amplitude (mm)	Jejunum	0.409 ± 0.111 (43)	0.438 ± 0.098 (43)	0.242
	Colon	0.559 ± 0.176 (23)	0.508 ± 0.15 (23)	0.164

**Table 5** Effects of *Saccharomyces cerevisiae* on propagating contractile clusters in stressed gut

Parameter	Tissue	Stressed	+ <i>cerevisiae</i>	P value ( $t$ test)
Velocity (mm/s)	Jejunum	1.763 ± 0.397 (23)	2.017 ± 0.480 (23)	0.0031
	Colon	1.647 ± 0.187 (23)	1.038 ± 0.222 (23)	< 0.001
Frequency (Hz)	Jejunum	0.016 ± 0.009 (23)	0.027 ± 0.007 (23)	< 0.001
	Colon	0.008 ± 0.002 (23)	0.008 ± 0.003 (23)	0.994
Peak amplitude (mm)	Jejunum	0.761 ± 0.316 (23)	0.559 ± 0.148 (23)	0.013
	Colon	0.640 ± 0.101 (23)	0.597 ± 0.103 (23)	0.190

**Table 6** Effects of *Saccharomyces boulardii* supernatant on propagating contractile clusters in stressed gut

Parameter	Tissue	Stressed	+ <i>boulardii</i> Snt	P value ( $t$ test)
Velocity (mm/s)	Jejunum	1.97 ± 0.39 (6)	2.585 ± 0.468 (6)	< 0.001
	Colon	1.588 ± 0.194 (6)	1.11 ± 0.383 (6)	0.038
Frequency (Hz)	Jejunum	0.020 ± 0.014 (6)	0.044 ± 0.027 (6)	0.067
	Colon	0.010 ± 0.001 (6)	0.007 ± 0.003 (6)	0.024
Peak amplitude (mm)	Jejunum	0.75 ± 0.285 (6)	0.737 ± 0.187 (6)	0.93
	Colon	0.65 ± 0.188 (6)	0.701 ± 0.09 (6)	0.59

(Figure 5 and Table 5). Velocity was increased by 14% (PS = 89%,  $P = 0.0031$ ) for jejunum and decreased by 37% (PS = 98%,  $P < 0.001$ ) for colon. Frequency was increased by 74% (PS = 95%,  $P < 0.001$ ) for jejunum, but there was only a 0.1% (PS = 50%,  $P = 0.994$ ) change for colon. Peak amplitude decreased by 27% (PS = 86%,  $P = 0.013$ ) for jejunum and 7% (PS = 72%,  $P = 0.190$ ) for colon. The Dmaps in Figure 5B and D show the regulation of jejunal motility by *S. cerevisiae*, with less potent effects on the colon. However the degree in which the contractions change in diameter appear to be lessened in the colon after addition of *S. cerevisiae*, and is apparent by the reduced frequency of red, strong contractions.

### ***S. boulardii* Snt stressed gut**

*S. boulardii* Snt decreased the effects of stress on PCC parameters, except for peak amplitude (Figure 6 and Table 6). Velocity increased by 31% (PS = 99.9%,  $P < 0.001$ ) for jejunum, but decreased by 30% (PS = 99.9%,  $P = 0.038$ ) for colon. Frequency increased by 114% (PS = 99.9%,  $P = 0.067$ ) for jejunum and decreased by 37% (PS = 99%,  $P = 0.024$ ) for colon. However, peak amplitude only decreased by 2% (PS = 56%,  $P = 0.930$ ) for jejunum and increased 8% (PS = 74%,  $P = 0.590$ ) for colon.

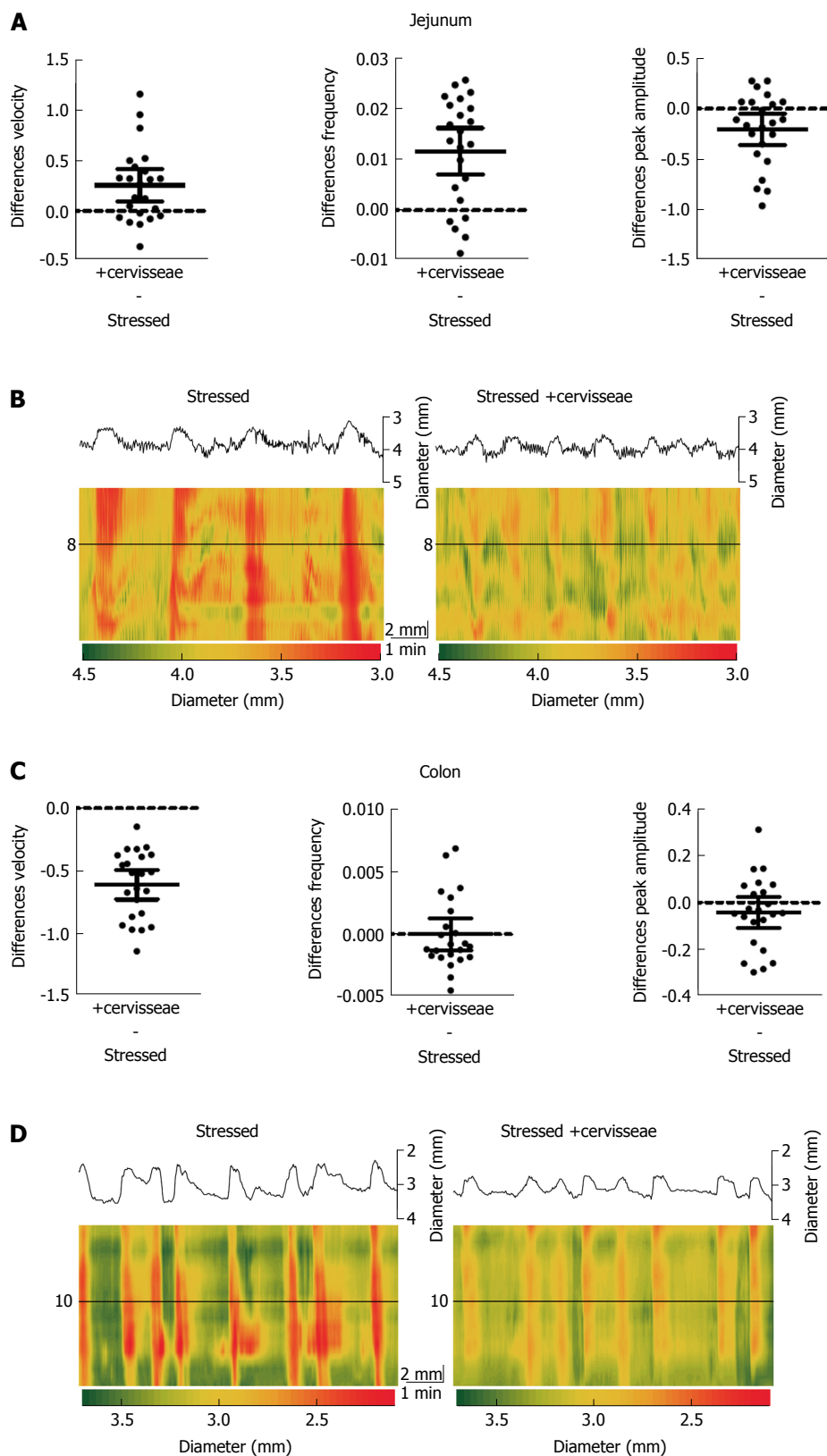
### ***S. cerevisiae* Snt stressed gut**

*S. cerevisiae* Snt reduced the effects of stress for colon velocity, jejunal frequency and peak amplitude for jejunum and colon (Figure 7 and Table 7). Effects on other parameters were minor. Velocity decreased by 7% (PS = 56%,  $P = 0.970$ ) for jejunum but decreased by 38% (PS = 99%,  $P = 0.002$ ) for colon. Frequency increased by 64% (PS = 83%,  $P = 0.003$ ) for jejunum and decreased by 2% (PS = 60%,  $P = 0.830$ ); also, peak amplitude decreased by 35% (PS = 99%,  $P = 0.0016$ ) for jejunum while it decreased by 31% (PS = 99%,  $P = 0.100$ ) for colon.

## **DISCUSSION**

We have used an *ex vivo* intestinal segment perfusion setup to show that *S. boulardii* or *S. cerevisiae* reverse much of the jejunal or colonic dysmotility induced by restraint stress. There is sometimes a potential for normal commensal, or otherwise beneficial, yeasts to adopt a pathological role in immune-compromised individuals. It is therefore of interest that the Snt from either *Saccharomyces* strains recapitulate much of the treatment effects of the live yeasts. Because the yeasts were applied intraluminally in *ex vivo* intestinal segments the treatment effect must have occurred

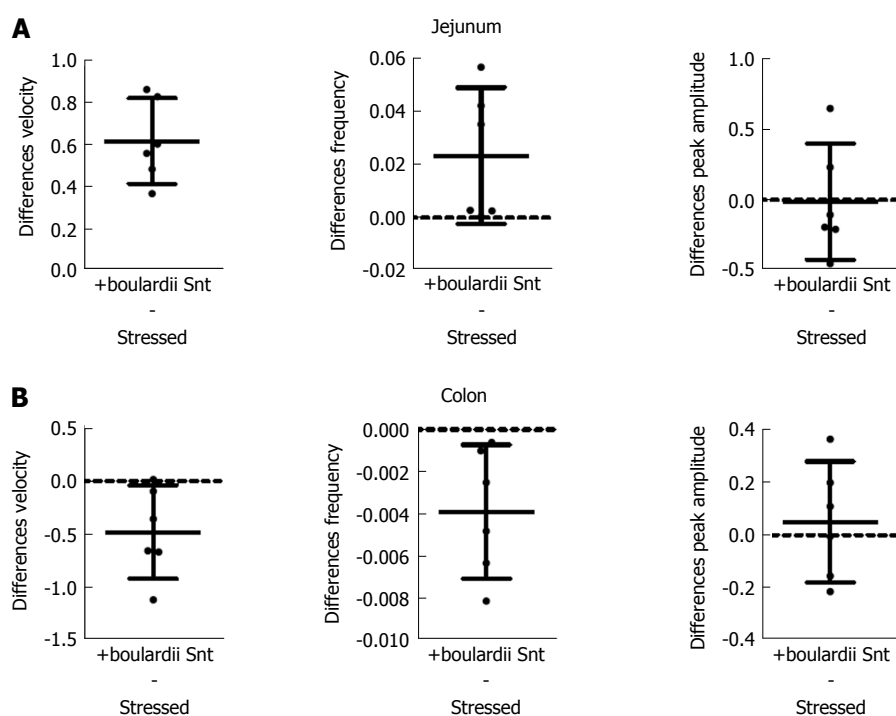




**Figure 5** *Saccharomyces cerevisiae* helped to reduce dysmotility in the stressed gut, with most visible effects on the jejunum. A and C: *Saccharomyces cerevisiae* (*S. cerevisiae*) increased stressed jejunal propagating contractile clusters velocity and decreased velocity in the stressed colon. *S. cerevisiae* increased jejunal frequency and slightly decreased peak amplitude in both the colon and jejunum. 95% CIs straddled 0 for mean paired differences in colonic frequency; B and D: Dmaps representing the stressed gut before and after addition of *S. cerevisiae*. *S. cerevisiae* helped to restore jejunal motility after stress. *S. cerevisiae* had less potent effects on the stressed colon, but helped to ease some of the dysmotility.

**Table 7** Effects of *Saccharomyces cerevisiae* supernatant on propagating contractile clusters in stressed gut

		Stressed	+ <i>cerevisiae</i> Snt	<i>P</i> value ( <i>t</i> test)
Velocity (mm/s)	Jejunum	0.713 ± 0.109 (6)	0.466 ± 0.122 (6)	0.97
	Colon	1.639 ± 0.18 (6)	1.018 ± 0.245 (6)	0.002
Frequency (Hz)	Jejunum	0.018 ± 0.004 (6)	0.029 ± 0.004 (6)	0.003
	Colon	0.0076 ± 0.001 (6)	0.0074 ± 0.001 (6)	0.83
Peak amplitude (mm)	Jejunum	0.713 ± 0.109 (6)	0.466 ± 0.122 (6)	0.0016
	Colon	0.741 ± 0.177 (6)	0.510 ± 0.202 (6)	0.10



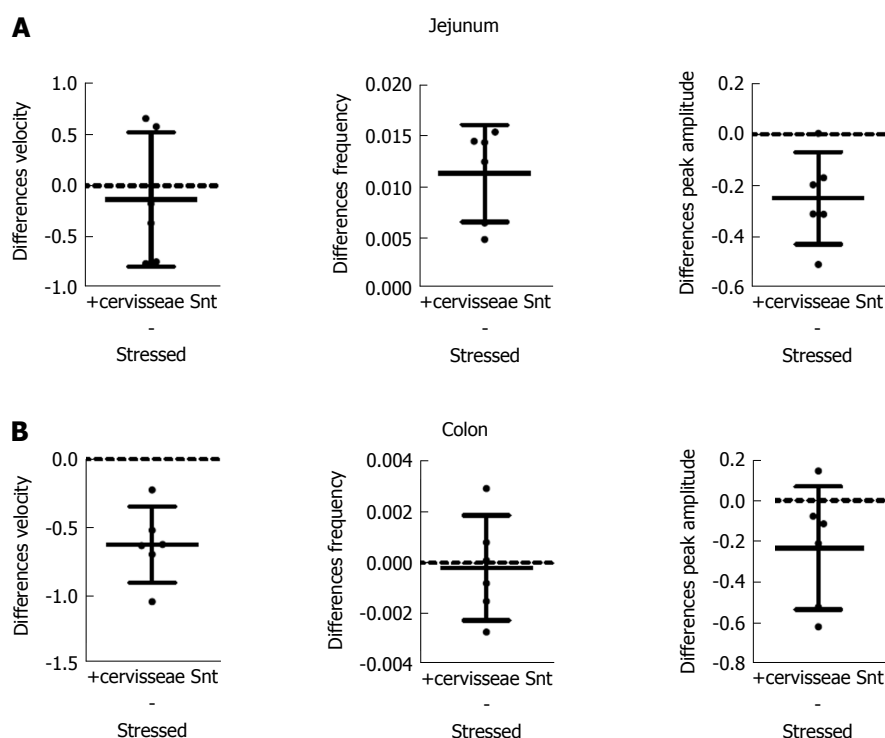
**Figure 6** Addition of *Saccharomyces boulardii* supernatant to the stressed lumen recapitulated much of the effect of the *boulardii* yeast. **A:** *Saccharomyces boulardii* (*S. boulardii*) Snt increased PCC velocity and frequency in the stressed jejunum. 95% CIs straddled 0 for mean paired differences in jejunal peak amplitude, showing no effect; **B:** *S. boulardii* Snt decreased PCC velocity and frequency in the stressed colon. *S. boulardii* Snt had little effect (very little increase) on stressed colonic peak amplitude and again the 95% CIs straddled 0 for mean paired differences. PCC: Propagating contractile clusters.

locally within the intestine<sup>[13]</sup>. Therefore, it could not have involved the hypothalamic-pituitary-adrenal axis or other central structures. The relative short latency (approximately 10 min) for the onset of the therapeutic effect is consistent with a drug-like pharmacological mode of action, however, fast indirect modes of action involving immune cells cannot be excluded, although we consider the latter possibility to be less likely than the former.

There have been previous reports of the acute *ex vivo* actions of bacterial, but not fungal microbes on intestinal motility (see discussion in<sup>[20]</sup>). Such effects appear to be region specific with different actions for small vs large intestine<sup>[20]</sup>. It is important to note that others have also reported a short (approximately 10 min) latency in onset for microbial effects on intestinal propulsive motility<sup>[18,26]</sup>, consistent with a drug-like pharmacological action of the microbes on the neuromuscular machinery. This supporting finding as well as the region-specific, local effect on the intestine leads

us to believe the effect is most likely pharmacological in action.

Wrap restraint has been reported to increase the contractile amplitudes in the small intestine and colon, while decreasing frequency in small intestine and increasing frequency in colon<sup>[13,27]</sup>. As far as we are aware the only other publication to date referring to treatment effects of beneficial microbes on stress-induced dysmotility is West *et al*<sup>[13]</sup> in which *Lactobacillus rhamnosus* JB-1<sup>TM</sup> reversed the effects of prior stress. *S. boulardii* or *S. cerevisiae* were also effective in reversing most, but not all of the stress-induced dysmotility. *S. boulardii* was particularly effective in reversing the effects of stress in both the jejunum and colon. *S. boulardii* also had similar effects in the unstressed gut as the stressed, increasing PCC frequency and velocity in the jejunum and decreasing PCC frequency and velocity in the colon. These effects on the unstressed gut were predictive of the restoration of the stressed gut towards unstressed



**Figure 7** Addition of *Saccharomyces cerevisiae* supernatant to the stressed lumen had some therapeutic effect, but not across parameters. A: *Saccharomyces cerevisiae* (*S. cerevisiae*) Snt had little effect on stressed jejunal PCC velocity. *S. cerevisiae* Snt increased stressed jejunal PCC frequency and decreased peak amplitude; B: *S. cerevisiae* Snt decreased both stressed colonic PCC velocity and peak amplitude. There was no effect on stressed colon PCC frequency.

measures.

*S. boulardii* and *S. cerevisiae* are nearly identical genetically, but differ in resistance to temperature and acidic stressors and growth characteristics<sup>[2]</sup>. Similarly, the treatment effects of *S. boulardii* and *S. cerevisiae* were not identical, indicating that despite their genetic similarity the functional interaction of these microbes or their Snt with the host intestine is functionally different and region specific. There is clearly a need to identify the bioactive molecules released by the yeasts and those that mediate the motility modifying effects on the host tissue. This is likely a difficult task since there is no reason to suppose that only a single molecule is the mediator for anyone strain; yeasts like other microbes produce a multitude of molecules, of which any combination could potentially be effective. A similar logic applies to bacterial microbes with motility modifying effects. Previous research on *Lactobacillus rhamnosus* JB-1™ isolated the molecule-containing microvesicles of the bacteria and tested them for their individual effect. Application of the microvesicles to the gut epithelium replicated the effects of the JB-1™ bacteria on enteric neurons<sup>[28]</sup>. In similar fashion, further isolating components of the Snt and evaluating their effects may help to narrow down the underlying bioactive molecules.

The results of the present paper suggest that there may be other yeasts with potential therapeutic actions in animal models of stress. Our *ex vivo* perfusion setup may provide a relatively simple, though not

high throughput, method to screen yeasts or fungi for beneficial effects on the host intestine. Previously discussed results of *S. cerevisiae* and *S. boulardii* clinical trials further support the therapeutic potential of *Saccharomyces* yeasts in humans<sup>[7,11]</sup>. We predict that the beneficial or probiotic potential of fungi and yeasts is set to expand with increased research, initially in animal models followed by human trials.

## COMMENTS

### Background

Stress has adverse effects on intestinal motility causing irregular propagating contractile clusters (PCCs) that decrease motility in the jejunum and increase motility in the colon. *Saccharomyces* yeasts have been shown to have potential beneficial and probiotic effects in the intestine and in the treatment of some gastrointestinal disorders.

### Research frontiers

Most stress and probiotic research studies focus on the prevention of stress-related symptoms on the intestinal tract. The use of probiotics, including yeasts, is an emerging treatment option for gastrointestinal disorders, particularly in exchange for antibiotics.

### Innovations and breakthroughs

This is first evidence of *Saccharomyces* yeasts acting in the pre-clinical treatment of stress-related gut dysmotility. It is also the first evidence of *Saccharomyces* supernatant (yeast microbes removed) having a beneficial effect on gut motility and the treatment of stress.

### Applications

The data suggests that *Saccharomyces* yeasts may be potential therapeutic

treatments for stress-related dysmotility in the intestine. Additionally, *Saccharomyces* supernatants cause similar effects as their respective yeasts, and might also play a therapeutic role. However, more animal experiments are needed to better support these results.

### Terminology

PCCs or propagating contractile clusters are sweeping bands of intestinal contraction that move in the oral to anal direction and are likely stimulated by the ENS.

### Peer-review

The authors investigated the possible effects of *Saccharomyces boulardii* or *Saccharomyces cerevisiae* on reducing the stress-related intestinal dysmotility. The study is well designed and well implemented. The manuscript should be accepted for publication with the following revision.

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