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Determinants in Microbial Colonization of the Murine Gastrointestinal Tract: pH, Temperature, and Energy-Yielding Metabolism of *Torulopsis pintolopesii*

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Torulopsis pintolopesii is an indigenous yeast that colonizes the secreting epithelia in the stomachs of mice and rats. A wild-type strain of this microbe was isolated and identified. To attempt to learn characteristics of the yeast that are advantageous to it in colonizing its natural habitat in vivo, we examined some aspects of its nutrition and energy-yielding metabolism and some environmental conditions that influence its growth in vitro. The yeast appeared to be limited in the compounds it can utilize as carbon and nitrogen sources. It grew best at 37°C and did not grow at 23 or 43°C. It grew optimally at neutral pH but could grow aerobically at pH values as low as 2.0 and anaerobically at pH values as low as 3.4. As assessed by measurements of growth rates and yield coefficients, it grew better aerobically than anaerobically. When grown aerobically, it had a cyanide-sensitive system for taking up O_2 and tested positively for cytochrome *c* oxidase activity. A petite mutant strain isolated from the wild-type strain had a growth rate and yield coefficient when incubated aerobically that were essentially the same as those of the wild-type parent grown anaerobically. Likewise similar to the wild-type parent grown anaerobically, the petite strain, though incubated aerobically, did not take up O_2. Yeast-free mice associated with either the wild-type or the petite mutant strain were colonized at essentially the same rates and to similar final population levels by both strains. The yeast's capacity to respire may be of little advantage to it in its natural environment. By contrast, its abilities to grow best at 37°C and to grow at low pH values are undoubtedly advantageous characteristics in this respect. The limitations in its carbon and nitrogen nutrition are difficult to evaluate as ecological factors in its colonization of the natural habitat.

Indigenous microorganisms are known to form layers on the surfaces of the epithelia in the gastrointestinal tracts of animals of numerous types (12, 14). Little is known, however, about the mechanisms by which the microbes involved enter, remain, and obtain energy for growth in their epithelial habitats (14).

For technological reasons largely, the environmental and nutritional conditions influencing microbial growth in epithelial microhabitats in the gastrointestinal tract are difficult to study. Most epithelial habitats in the tract exist in layers of mucus on the glycocalyces directly overlying epithelial cells. Such areas could differ dramatically from habitats in the lumen in conditions influencing survival and growth of microorganisms (12, 14). Thus, findings from determinations of various parameters of environment and nutrition in lumen content (for example, pH or glucose content) may or may not be indicative of conditions on the epithelial surfaces.

We are interested in learning the nutritional and environmental conditions for which microorganisms are adapted in the various epithelial habitats in the murine gastrointestinal canal. One such habitat, the surface of the secreting epithelium of the stomach, is colonized in adult conventional mice by a particular yeast, *Torulopsis pintolopesii* (11, 15). This yeast is found in nature only in the tracts of rodents or in soil or structures in areas frequented by such animals (10, 23). Its populations do no obvious harm to their animal hosts. They induce no unusual inflammatory response in the gastrointestinal mucosa in colonized animals and are not pathogenic in mice when injected intravenously or intraperitoneally even at high levels (D. C. Savage, unpublished data). The yeast must be highly adapted, therefore, for growth in the murine gastrointestinal tract.

The population levels of the yeast in the rodent tract are always highest in the region of the

gastric secreting mucosa (15). We hypothesize that the yeast is best adapted for growth on the secreting epithelium. To attempt to test that hypothesis, we studied some aspects of its nutrition and energy-yielding metabolism and some of its environmental requirements.

MATERIALS AND METHODS

Animals. Four-month-old specific pathogen-free female and 41-day-old male Crl:COBS CD-1 (ICR)BR mice free of *T. pintolopesii* and female C57BL/6NCrlBR mice with the yeast colonizing their stomachs (Charles River Breeding Laboratories, Wilmington, Mass.) were housed in barrier cages with Ab-Sorb-Dri (Allied Mills, Chicago, Ill.) as bedding. They were fed autoclave-sterilized Lab-Blox (Allied Mills) and given acidified tap water (0.001 M HCl) ad libitum.

Isolation and identification of yeast strains. A yeast strain, designated wild type, was isolated from the stomach of a C57 mouse on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) supplemented with 40,000 U of penicillin and 0.04 g of streptomycin per liter. A petite mutant strain was isolated from the wild-type strain growing on a plate of the Sabouraud medium (containing antibiotics) that had been covered with triphenyltetrazolium chloride overlay (1). The strains were grown routinely in yeast-peptone-glucose (YPG) broth medium and stored in the lyophilized state or on YPG agar slants at 4°C.

The wild-type strain was identified by procedures described by van Uden and Vidal-Leiria (24) and by Kockova-Kratochvilova (5). Deoxyribonucleic acid (DNA) for determination of the guanosine plus cytosine content of the yeast was isolated (19) from the wild-type strain grown in YPG broth. Forty micrograms of the DNA mixed with 24 μg of *Micrococcus lysodeikticus* DNA (for a standard) in CaCl-tris(hydroxymethyl)aminomethane buffer solution was centrifuged in a CsCl gradient on a Spinco model E ultracentrifuge at 44,000 rpm for 17 h, until the system reached equilibrium. A microdensitometer tracing was made, and the buoyant density was calculated from the equation:

$$e = e_o + 0.0089 \left(\frac{1.4(r)}{y} + 5.81\right)^2 - \left(\frac{1.4(r_o)}{y} + 5.81\right)^2$$

where e is the buoyant density of yeast DNA, e_o is the buoyant density of standard DNA (1.731), y is the cell length as measured by microdensitometer tracing, r_o is the distance from the top of the cell to the standard band, and r is the distance from the top of the cell to the sample band. The value of e obtained was then used to determine the moles percent guanine plus cytosine in the DNA from the equation (18) $\rho = 0.0988(GC) + 1.660$ g/cm^3, where ρ is the density of the yeast DNA and GC is the moles percent guanine plus cytosine.

Nutritional tests. Tests for assimilation of carbon sources were made with broth media containing 1% peptone (Difco), 0.5% yeast extract, and one of several compounds (listed below) at concentrations of 2%. Fermentation tests were made with the same media but with 0.08% bromothymol blue added as an indicator. Nitrogen assimilation tests were conducted in yeast nitrogen base (Difco) with various nitrogenous compounds added.

Environmental tests. For tests of the effects of pH on its growth, the wild-type strain was grown aerobically and anaerobically in a defined medium, basal medium (2), adjusted to various pH values. Anaerobic incubation was accomplished in an anaerobic chamber (Coy Manufacturing Co., Ann Arbor, Mich.) with a nitrogen-hydrogen (90:10) atmosphere. For tests of the effects of temperature on its growth, the microbe also was grown in basal medium adjusted to pH 7.0.

Yield coefficient determination. Yield coefficients were determined for the wild-type and petite mutant strains. Wild-type cells were grown both aerobically and anaerobically in a broth medium containing 0.5% yeast extract, 1% peptone (Difco), 0.02% ergosterol, 0.5% Tween-80, 0.05% resazurin, and D-glucose at various growth-limiting concentrations, ranging from 0.2 to 2.0 mg/ml. Petite mutant cells were grown in the same media incubated aerobically. For aerobic incubation, flasks containing inoculated media were shaken vigorously on a rotary shaker at 37°C. For anaerobic incubation, flasks containing the media were stoppered with cotton and placed into the anaerobic chamber (Coy) containing a nitrogen-hydrogen (90:10) atmosphere until the resazurin in the media was reduced. They were then inoculated, stoppered with rubber stoppers, removed from the chamber, and shaken on the rotary shaker at 37°C. All cultures were incubated for 4 days (maximum growth was always reached within 24 to 48 h; lysis of the yeast cells could not be detected by direct cell count within 48 h thereafter), after which the cells were harvested, washed three times with water, and dried to constant weight in an oven at 100°C.

End product analysis. Wild-type cells were grown aerobically and anaerobically in broth containing 0.5% yeast extract, 1% peptone (Difco), 0.5% Tween-80, 0.02% ergosterol, and 0.177% D-glucose (complex medium) or in basal medium (2) containing 0.5% Tween-80, 0.002% ergosterol, and 0.177% D-glucose (defined medium). Petite mutant cells were grown aerobically in the complex and basal media. The glucose was added to the media after autoclaving. All media were incubated at 37°C on a rotary shaker as described above for yield coefficient determinations. Media in which cells were growing aerobically were exposed to a continuous flow of sterile air. The effluent air was passed through a solid CO_2 trap. The condensate so generated was added to the medium from which cells had been removed before end product analysis. Growth was followed by measurement of optical density at 580 nm on a Spectronic 20 colorimeter (Beckman Instruments, Inc., Fullerton, Calif.). End products were assayed on cell-free medium after growth had stopped. Residual glucose was assayed by the Nelson test (9). Ethanol was assayed with alcohol dehydrogenase (Sigma Chemical Co., St. Louis, Mo.). Volatile and nonvolatile fatty acids were tested for by gas-liquid chromatography (Dohrman Envirotech, Mountain View, Calif.) against known standards.

Growth rate. Growth rates of the wild-type strain incubated aerobically and anaerobically were deter-

mined in the basal medium (2) with 0.5% Tween-80 and 0.02% ergosterol added. For the same purpose, the petite mutant strain was grown in the medium incubated aerobically. Cultures were grown aerobically in 250-ml side arm flasks with reaction volumes of 20 ml. The flasks were shaken on a rotary shaker at 37°C. Cultures were incubated anaerobically at 37°C in tubes closed with rubber stoppers. Growth was estimated as changes in optical density (determined as described above) and translated into viable population levels with standard curves.

Cytochrome *c* oxidase activity. Cytochrome *c* oxidase was tested for in yeast cells grown aerobically and anaerobically in broth medium containing 2% D-glucose, 1% peptone (Difco), 0.5% yeast extract, 0.5% Tween-80, 0.002% ergosterol, and 0.05% resazurin incubated on a rotary shaker at 37°C. The cells were harvested by centrifugation and broken in a French pressure cell at 1,000 lb/in^2. The broken cells were subjected to a number of differential centrifugations. The fraction that sedimented at 25,000 × *g* but stayed in the supernatant at 5,000 × *g* was used for the assay (22). Reagents included 0.1 M phosphate buffer (pH 7), reduced 0.1% cytochrome *c*, and the cell extract. Cytochrome *c* was reduced with sodium hydrosulfite crystals. Blanks consisted of whole cells and buffer. Protein determinations were made of the extract with the Folin-Lowry assay (7).

Oxygen uptake. Oxygen uptake by the yeast strains was measured with an oxygen meter (model 54; Yellow Springs Instrument Co., Yellow Springs, Ohio). The reaction volume was 2.54 ml. A 2.15-ml amount of oxygenated 0.01 M phosphate buffer (pH 6.8), 0.25 ml of 6-mg/ml D-glucose, and 0.1 ml of whole cells were added to the reaction vessel. The control was a reaction vessel containing only the oxygenated buffer. The oxygenated buffer was prepared by stirring the solution vigorously at 37°C. The effects on oxygen uptake by the strains of rotenone (0.01 M), amobarbital (0.05 M), sodium azide (2.5 M), and potassium cyanide (0.07 M) were tested. These reagents were added to the reaction mixture in place of the buffer. Estimates of the number of yeast cells in the whole cell preparation were made by serial dilution and spread plate techniques.

Yeast association of mice. The petite mutant and wild-type strains were grown in YPG for 24 h. In the first experiment, 50 4-month-old, yeast-free CD-1 specific pathogen-free male mice were divided into two groups of 25 each. Mutant cells were poured into the acidified drinking water of one group, and wild-type cells were poured into that of the other group. Yeasts remained in the water for 24 h, after which the water was changed. Ten mice were kept yeast free as controls. At 24, 48, 72, 120, and 240 h after they were exposed to the yeasts, 5 mice from each group were killed (chloroform). Their stomachs were removed, rinsed free of contents with phosphate-buffered saline, weighed aseptically, and dropped into a grinding tube containing sufficient sterile water to make a 1:10 dilution. The tissue was ground to form a homogeneous suspension with a tissue grinder (Tri-R Instruments, Inc., Rockville Centre, N.Y.). Estimates of the population levels of the yeasts in the suspensions were made by serial dilution and spread plate techniques on Sabouraud agar with 0.5% yeast extract, penicillin, and streptomycin added or on an agar medium consisting of 1% peptone (Difco), 0.5% yeast extract, and 2% carbon source (sodium lactate, starch, D-fructose, D-glucose, D-lactose, D-mannose, D-galactose, sucrose, arabinose, or maltose). To test reversion of petites to the parental form, all the colonies were subjected to triphenyltetrazolium chloride overlay.

In the second experiment, 24 4-month-old, yeast-free CD-1 specific pathogen-free male mice were divided into two groups of 12 each. Again, one group was given mutant cells of the yeast, whereas the other was given wild-type cells as described. In this experiment, however, all of the animals were killed and sampled for *T. pintolopesii* 240 h after receiving the strains. The sampling and culture procedures were accomplished as described for the first experiment.

RESULTS

Identification of strains. Colonies of the wild-type strain of *T. pintolopesii* growing on Sabouraud agar (Difco) supplemented with penicillin and streptomycin were smooth and well delineated. After 24 h of growth they were about 0.1 mm in diameter, and after 48 h they were about 0.9 mm in diameter. Rudimentary pseudomycila were present in most cases when the microbe was grown in YPG. Occasionally, an arthrospore was observed. No ascospores were observed on corn meal agar or potato plugs (24). Individual cells varied in size; giant cells were observed infrequently. When the yeast was grown on media containing D-glucose at growth-limiting concentrations, small mycelial forms were observed. These forms were similar to some observed in vivo in stomachs of mice colonized by the yeast (20). No basidiospores were observed. The microbe reproduced by asexual budding.

Growth of the organisms in YPG was inhibited by ethanol, sodium lactate, and sodium tartrate at concentrations greater than 4, 2, and 2%, respectively. Likewise, growth in the medium was inhibited by cycloheximide at concentrations as low as 0.004 mg/ml. However, the organisms grew well in the medium with chloramphenicol added, even at concentrations of 4 mg/ml. The yeast could not grow in YPG containing 10% NaCl but grew slightly in the medium containing 5% NaCl. It produced acid when growing in YPG supplemented with glucose, hydrolyzed esculin to a minor degree, and was oxidase positive. It did not produce starch; hydrolyze fat, gelatin, or casein; ferment lactose; reduce nitrate; or produce indole, H_2S, or acetoin. The density of its DNA as revealed by bouyant density centrifugation was 1.69 g/cm^3. As calculated, the guanine plus cytosine content of the DNA was 30.4 moles percent. These findings indicated that the microbe was a strain of

T. pintolopesii. The guanine plus cytosine content compared well with that of 32.4 moles percentage for *T. pintolopesii* DNA reported by Nakase and Komagata (8).

Influence of temperature and pH on growth. The wild-type strain grew in basal medium at 30 and 37°C (final population levels after 48 h of incubation were approximately 3 $\times 10^6$ and 1×10^7 cells per ml, respectively), but not at all at 23 or 43°C. It grew at pH values from about 2.0 to 9.0 in media incubated aerobically but only at pH values from about 3.4 to 7.4 in media incubated anaerobically (Table 1).

Carbon and nitrogen nutrition. D-Glucose, D-mannose, and D-fructose were fermented and assimilated by the wild-type strain in the media used. The following compounds were neither assimilated nor fermented: D-mannitol, D-maltose, D-galactose, lactose, sucrose, glycerol, sodium pyruvate, sodium lactate, ethanol, D-(+)-arabinose, D-(+)-trehalose, sodium acetate, D-sorbitol, D-(+)-cellobiose, dulcitol, ribose, xylose, raffinose, inositol, L-rhamnose, starch, sodium gluconate, sodium citrate, salicin, sodium succinate, ethylene glycol, erythritol, and malate.

The organisms assimilated as nitrogen sources only $(NH_4)_2SO_4$, L-asparagine, L-glutamine, sodium-L-glutamate and DL-methionine. The final population levels after 48 h of incubation in yeast nitrogen base with the nitrogen source added were 7.5×10^6 cells per ml for $(NH_4)_2SO_4$, 9.0×10^6 cells per ml for L-asparagine, 9.2×10^6 cells per ml for L-glutamine, 8.9×10^6 cells per ml for L-glutamate, and 8.0×10^6 cells per ml for L-methionine. KNO_3, L-glycine, L-lysine, L-phenylalanine, and L-tryptophan were not assimilated as nitrogen sources by the organisms.

Yield, growth rate, oxygen uptake. Wild-type cells of the yeast grown aerobically had greater yield coefficients and growth rates and took up O_2 at faster rates than did wild-type cells grown anerobically or petite mutants incubated aerobically (Table 2). Uptake of oxygen by the wild-type strain was inhibited completely by KCN and sodium azide but was not affected by rotenone or amobarbital at the concentrations tested. The petite mutant incubated aerobically and the parental wild-type cells grown anaerobically behaved almost identically in the tests of growth rate, yield and O_2 uptake. The petite cells were not tested for cytochrome *c* oxidase.

TABLE 1. *Growth of T. pintolopesii wild type in media adjusted to various pH values*

Incubation	pH		No. of cells $(\times 10^{-6})$/ml[a]
	Before[b]	After[c]	
Aerobic	9.2	8.7	8.0
	8.6	8.1	8.5
	7.4	7.2	9.4
	7.0	6.4	10.0
	5.5	5.3	10.2
	4.7	4.2	10.7
	3.4	3.1	9.8
	2.5	2.4	9.5
	2.0	1.9	9.0
	1.6	1.5	4.5
	1.1	1.1	NG[d]
Anaerobic[e]	9.2	9.0	4.1
	8.6	8.4	4.8
	7.4	7.3	6.1
	7.0	6.6	9.5
	6.5	5.4	8.3
	4.7	4.4	8.5
	3.4	3.1	6.7
	2.5	2.4	3.2
	2.0	2.0	NG
	1.6	1.6	NG
	1.1	1.1	NG

[a] Estimated as optical density at 580 nm and translated from standard curves.
[b] pH in basal medium before incubation.
[c] pH in medium after incubation for 48 h at 37°C.
[d] NG, No growth.
[e] Incubated in an atmosphere of N_2–CO_2 (90%:19%).

TABLE 2. *Yield coefficient, doubling times, oxygen uptake activities, and cytochrome c oxidase activities of the wild-type strain of T. pintolopesii grown aerobically and anaerobically and a petite mutant strain grown aerobically*

Strain	Growth	Test			
		Yield coefficient (g[dry wt]/mol of glucose)[a]	Growth rate (doubling time [min])[b]	O_2 uptake activity (nmol/min per mg of protein)[c]	Cytochrome *c* oxidase activity (nmol of cytochrome *c* oxidized/min per mg of protein)
Wild type	Aerobic	76	98	111 ± 15	32
Wild type	Anaerobic	23.3	164	2 ± 1.5	0
Petite	Aerobic	21.6	158	0	NT[d]

[a] Yield coefficients were determined three times and were essentially the same each time.
[b] Doubling times were determined twice and were essentially the same each time.
[c] Arithmetic mean ± standard deviation.
[d] NT, Not tested.

End products of fermentation. Ethanol and some acetate were detected in fluids from cultures containing D-glucose as a carbon and energy source in which the wild-type strain of *T. pintolopesii* had grown aerobically or anaerobically. No volatile fatty acids other than acetate or nonvolatile compounds were detected. No effort was made to estimate accurately the yield from glucose of the two end products. We did note, however, that the wild-type strain produced much more ethanol than acetate when growing aerobically or anaerobically and much more ethanol per amount of glucose utilized when growing anaerobically than when growing aerobically. The petite mutant derivative also produced only ethanol and acetate from glucose.

In vivo yeast population. Yeasts recovered from mice associated with the wild-type *T. pintolopesii* produced colonies that turned red when covered with triphenyltetrazolium chloride overlay and grew only on media containing D-glucose, D-fructose, or D-mannose. Animals associated with the petite variant yielded on culture yeasts that did not give red colonies under triphenyltetrazolium chloride overlay and grew on media containing only D-glucose, D-fructose, or D-mannose. The parental and petite variants colonized their hosts at essentially the same rates and to similar maximum population levels (Table 3).

DISCUSSION

Few of the microbial types indigenous to the mammalian gastrointestinal tract are able to generate energy by aerobic respiration. Indeed, as is well known, most of the bacterial species present in the ecosystem are strictly anaerobic (14). This fact has fostered the view that the gastrointestinal ecosystem of mammals is basically an anaerobic one and that to be regarded as autochthonous to the system a microorganism must be capable of growth under anaerobic conditions (14). *T. pintolopesii* satisfies that criterion, growing adequately in media incubated anaerobically. It grows best under aerobic conditions, however, and, as has been reported (24), has the capacity to utilize O_2 as a terminal electron acceptor. Thus, it is facultative in its energy-yielding metabolism.

In the tracts of adult laboratory rodents, numerous microbial types, even some oxygen-intolerant anaerobic species of bacteria (13, 21), are known to associate intimately with the mucosal epithelia in the habitats they occupy. To us, it is a paradox that habitats near epithelial cells ostensibly aerobic in their energy-yielding metabolism can be occupied by anaerobic bacteria, especially oxygen-intolerant types. Since such microbes do occupy epithelial habitats in naturally colonized adult animals, however, the environment on the surface of the epithelial cells must have relatively low oxidation-reduction potential. Moreover, little if any molecular O_2 must enter the areas.

Before they are colonized by microorganisms, however, habitats in the gastrointestinal tract must have a somewhat higher oxidation-reduction potential than after they are colonized. During succession of the gastrointestinal microbiota in neonatal mice, aerotolerant metabolic anaerobes (lactobacilli, streptococci) and facultative anaerobes (*Escherichia coli*) colonize the tract before oxygen-intolerant anaerobes appear, especially those found in layers on the large bowel epithelium (16, 17). In fact, *E. coli* and streptococci can be found in microcolonies on the co-

TABLE 3. *Colonization of specific pathogen-free mice associated with wild-type and petite mutant strains of T. pintolopesii*

Expt	Time[a] (h)	Yeast not given (control)		Wild-type strain		Petite strain	
		No. of mice	Population level	No. of mice	Population level[b]	No. of mice	Population level[b]
1	24			5	6.1×10^4	5	0.2×10^4
	48			5	6.7×10^5	5	4.1×10^5
	72			5	4.1×10^6	5	2.7×10^6
	120			5	2.7×10^7	5	1.2×10^7
	240	10	NC[c]	5	1.6×10^7	5	1.6×10^7
2	240	12	NC[c]	12	$(1.0 \pm 0.2) \times 10^7$	12	$(0.6 \pm 0.2) \times 10^7$

[a] After animals were exposed to cultures of either the wild-type or the petite strain.

[b] For experiment 1, data are given in each case as the arithmetic mean of the number of viable yeast cells per gram of wet stomach tissue. For experiment 2, data are given in each case as the arithmetic mean ± standard deviation of the number of viable yeast cells per gram of wet stomach tissue. As assessed by Student's t test, the mean value for the wild-type strain differs from that for the mutant strain, but at a low level of significance ($P > 0.05$).

[c] NC, None cultured.

lonic epithelium in the baby mice before the anaerobes appear (16). In addition, oxygen-intolerant bacteria that colonize the epithelium of the large bowel in mice fail to colonize the tract of a germ-free rodent unless that tract is colonized first by oxygen-tolerant organisms. However, the latter microorganisms may be aerotolerant strains of anaerobic bacteria (13). Undoubtedly, these oxygen-tolerant bacteria serve, at least in part, to lower somewhat the oxidation-reduction potential in the lumen and in the epithelial habitats.

These concepts are supported by the findings reported in this paper. The wild-type parental strain of *T. pintolopesii* colonized the gastric nonsecreting epithelium at about the same rate or only slightly more rapidly than did the petite mutant derivative and reached a final population level only slightly higher than that of the mutant. As expected (3), the petite mutant does not revert to wild type in the colonized animals. When supplied with abundant O_2, the wild-type strain can multiply in vitro at a rate almost twice that of the mutant. Thus, had much O_2 been present in the habitat in vivo, presumably the wild type would have colonized it much more rapidly than did the mutant. By direct measurement, cells of the wild-type strain washed from mouse stomachs cannot be demonstrated to take up O_2 (J. E. Artwohl, M.S. thesis, University of Illinois, Urbana, 1976). Apparently, the ability of *T. pintolopesii* to generate energy by aerobic respiration is of little ecological advantage to it in its natural habitat.

If oxygen is not a significant factor for *T. pintolopesii* in colonizing its habitat on the gastric secreting epithelium, then factors other than O_2 must be powerfully selective against other microbial types colonizing the area. Unlike epithelial habitats in other areas of the rodent gastrointestinal tract, *T. pintolopesii* is usually the sole inhabitant of the gastric secreting epithelium in mice and rats (15). In ecological terms, then, the area must have some extremes of environment or nutrition that render it susceptible to colonization by only a limited number of microbial types.

Certainly, the HCl produced in the area could be highly selective against microbes not able to grow under conditions of low pH. The lumen pH in rat stomachs in the region of the *T. pintolopesii* habitat has been reported to be as low as 2.0 (6). Thus the yeast's ability to grow at low pH values must be advantageous to it in colonizing the habitat. Likewise, its ability to grow best at 37°C must be advantageous for colonizing a habitat in an animal that constantly regulates its temperature at about 37°C.

The ecological advantage of its carbon nutrition as studied is less clear to us, however, than are those of pH and temperature. In our media, the wild-type strain assimilated and fermented only glucose, mannose, and fructose of the monosaccharides tested and neither assimilated nor fermented any of the other carbon compounds to which it was exposed. The media were poised, however, at about neutral starting pH. The strain undoubtedly grows in its natural habitat at a pH lower than neutral. Thus, our findings at this point may not be conclusive. Nevertheless, some support for the findings has been obtained in earlier work. The yeast is dependent for nutrients upon constituents of its host's diet; starvation of mice for 48 h dramatically reduces the population levels of *T. pintolopesii* on the gastric epithelium in naturally colonized animals (20). Thus, it may be dependent, at least to some extent, on host enzymes to hydrolyze polymers in the diet (e.g., polysaccharides) to monosaccharides, many of which would be glucose or glucose derivatives. The yeast colonizes the tracts of mice or rats only after the animals are weaned (15), possibly because it cannot utilize as a carbon and energy source the lactose present in milk. Based upon our information at this time, however, we cannot evaluate the yeast's carbon nutrition as an ecological determinant.

Likewise, the yeast's nitrogen nutrition as studied is difficult to evaluate. In our tests it utilized NH_4^+ and certain amino acids as nitrogen sources. Ammonium ion has been recognized to be an important, if not the most important, source of nitrogen for microorganisms in gastrointestinal ecosystems and can be derived from urea produced by the host or from amino compounds in the host's diet (14). We have not yet tested our strains of *T. pintolopesii* for urease or ability to deaminate or transaminate amino acids. Presumably, they have the latter capacities, at least, since they utilize some amino acids as nitrogen sources. As with the issues of carbon nutrition studied, however, we cannot determine how these aspects of the yeast's nitrogen nutrition might be of an advantage to it in colonizing its native epithelial habitat.

When growing aerobically or anaerobically, the wild-type strain produces ethanol and perhaps also some acetate. As would be expected for a facultative fermenter, it converts more carbon into ethanol when growing anaerobically than when growing aerobically (4). The influence on the host of ethanol produced by the microorganisms growing in vivo in the stomach (if any) is problematic. Moreover, any discussion of a role for ethanol as an ecological determinant would be without foundation. Still, based upon

the information provided, we can conclude that *T. pintolopesii* is well adapted for growth in its native habitat on the secreting epithelium of the rodent stomach.

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