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Saccharomyces boulardii Enhances *N*-Terminal Peptide Hydrolysis in Suckling Rat Small Intestine by Endoluminal Release of a Zinc-Binding Metalloprotease

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ABSTRACT

Saccharomyces boulardii (*S. boulardii*), a biotherapeutic agent effective in acute and chronic enterocolopathies, produces trophic intestinal effects at least in part mediated by the endoluminal release of polyamines. However, the effects of the yeast on peptide hydrolysis have not yet been studied. The objectives of this study were to assess in suckling rats the endoluminal and mucosal aminopeptidase activities in response to *S. boulardii* treatment and to analyze their related mechanisms. Peptidase activities were assayed on yeast cells by using several L-amino acid-*p*-nitroanilide substrates in the pH range of 2 to 10. A marked hydrolytic activity was found for L-leucine-*p*-nitroanilide that peaked at pH = 8 (K_m = 0.334 mM, V_{max} = 44.7 μmol·min^{-1}·g^{-1} protein). *N*-terminal peptide hydrolysis was confirmed using as substrate L-Leu-Gly-Gly (K_m = 4.71 mM, V_{max} = 18.08 μmol·min^{-1}·g^{-1} protein). Enzyme reactions were inhibited in the presence of 1 mM Zn^{2+}. Oral treatment of sucklings with *S. boulardii* significantly enhanced jejunal and ileal mucosal leucine-aminopeptidase activities by 24 and 34%, respectively, over controls. In concordance, aminopeptidase activity was enhanced in jejunal and ileal endoluminal fluid samples by 47 and 105%, respectively. By use of an IgG-purified antibody raised against the zinc-binding domain common to metalloproteases, the yeast aminopeptidase was immunoprecipitated and detected as an heteromeric enzyme of 108 and 87-kD subunits. *S. boulardii*, when given orally to suckling rats, is able to significantly enhance hydrolysis of *N*-terminal oligopeptides in both endoluminal fluid and intestinal mucosa by the endoluminal release of a leucine aminopeptidase that appears to be a zinc-binding metalloprotease belonging to the M1 family of peptidases. **(*Pediatr Res* 51: 528–534, 2002)**

Saccharomyces boulardii (*S. boulardii*) is a nonpathogenic yeast exerting therapeutic properties in acute and chronic enterocolopathies, antibiotic-associated diarrheas, and enterotoxigenic *Clostridium difficile* overgrowth (1–4).

In human volunteers (5, 6) and in growing rats (5), several studies have documented that oral treatment with a lyophilized preparation of *S. boulardii* produces trophic intestinal effects including increases in the specific and total activities of brush-border membrane (BBM) enzymes (5), enhanced secretion of s-IgA in intestinal fluid (7), and enhanced production of the receptor for polymeric Ig in villus and crypt cells (7). In addition, after oral treatment of rats with *S. boulardii*, there is a marked stimulation of sodium-dependent D-glucose uptake into BBM vesicles with a corresponding accumulation of the BBM sodium D-glucose cotransporter-1 (SGLT-1, 75 kD) (8). These trophic effects are, at least in part, mediated by endoluminal release of polyamines, as yeast cells contain substantial amounts of putrescine, spermidine, and spermine (9, 10). In addition, polyamine concentrations in mucosa and endoluminal fluid were found to be increased in proportion to the amount of spermine and spermidine supplied by the yeast (8, 9).

In neonates and young infants, the quality of endoluminal proteolysis is of great importance, conditioning the absorption of completely or incompletely degraded proteins and antigens by the mucosal barrier whose permeability is increased (11). This is one of the underlying mechanisms inducing food protein intolerance. Likewise, it has been documented that young rats with immature intestinal functions and abnormal mucosal permeability to macromolecules are confronted with more intact food antigens that bind to the enterocyte surface (12, 13). In young suckling rats, intact cow's milk proteins are degraded much less than in older rats (13), resulting in increased binding of intact cow's milk antigens to BBM (14).

So far, the effects of *S. boulardii* on intestinal mucosal peptidase activity and endoluminal peptide hydrolysis have not yet been investigated. The objectives of the present study were

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to assess whether oral treatment with lyophilized *S. boulardii* enhances endoluminal and mucosal aminopeptidase activity in suckling rat small intestine and to analyze whether *S. boulardii* produces a protease that after being released into the small intestinal lumen could upgrade hydrolysis of peptides, implying a potential benefit in some situations in which upregulation of intestinal nutrient degradation could be advantageous.

METHODS

Media and culture conditions. *S. boulardii* cells were inoculated in YPD (yeast extract, 0.5%; peptone, 2%; glucose, 2%; DIFCO, Detroit, MI, U.S.A.) media and grown at 30°C with moderate shaking to exponential growth after three replication cycles (5×10^7 cells/mL).

To disrupt the external capsid, yeast cells were concentrated ($1.45–1.50 \times 10^{10}$ cells/mL) and shaken with beads (0.45-μm diameter) in six pulses of 30 s separated by 1 min under cold CO_2 flux by using an MSK pulse apparatus (Braun, Paris, France). Viability tests revealed that approximately 100% of cells were viable with an efficiency of disrupted capsids of 96.7%. After stabilization in 0.1 M phosphate buffer (pH = 7), particulate components were removed by centrifugation (500 $\times$ *g* for 15 min at 0°C), and the supernatants were stored at −170°C in liquid nitrogen until analyzed.

Animals and treatments. The present study was approved by the Animal Welfare Committee du Fonds de Recherche Scientifique Médicale. Litters of Wistar rats were acclimatized in an air-conditioned room at 21 ± 1°C with a 12-h light-dark cycle. Immediately after birth, each litter was reduced to six pups per lactating mother to equalize conditions of nursing and feeding. *S. boulardii* was prepared in a lyophilized form (100 mg per flask, biologic activity 2.9×10^9 viable cells/mL) by the manufacturer (Laboratoires Biocodex, Montrouge, France). As previously reported (5), we used a dose of 0.5 mg of lyophilized yeast cells per gram body weight per day. The appropriate dose was administered in 0.5 mL saline by nasogastric intubation twice daily from d 11 to 14 postpartum. Control groups were treated according to the same schedule and received equal volumes of saline instead of yeast cells. Six to 10 animals per group were studied. The suckling period was chosen for the experiments because at that time the activity of rat BBM aminopeptidase is low (15, 16) and is mainly located in crypt cells (16).

Preparation of tissues. On d 14 postpartum, rats were killed rapidly by decapitation, and the small intestine from the pylorus to the ileocecal valve was immediately excised. After being rinsed in 0.9% cold saline, the total length was measured and divided into two equal segments. The proximal half was considered the jejunum and the distal half, the ileum. The mucosa from each segment was scraped off, weighed, and frozen in liquid nitrogen as described (9).

Collection of endoluminal fluid. For the collection of intestinal fluid, jejunal and ileal segments were flushed with 2 mL of cold 0.9% saline. The collected fluid was centrifuged (500 $\times$ *g*, 5 min), and the supernatants were pooled and filtered through a 0.2-μm membrane filter (Millipore) to discard yeast cells in suspension (9).

Enzyme assays. Aminopeptidase activity was assayed on suspensions of *S. boulardii* broken cells, culture media, intestinal mucosa, and intestinal fluid according to Maroux *et al.* (17) by using several substrates: L-alanine-*p*-nitroanilide, L-leucine-*p*-nitroanilide, L-methionine-*p*-nitroanilide, L-proline-*p*-nitroanilide, and L-lysine-*p*-nitroanilide in a 2-aminomethyl-propandiol buffer. Unless otherwise indicated, assays were performed at 37°C, pH = 8, for 30 min. One unit equals 1 μmol of *p*-nitroanilide formed per min/g protein. Protein content was determined by the method of Lowry *et al.* (18).

Peptide hydrolysis. To determine the hydrolytic activity of the yeast enzyme against peptides, *N*-terminal hydrolysis of the tripeptide L-Leu-Gly-Gly (4.5 mM) was assayed in a Tris-HCl 100-mM buffer (pH, 8; 37°C) in the presence of broken yeast cell suspensions at two dilutions, 1/50 and 1/500, according to the method published by Vaeth and Henning (19). After an incubation period of 30 min, the assay mixture was centrifuged, and leucine concentration was measured in the deproteinized (sulfosalicylic acid) supernate (600 μL). Leucine concentration was determined by an automated amino acid analyzer (Pharmacia LKB, Antwerpen) using the Biochromic 20 software. Results are expressed in micromoles of leucine formed per gram cell protein.

Immunoprecipitation and immunoblotting. To demonstrate the production by *S. boulardii* cells of a metalloprotease, a peptide corresponding to a highly conserved sequence of 36 amino acids was synthesized (Table 1). This sequence includes the zinc-binding domain signature common to all zinc-binding metalloproteases of family M1 (VVQHELAHQW). Rabbits were immunized with peptide AA 298 to 333 corresponding to the sequence of aminopeptidase II from *S. cerevisiae* (Table 1). A polyclonal antiserum was generated, and IgG were purified. One hundred μL of protein A sepharose-4B beads (50% suspension) (Pharmacia, LKB, Antwerpen) was mixed with 1.5 mL of purified antisera during 4 h at 4°C under gentle continuous rotation. After centrifugation (500 $\times$ *g*, 3 min), the supernate was discarded and the beads coated with the antibodies (100 λ) were mixed with 1 mL of *S. boulardii* cell suspension (viable cells, 1.5×10^{10} mL), 1 mL of 0.1 N NaOH, and 2 mL of immunoprecipitation buffer containing Tris-HCl 100 mM (pH = 8), EDTA 1 mM, PMSF 1 mM, leupeptin 1 μg/mL, and aprotinin 1 μg/mL. After overnight incubation at 4°C, under continuous rotation, the beads were recovered by centrifugation (500 $\times$ *g*, 3 min) and washed once with a washing buffer [Tris-HCl 50 mM (pH = 7.4), NaCl 100 mM, SDS 0.1%, Triton-X 100 %], followed by six washes with the same buffer without NaCl. After aspirating the excess of buffer, immunoprecipitated proteins were solubilized in 30 μL of Laemmli's buffer (20) and boiled at 100°C for 5 min. Immunoprecipitated proteins were separated by electrophoresis (7.5% SDS-PAGE) and electrotransferred to polyvinilydene difluoride (PVDF) membranes for 90 min at 50 V as described by Towbin *et al.* (21). Using ^{125}I-labeled protein A (Amersham, Ghent), bound antimetalloprotease antibodies were detected by autoradiography using 24 $\times$ 30-cm Fuji film (St. Nicolas, Belgium) at −70°C for 12 to 72 h (22).

Table 1. *Comparison of identities and zinc-binding domains among human, rat, and* S. cerevisiae *M1 family of aminopeptidases*

Enzyme	Longest sequence with identities	No. of identities
Human intestinal aminopeptidase N (EC 3.4.11.2)	RVVTVIAHELAHQWFGNLVTIEWWNDLWLNEGFASY | | | | | 380 385 395 405 415	(35/35)
Rat intestinal aminopeptidase N (EC 3.4.11.2)	RVVTVIAHELAHQWFGNLVTVDWWNDLWLNEGFASY | | | | | 379 384 394 404 414	(33/35)
Alanine/arginine aminopeptidase (I) yeast, S. cerevisiae (EC 3.4.11.-)	RVAEVIQHELAHQWFGNLVTMDWWEGLWLNEGFATW | | | | | 293 298 308 318 328	(26/35)
Aminopeptidase II (YSCII) yeast, S. cerevisiae (EC 3.4.11.-)	RVAEVVQHELAHQWFGNLVTMDWWEGLWLNEGFATW | | | | | 298 303 313 323 333	(25/35)

_____ zinc-binding domain signature.

Calculations and statistics. All data are given as mean ± SD. If not indicated, SD represents less than 5% of the mean. Differences between controls and *S. boulardii*-treated rats were tested for statistical significance ($p < 0.05$) by using the nonparametric Mann-Whitney *U* test. K_m and V_{max} were calculated using the Prism 3.0 (Graphpad) automated program. Protein band intensities were quantified by densitometry using an image densitometer (CS-690, Bio-Rad). Relative abundance was expressed in arbitrary volume units (OD × mm^2). Relative molecular weights were estimated by densitometry and comparative migration with proteins of known molecular weights.

RESULTS

Aminopeptidase activity in suspensions of* S. boulardii *cells and in culture media. Figure 1 represents changes in the specific activity of yeast aminopeptidase measured in 1.5 × 10^{10}/mL viable cells using different substrates (L-alanine, L-leucine-, L-methionine-, L-lysine-, and L-proline-*p*-nitroanilide) at different pH (2–10) in the incubation media at 37°C. No activity was detected when L-alanine-*p*-nitroanilide was used as substrate, regardless of the pH of the incubation medium. L-Methionine-*p*-nitroanilide revealed some activity at low pH with a peak at pH 3 but very low activities in neutral or basic pH ranges. L-Lysine- and L-proline-*p*-nitroanilide produced intermediate activities over the whole pH range, which peaked at pH 4. L-Leucine-*p*-nitroanilide provided some activity in the acid range, which raised sharply at pH 6 to reach a major peak of activity at pH 8. All these substrates were used at the same concentration (4.5 mM). Addition in excess of 1 mM $ZnCl_2$ to the medium abolished the reaction (data not shown), demonstrating that the enzyme is a zinc-binding protease. Because L-leucine-*p*-nitroanilide provided the highest activity at pH 8, all subsequent enzyme assays were performed with this substrate. In comparison, human and rat aminopeptidase N, two membrane metalloproteases belonging to the M1 family, are most active at pH 8 (23). Their activities are routinely measured using L-alanine or L-leucine-*p*-nitroanilide substrates, with a K_m of 2.86 mM for L-alanine (24) and 0.647 mM for L-leucine substrates, respectively (25). Using L-leucine-*p*-nitroanilide as substrate, K_m of the leucine aminopeptidase of *S. boulardii* cells (Fig. 2, left panel) was 0.334 ± 0.03 mM with a V_{max} of 44.7 ± 1.16 μmol of *p*-nitroanilide formed per min/g cell protein. As shown in Figure 2, the reaction was

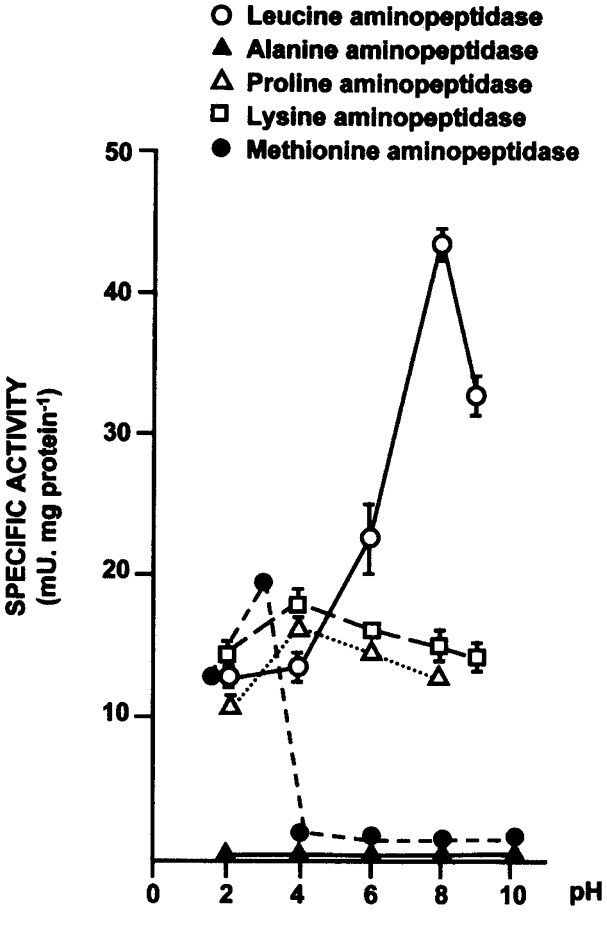

Figure 1. Changes in specific enzyme activities measured in *S. boulardii* cell suspension according to different *N-x-p*-nitroanilide substrates (4.5 mM) at different pH of the incubation media. Each value represents mean ± SD of five individual assays. If not depicted, SD represents less than 5% of the mean.

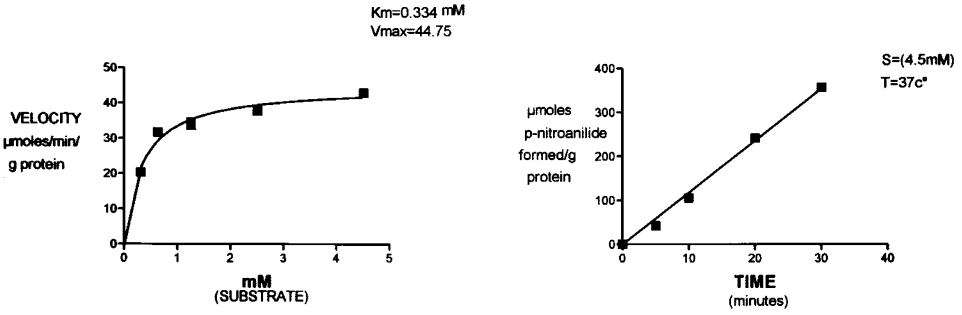

Figure 2. Kinetic parameters of leucine aminopeptidase measured in *S. boulardii* cell suspension using L-leucine-*p*-nitroanilide as substrate. (*Right panel*) Enzyme reaction is linear with time. (*Left panel*) Velocity according to substrate concentration. Each point represents the mean of three to four individual measurements.

linear with time (right panel) and enzyme concentration (not shown).

Before growth of *S. boulardii* in its appropriate YPG culture medium, no activity of leucine aminopeptidase could be detected (Fig. 3, left panel), whereas after exponential growth, a small amount of enzyme activity was measured into the medium, corresponding to a release of approximately 1/5000 of the total enzyme activity produced by the total mass of yeast cells in culture (Fig. 3, left and central panels). The specific activity of leucine aminopeptidase expressed per gram of yeast cell protein averaged 43 ± 2 U/g protein (Fig. 3, central panel).

Peptide hydrolysis. To determine whether the leucine aminopeptidase detected in *S. boulardii* cells hydrolyses the *N*-terminal amino acid of oligopeptides, the tripeptide L-Leu-Gly-Gly was used *in vitro* as substrate (4.5 mM) in the presence of yeast cells. As shown in Figure 4, the amount of leucine produced per gram of cell protein was linear with time and enzyme concentration (dilutions 1/50 and 1/500). Kinetic analysis at different peptide concentrations revealed a lower affinity of the enzyme for oligopeptides than for nitroanilide derivatives because a K_m of 4.71 ± 0.47 mM was estimated with a V_{max} of 18.08 ± 0.85 µmol L-leucine formed per min/g cell protein. The same observation was reported for the affinity of rat aminopeptidase N to L-leucine-*p*-nitroanilide (K_m = 0.647 mM) (25) and L-Ala-Gly (K_m = 3.4 mM) (26).

Effects of* S. boulardii *on suckling rat small intestine. There was no difference between treated and control groups regarding initial and final body weight, intestinal length, and jejunal and mucosal weight. Changes in jejunal aminopeptidase activity using L-leucine-*p*-nitroanilide as substrate are shown in Figure 5. Compared with control pups, *S. boulardii*-treated rats exhibited significant ($p < 0.01$) increases in the specific (24% *versus* controls) and total activities of aminopeptidase whether the total activity was expressed per centimeter of gut length (23% *versus* controls) or per total jejunum (22% *versus* controls). Similarly, the specific and total activities of ileal aminopeptidase were enhanced in *S. boulardii*-treated rats by 34 and 39%, respectively, compared with saline-treated controls (Fig. 6). It is noteworthy that the total activity of aminopeptidase measured in 1.5 × 10^{10} viable *S. boulardii* cells (Fig. 3, middle panel) was 2-fold higher than the total activity of the enzyme measured in ileal mucosal samples (Fig. 6).

To demonstrate that the increase in jejunal and ileal mucosal aminopeptidase activities was related to the release of a leucine

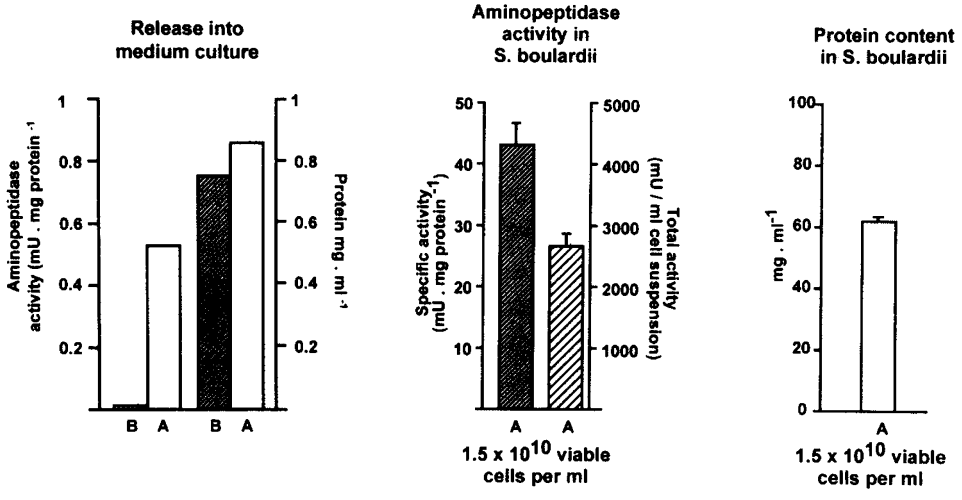

Figure 3. (*Left panel*) Detection of leucine aminopeptidase activity is figured on the left side and protein concentration in the culture media of *S. boulardii* cells (2 × 10^{10} cells/mL) on the right side before (*B, stippled bars*) and after (*A, open bars*) exponential growth. Values represent mean ± SD of 10 individual assays. (*Center panel*) Specific (*closed bars*) and total (*stippled bars*) activities of leucine aminopeptidase measured in samples of *S. boulardii* cells (2 × 10^{10} viable cells/mL). Values are mean ± SD of 10 individual assays. (*Right panel*) Protein content of *S. boulardii* cells (2 × 10^{10} viable cells). Values are mean ± SD of 10 individual assays.

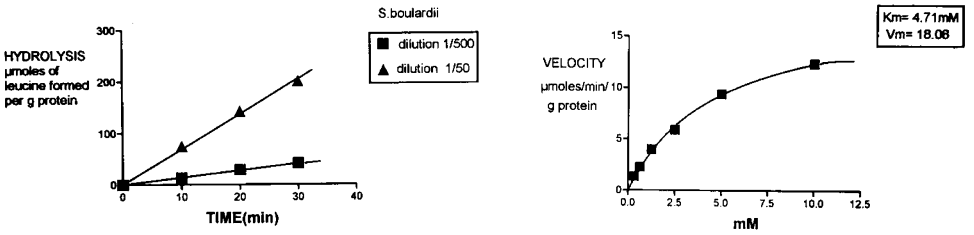

Figure 4. Kinetic parameters of Leu-Gly-Gly peptide hydrolysis measured in *S. boulardii* cell suspensions using L-Leu-Gly-Gly peptide as substrate. (*Left panel*) Enzyme reaction is linear with time and enzyme concentration. (*Right panel*) Velocity according to substrate concentration. Each point represents the mean of three to four individual measurements.

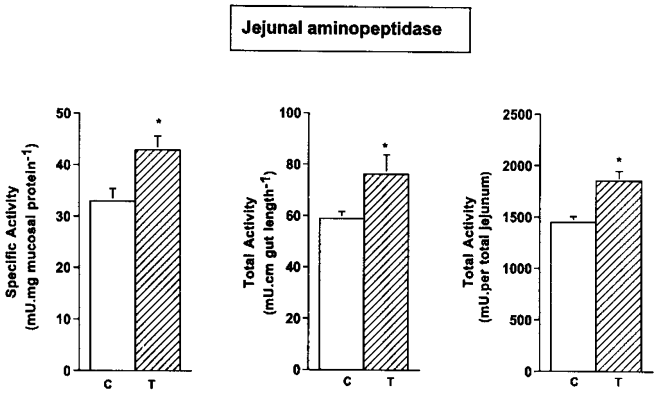

Figure 5. Changes in the specific and total activities of aminopeptidase measured in the jejunal mucosa of suckling pups (d 14 postpartum) treated with a lyophilized preparation of *S. boulardii* (0.5 mg/g body weight) or with saline. Each value represents mean ± SD of nine individual animals. $*p < 0.05$; $**p < 0.01$.

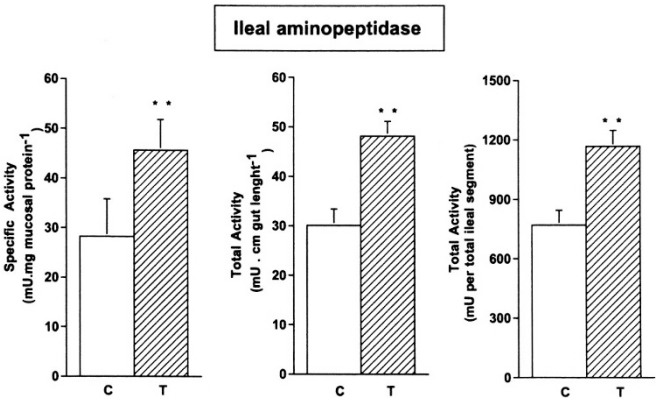

Figure 6. Changes in the specific and total activities of aminopeptidase measured in the ileal mucosa of suckling pups (d 14 postpartum) treated with a lyophilized preparation of *S. boulardii* (0.5 mg/g body weight) or with saline. Each value represents mean ± SD of nine individual observations. $**p < 0.01$.

aminopeptidase by yeast cells, the enzyme activity was determined in jejunal and ileal endoluminal fluid samples collected and filtered from sucklings treated with *S. boulardii* or with equivalent volumes of saline. The results are shown in Table 2. Compared with control rats, leucine aminopeptidase activity was enhanced by 47% ($p < 0.05$) in the jejunal fluid and by 105% ($p < 0.01$) in the ileal fluid of *S. boulardii*-treated rats without changes in protein concentration.

***Immunoprecipitation of aminopeptidase from* S. boulardii.** To identify the enzyme of *S. boulardii* cells, we immunoprecipitated the corresponding protein from a pool of yeast cells (1.5×10^{10} cells/mL) containing 62 mg protein/mL using an IgG-purified polyclonal antibody raised against a sequence of aminopeptidase II from *S. cerevisiae* (Table 1). The autoradiography shown in Figure 7 demonstrates two single bands of 108 and 87 kD, respectively (relative molecular weights). Relative abundance (OD × mm^2) of the 108-kD protein was estimated to be 1.74 and that of the 87-kD protein 11.54.

DISCUSSION

Among the major types of proteases, metalloproteases are the most diverse with more than 30 families identified to date (27). Of these, approximately half contain the HEXXH motif, which has been shown to form part of the metal-binding domain (27). Zinc-binding metalloproteases can be grouped together as a superfamily known as the metzincins on the basis of their sequence similarity (27). Family M1 includes bacterial aminopeptidase N (EC 3.4.11.2), mammalian aminopeptidase N (EC 3.4.11.2), mammalian glutamyl aminopeptidase (EC 3.4.11.7) or aminopeptidase A, leukotriene A-4 hydrolase (EC 3.3.2.6) (28), alanine/arginine aminopeptidase from *S. cerevisiae* (gene AAP1) (29), and aminopeptidase II (gene APE2) from *S. cerevisiae* (30). The similarities between the sequences of human, rat, and yeast aminopeptidases that contain the

Table 2. *Aminopeptidase activity in jejunal and ileal fluids (2 mL) collected from suckling rats (d 14) treated with* S. boulardii *or saline*

	Controls	*S. boulardii* treated	Δ%[a]	P[b]
No. of rats	9	9		
Jejunal aminopeptidase (mU/mg protein)	5.21 ± 0.47[c]	7.69 ± 1.84	+47	< 0.05
Protein (mg/mL)	1.59 ± 0.06	1.57 ± 0.05	−2	ns[d]
Ileal aminopeptidase (mU/mg protein)	17.27 ± 0.58	35.50 ± 6.90	+105	< 0.01
Protein (mg/mL)	0.93 ± 0.14	0.92 ± 0.15	−2	ns

[a] Variation in percentage of treated group compared with control group.
[b] Probability *vs* controls.
[c] Data are mean ± SD.
[d] ns = not significant.

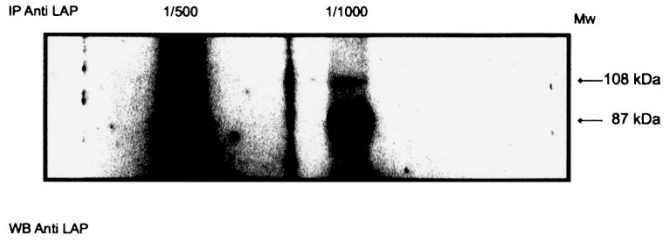

Figure 7. Autoradiography of immunoprecipitated proteins of *S. boulardii* immunoblotted with a specific antibody recognizing the zinc-binding domain of aminopeptidases of family M1. Note the presence of two subunits at 108 and 87 kD (relative molecular weights), respectively. Abundance of the 108-kD subunit is 1.74 (OD U × mm^3), whereas the abundance of the 87-kD subunit is 11.54 (OD U × mm^2). *Left lane*, antibody dilution for Western blot was 1/1000; *right lane*, antibody dilution was 1/500.

zinc-binding motif are shown in Table 1. Alanine/arginine aminopeptidase and aminopeptidase II from *S. cerevisiae* have been identified by genomic DNA sequencing, but the corresponding proteins have not yet been characterized (29, 30).

In the present study, we found that cultured *S. boulardii* expressed a peptidase activity that was highest at neutral or slightly basic pH for leucine-bound substrates. K_m of the enzyme for L-leucine-*p*-nitroanilide was 0.336 mM, which is lower than the K_m of rat aminopeptidase N for the same substrate (0.647 mM). In addition, the enzymatic reaction was inhibited in the presence of an excess of Zn^{2+}, which suggests that this peptidase belongs to the M1 family of zinc-binding metalloproteases. Aminopeptidase II from *S. cerevisiae* is believed to be involved in the cellular supply of leucine from externally offered leucine-containing di- or tripeptide substrates (30). We found similar properties of *S. boulardii* peptidase against the peptide L-Leu-Gly-Gly. *In vitro*, leucine was hydrolyzed proportionally to the amount of enzyme and to the duration of the reaction. K_m of the enzyme for L-Leu-Gly-Gly was 4.71 ± 0.47 mM, indicating a lower affinity for oligopeptides than for nitroanilide derivatives. Similar findings have been published for microvillus aminopeptidase N (25, 26). In a rat model of small bowel resection, Zaouche *et al.* (31) found that rats treated with *S. boulardii* exhibited an increase in aminopeptidase-N activity, but the reason for this increase remained unknown.

In infants and suckling pups, mucosal aminopeptidase activity is very low and progressively increases during the weaning period to reach peak levels at the end of the first month postpartum (15, 16). Compared with age-matched controls, administration to suckling rats of a lyophilized preparation of *S. boulardii* significantly enhanced leucine aminopeptidase activity in both the jejunal and ileal mucosa as well as in endoluminal fluid, implying that the yeast peptidase activity was preserved in lyophilized preparations, resisted gastric acidity and bilipancreatic secretions, and was released in endoluminal fluid, presumably by disruption of the capsid by glycanases produced by the microflora. Figure 1 shows that without capsid, approximately 25% of the enzyme activity was preserved in the acid range between pH 2 to 4 using L-leucine-*p*-nitroanilide as substrate. Concordant findings were published by Pothoulakis *et al.* (32) who demonstrated that *S. boulardii* secretes *in vivo* a protease of approximately 54 kD that was resistant to acidity and was able to inhibit binding of purified [^{3}H]-labeled *C. difficile* enterotoxin A to rabbit ileal BBM by 37%, to reduce enterotoxin-induced fluid secretion by 55% in rat ileal loops, and to decrease mannitol permeability by 93% (32, 33).

The autoradiography shown in Figure 7 demonstrates that two single bands of 108 and 87 kD, respectively (relative molecular weights), were detected. These proteins presumably represent subunits of the yeast aminopeptidase because, in rat and human BBM, aminopeptidase N is a tetrameric (enzyme I) or dimeric (enzyme II) enzyme with α- and β-subunits (α_1 α_2) (α = 91 kD) or (α_1 β_1) (β = 71 kD) (23). The 87-kD subunit was 6.5-fold more abundant than the 108 kD.

Preliminary reports indicate that oral administration of bacterial or yeast biotherapeutic agents to infants may have a preventive effect on the development of chronic persistent or chronic protracted diarrhea after acute gastroenteritis, presumably by intestinal immune reactions against food allergens (3, 34). For instance, *Lactobacillus casei* strain GG, given orally to children with Crohn's disease, significantly increases the number of cells secreting specific IgA to β-lactoglobulin and to casein (35). In the case of oral treatment with *S. boulardii*, several mechanisms may be involved including immunostimulatory effects of the gut-associated-lymphoid tissue (GALT) system with enhanced secretion of specific s-IgA (7), inhibition of toxin binding to enterocyte receptors by proteolysis (32), and endoluminal production of polyamines (9) that decrease intestinal permeability and stimulate BBM enzymes (5) and transporters (8). In the present study, we show that the endoluminal release of an aminopeptidase by *S. boulardii* upgrades endoluminal *N*-terminal hydrolysis of oligopeptides that could be potentially important in preventing reactions to food antigens when mucosal permeability is increased. Further work is warranted to confirm this mechanism in infants and children.

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