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Ecological Determinants in Microbial Colonization of the Murine Gastrointestinal Tract: Adherence of *Torulopsis pintolopesii* to Epithelial Surfaces

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Torulopsis pintolopesii is a yeast indigenous to the gastrointestinal tracts of conventional mice and rats from many colonies. In such natively colonized animals, the organism forms layers on the surface of the epithelium in the secreting portion of the stomach and can be cultured from all areas of the gastrointestinal tract. When given in water or food to germfree mice or specific pathogen-free mice possessing an indigenous microbiota free of yeast, *T. pintolopesii* also can be cultured from all areas of the tract at population levels ranging from 10^5 to 10^8 cells per g (wet weight). Likewise, as in its native hosts, the organism forms layers on gastric surfaces in the associated animals. The layers form on the secreting surface in both the specific pathogen-free and monoassociated ex-germfree mice. In the latter animal, however, a layer of yeast also forms on the nonsecreting gastric surface. In tests of its capacity to adhere to gastrointestinal surfaces in vitro, the organism adheres to epithelia from all areas of the mouse tract. These findings support an hypothesis that the capacity of *T. pintolopesii* to adhere to epithelial surfaces may be only one determinant influencing it to form layers on the gastric secreting surface in its native hosts.

Indigenous microorganisms of many types are known to associate intimately with epithelial surfaces in the gastrointestinal tracts of animals (8). One such microorganism, *Torulopsis pintolopesii*, forms layers on the secreting epithelium in the stomachs of mice and rats from many colonies housed under conventional conditions (11). The organism, a yeast, appears to us to be adapted well for colonizing its habitat on the gastric secreting surface (1). Under certain conditions, however, it forms layers on both the nonsecreting and secreting surface in the murine stomach. Normally, the gastric nonsecreting epithelium in conventional mice and rats is colonized by lactic acid bacteria, principally *Lactobacillus* species (7, 10, 12). If such animals are given antibacterial drugs in their water, then the lactic acid bacteria disappear from the keratinized surface of the nonsecreting epithelium. The yeast then forms layers on that surface as well as the secreting surface (7). Thus, the organism has some versatility in its capacity to colonize epithelial habitats in the murine stomach.

We have been exploring various physiological characteristics of *T. pintolopesii* in an effort to learn the factors that suit it for colonizing epithelial surfaces (1). One such factor could be a capacity of the organism to adhere to substances on the surfaces (3, 9). Lactobacilli that normally colonize the nonsecreting epithelium adhere to the surfaces of keratinized cells in that epithelium (10, 14). Acidic polysaccharides may mediate that adherence (2, 9). When it colonizes the keratinized surface, *T. pintolopesii* may adhere to the cells by mechanisms similar to those by which the lactobacilli adhere (9). Little is known, however, about the specific mechanisms by which either microbial type adheres to the keratinized cells. Even less is known about the mechanisms by which *T. pintolopesii* remains in its native habitat on the secreting epithelium.

We have adapted procedures reported by others (3-5) to develop models with which to study in vitro the mechanisms by which the organism adheres to epithelial surfaces. Pieces of gastrointestinal mucosa from mice were exposed in vitro to yeast cells grown in culture media. In one model, the cells adhering to the tissues were assayed by culture methods. In another, the yeast cells were labeled with a radioisotope. In that case, the number adhering to the tissues was assayed by liquid scintillation spectrometry. In addition, mice not colonized by the organism were associated with it and then used in a study of the areas in the tract colonized by the organism. In this paper, we report that the capacity

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of the microbe to adhere to substances on epithelial surfaces may be only one factor influencing it specifically to form layers on the gastric secreting surface in its native host.

MATERIALS AND METHODS

Microorganisms. The yeast strain used in this study was *T. pintolopesii* strain 108-1 originally isolated from the stomach of a conventional C57Bl mouse (1). It was grown in Sabouraud dextrose broth (Difco) or a chemically defined medium (1). The latter medium was sterilized with filters (0.45-μm pore size; Millipore Corp.). Cultures in flasks with cotton stoppers were incubated at 37°C without agitating unless stated otherwise.

If cultured under ordinary conditions, a population of *T. pintolopesii* 108-1 contains large numbers of mutant cells able to form petite colonies (1). To reduce the proportion of such mutant cells, prewarmed medium was used both in transfer and incubation of the yeast. In addition, all containers were wrapped with aluminum foil so as not to expose the microbes to light. Cultures were kept in a refrigerator until used. The proportion of cells able to form petite colonies in any culture was checked on Sabouraud dextrose agar (Difco). Cell suspensions containing less than 1% of mutant cells were used as inocula in all experiments.

Labeling of *T. pintolopesii* 108-1 with [^{3}H]methionine. [^{3}H]methionine (specific activity, 8.8 Ci/mmol) was purchased from Amersham Corporation (Arlington Heights, Ill.). This compound was added to the chemically defined medium (1) to a final concentration of 1 μCi/ml of medium. A 0.5-ml inoculum containing 10^8 yeast cells in stationary phase was added to 50 ml of medium in a 250-ml flask. The inoculated media were incubated at 37°C without shaking for 36 to 46 h. Growth was checked with a Klett-Summerson photometer. The total amount of radioactivity in each incubated culture was assayed with 10 20-μl samples, each of which was spotted onto a Selectron nitrocellulose filter of pore size 0.45 μm (Schleicher & Schuell Co., Keene, N. H.) pretreated with 5% trichloroacetic acid. The amount of radioactivity associated with material precipitable with trichloroacetic acid was assayed with 20-μl samples removed from the culture. Each of 10 such samples was added to 5 ml of cold 5% trichloroacetic acid. The solutions were incubated on ice for 45 min and then passed through Selectron filters. The precipitates retained on the filters were washed with 15 ml of cold 5% trichloroacetic acid. Yeast cultures were also grown in defined media not containing [^{3}H]methionine. These cultures were incubated to stationary phase. Ten 20-μl samples from them were also spotted on filters for use as background activity controls.

All filters were air dried overnight in scintillation vials. Thereafter, into each scintillation vial containing the dried samples were added successively 0.1 ml of water, 0.2 ml of 70% perchloric acid, and 0.4 ml of 30% H_2O_2. The vials were tightly capped and heated in a 70 to 80°C water bath overnight or until the filters were digested. After the solutions came to room temperature, 10 ml of Aquasol-II (New England Nuclear) was dispensed into the vials. During this process, the vials were protected from fluorescent light. Radioactivity in the vials was assayed with an Isocap 300 liquid scintillation counter. Replicate samples for background activity were counted before and after the experimental samples. If the mean background counts per minute of each set of background samples was the same, then the conditions of chemi- and photoluminescence were assumed to be the same in all experimental samples. Quench correction for each experimental sample was performed. [^{3}H]toluene (Packard Instruments) was added as an internal standard. The disintegrations per minute in each vial were calculated from the following equation: A_u = disintegrations per minute/vial = $[(A_s)(R_u - R_b)]/(R_t - R_u)$, where A_s = disintegrations per minute of [^{3}H]toluene added; R_u = counts per minute of the sample; R_t = counts per minute of sample with added [^{3}H]toluene; and R_b = counts per minute of the background sample.

The percentage of total radioactivity in the cultures incorporated into material precipitable with trichloroacetic acid was calculated as follows: percent incorporation = [(trichloroacetic acid-precipitable disintegrations per minute per milliliter)/(total disintegrations per minute per milliliter)] × 100%.

The mean level of the population of yeast cells in a labeled culture was determined with six Petroff-Hausser cell counts. The mean total cell count was calculated to determine cells per milliliter of culture. An assumption was made that all radioactivity incorporated into material precipitable with trichloroacetic acid was in yeast cells, and the relationship between cells and radioactivity was expressed as follows: number of cells/disintegrations per minute = (mean number of cells per milliliter)/(mean trichloroacetic acid-precipitable disintegrations per minute per milliliter).

Animals. Specific pathogen-free (SPF) mice, Crl: COBS CD-1(ICR)BR strain, originally purchased from Charles River Breeding Laboratory, Inc. (Wilmington, Mass.), were maintained in a colony in our laboratory. These particular SPF mice were free of *T. pintolopesii* undoubtedly because of the methods by which they were derived and maintained by the supplier. The mice were fed ad libitum commercial food (Allied Mills, Chicago, Ill.) autoclaved at 121°C for 30 min and given acidified (0.001 N HCl) or sterile water to drink. Six- to twenty-week-old SPF mice were used in the studies. Germfree (GF) mice, Crl:CD-1(ICR)GN strain, 27 to 36 weeks old, were also originally purchased from Charles River. They were raised in vinyl isolators sterilized with peracetic acid, fed ad libitum food sterilized in the same way as that received by SPF mice, and given sterile water to drink.

Assay of radioactive yeasts adhering to tissues. Suspensions of yeast cells were prepared from cultures grown in defined medium containing [^{3}H]methionine and harvested by centrifugation at 7,800 × *g* for 10 min. The cells were washed eight times with phosphate-buffered saline (PBS) (Na_2HPO_4, 0.12%; $NaH_2PO_4 \cdot H_2O$, 0.022%; NaCl, 0.85% [pH 7.4]) and suspended in PBS to a concentration of about 3×10^8 cells per ml (optical density 0.5 at 660 nm, Spectronic 20, Bausch and Lomb). The suspensions were used immediately.

SPF mice were killed with CO_2. Six pieces of tissue (approximate wet weight of one piece: nonsecreting

epithelium, 9 to 14 mg; secreting epithelium, 15 to 20 mg; upper small intestine, 35 to 40 mg; lower small intestine, 32 to 35 mg; cecum, 25 to 35 mg; colon, 30 to 41 mg) were placed into small tubes and incubated at 37°C for 10 min with 1 ml of the suspension (3×10^8 cells per ml) of *T. pintolopesii* 108-1 labeled with [^{3}H]methionine. After incubation, the tissue pieces were washed with five changes of PBS, placed separately into scintillation vials, dried, digested, and prepared for liquid scintillation assay as described above for the filters used in assessing incorporation of radioactivity into the yeast cells. Likewise, the samples were assayed for radioactivity and corrected for quench as described for the filters. In some experiments, 0.1-ml samples of the last of the five changes of PBS used to wash each tissue piece also were spotted on filters that were dried, digested, and counted for radioactivity as described above. Such counts were never above background levels. The level of radioactivity associated with the tissues was calculated from the following formula: disintegrations per minute per milligram, wet weight, of tissue = [(disintegrations per minute per vial)(mean percent incorporation)/(milligrams, wet weight, of tissue per vial)]. The number of yeast cells adhering to the tissues was calculated from the following formula: number of yeast cells/milligram, wet weight, of tissue = (number of yeast cells/disintegrations per minute)(disintegrations per minute/milligram, wet weight, of tissue).

In some experiments, a control was performed to detect any radioactive material leaking from the labeled yeast cells into the suspending buffer. Any such material could bind to the tissues during their exposure to the yeast cell suspension and be detected in the liquid scintillation counter. If so detected, the extracellular label would be interpreted to be present in intact yeast cells. Thus, the number of such cells adhering to the tissues would be estimated to be higher than the number actually on the tissues. Therefore, after the tissues had been removed from labeled yeast suspensions, the suspensions were centrifuged (7,800 $\times$ *g* for 20 min) to remove remaining yeast cells. The total amount of radioactivity and the amount precipitable with trichloroacetic acid were then estimated by the method described above in 20-μl samples of the supernatant solution free of yeast cells. Estimates of the level of background radiation were made from samples of supernatant solutions from unlabeled yeast cell suspensions also as described above. Pieces of intestinal tissues (obtained as described) were incubated at 37°C for 10 min in the supernatant solution from the labeled yeast suspensions. Thereafter, the tissues were washed, dried, and processed for assay by liquid scintillation counting as described. In tabulated data (Table 2), the amount of radioactivity in disintegrations per minute detected with such tissue pieces was subtracted from that detected with pieces exposed to the suspension of yeast cells.

Association of animals with the yeast. Cultures (48 to 68 h) of *T. pintolopesii* 108-1 grown in the defined medium were harvested, washed twice, and suspended in sterile saline. The concentration of the suspensions was 2×10^8 cells per ml. Twenty milliliters of such a suspension was poured on the animals' food or into their drinking water. Six-week-old female SPF mice associated with the yeast and control SPF mice free of the yeast were housed in cages with paper tops in the laboratory. Twenty-seven-week-old, female GF mice housed in an isolator also were associated with the yeast. Comparable control GF animals were housed in a separate isolator.

Examination for yeast in the gastrointestinal tracts of associated animals. Mice were killed with chloroform. The following parts of their digestive tracts were removed: nonsecreting and secreting areas of the stomach; upper, middle, and lower small intestine; cecum; and colon. The contents of the stomach and cecum also were harvested. Tissues were washed with PBS, blotted with sterile paper, and then either processed by histological methods for microscopic examination or weighed and homogenized with glass-Teflon homogenizers. Homogenates were diluted serially with 0.05 M phosphate buffer containing 0.1% agar, and spread on Sabouraud dextrose agar plates containing 40,000 U of penicillin and 0.04 g of streptomycin per liter (11). Colonies were counted after 48 h of incubation at 37°C.

Examination for yeast on tissues exposed to the organism in vitro. SPF or GF mice were killed with chloroform. The gastrointestinal tracts were removed immediately. Stomach (secreting and nonsecreting) and cecum walls were divided into small pieces (ca. 5 mm square). One-centimeter segments of the small intestine (upper, middle, and lower) and colon 3 cm distant from the ileocecal junction were cut longitudinally. Each tissue was washed with PBS three times. Five to six small pieces of each tissue were put into small tubes containing 1 ml of a suspension containing 3×10^8 cells of the yeast per ml. The tube was then incubated at 37°C for 10 min. After 5 min, the tube was gently agitated. After the incubation, the tissues were washed with five changes of PBS, blotted with sterile paper, and processed by histological methods for microscopic examination.

Histology. Tissues were mounted with 2% methylcellulose in 0.9% NaCl at −20°C in a Microtome-Cryostat (International Equipment Company, Needham, Mass.). Sections cut at 8 μm were stained by the Gram method modified for tissues (12) and examined with a Zeiss research microscope.

RESULTS

Colonization by *T. pintolopesii* 108-1 of the gastrointestinal tract of SPF and GF mice in vitro. SPF or GF mice were associated with *T. pintolopesii* 108-1. Eight to ten days after being given the yeast, the associated animals and control SPF and GF mice were killed. The gastrointestinal tracts of the animals were removed. Pieces from various areas of each tract were either processed for histological examination or cultured for estimation of the population levels of the yeast.

In the SPF mice associated with the organism, a thick layer of the yeast was observed microscopically only on the secreting epithelium of the stomach (Table 1). Similarly, a layer of the microbe was observed on the gastric secreting

TABLE 1. *Layers of T. pintolopesii 108-1 on the epithelial surfaces in the digestive tracts of mice associated with the yeast strain*[a]

Mice[b]	No. of mice tested	No. of mice with yeast on:[c]					
		Sec	NS	US	LS	Cec	Col
SPF	5	0[d]	5	0	0	0	0
GF	5	5	5	0	0	0	0

[a] Detected by examination by light microscopy of thin sections of mucosa frozen with contents intact.

[b] SPF, Specific pathogen-free CD-1 (free of yeast but have layers of lactobacilli on the nonsecreting gastric epithelium); GF, germfree CD-1 (no living microorganisms in their gastrointestinal tracts).

[c] Sec, Gastric secreting; NS, gastric nonsecreting; US, upper small intestine; LS, lower small intestine; Cec, cecum; Col, proximal colon.

[d] Number of mice with layers of yeast on the epithelial surface 8 to 10 days after being exposed to cultures of the microorganism.

epithelium in all the gnotobiotic mice monoassociated with the yeast. In these ex-GF animals, however, a layer of yeast cells was seen also on the keratinized, gastric nonsecreting epithelium. Such layers were not seen in areas of the tracts other than the stomach in either the SPF or gnotobiotic animals (Table 1).

The organism could be cultured from all areas of the tracts of both SPF and GF animals associated with it (Fig. 1). In the associated SPF mice, however, the levels were highest on the gastric secreting epithelium and in the cecal contents and colons. In the gnotobiotic mice, by contrast, except for the upper small bowel and the cecum wall, the levels were high throughout the tract.

These findings (Fig. 1) confirm the microscopic observations (Table 1). High population levels of the organism (>10^7 cells per g [wet weight]) were detected when mucosal walls with visible layers on them were cultured separately from lumenal contents (the secreting gastric wall in SPF mice and the secreting and nonsecreting gastric walls in GF mice). By contrast, low levels (ca. 10^5 cells per g [wet weight]) were detected when mucosal walls without layers on them were cultured separately from lumenal contents (nonsecreting gastric wall in SPF mice and cecal wall in both SPF and GF animals). When the walls and contents were cultured together, however, as was the case for all samples of the small intestine and the colon from animals of both types, then the population levels were generally high.

When the wall lacks a yeast layer, estimates of population levels of the organism from samples of content and wall together should reflect the level in the contents. In addition, all samples of lumenal content in the stomach and areas distal to it should reflect the levels on the gastric wall. This phenomenon is evident in the samples from the GF mice. In those from the contents of the nonsecreting side of the stomach and the small bowels of SPF mice, however, the population levels are somewhat lower than expected values. This observation may indicate some repression of the yeast population in the lumen by the lactic acid bacteria in those animals (7).

Microscopic observation of *T. pintolopesii* 108-1 adherence. Gastrointestinal mucosal tissues from seven areas from stomach to colon of 10 each SPF and GF mice were incubated with yeast suspensions and then washed, frozen, and sectioned. The sections were stained and examined microscopically. The yeast adhered to all of the epithelial surfaces obtained from both SPF and GF mice. Few, if any, yeast cells were

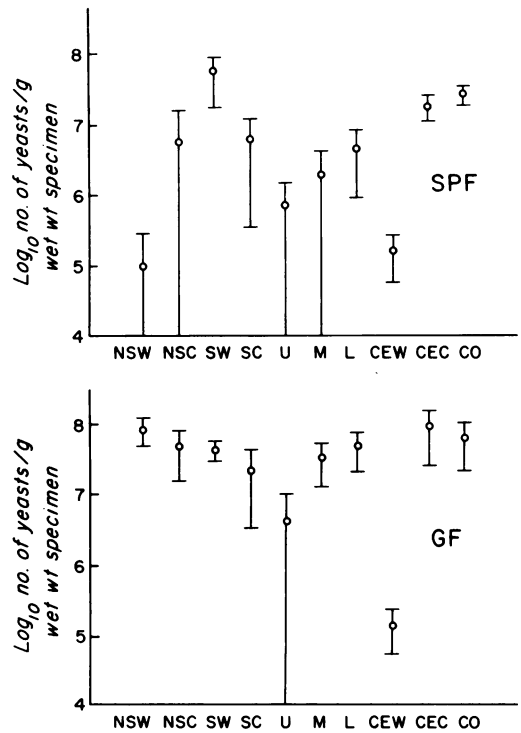

FIG. 1. *Population levels of T. pintolopesii 108-1 in the digestive tracts of SPF or GF mice associated with the yeast for 8 to 10 days. NSW, Nonsecreting wall of the stomach; NSC, contents of nonsecreting area of the stomach; SW, secreting wall of the stomach; SC, contents of secreting area of the stomach; U, upper small intestine; M, middle small intestine; L, lower small intestine; CEW, cecum wall; CEC, cecum contents; CO, colon. Values (circles) are arithmetical means ± standard deviations (bars); n = 5 mice.*

seen adhering to the serosal surface of the mucosa taken from any area of the tract. A layer of lactobacilli covered approximately 80% of the keratinized epithelium from the stomachs of SPF mice used in the experiment. The yeast cells attached to small areas of the keratinized epithelial surfaces that were not occupied by resident lactobacilli. No lactobacilli were present on the keratinized epithelium from the stomachs of GF mice. In this case, yeast cells were seen adhering to as much as 30% of the keratinized epithelium. The yeast cells attached to as much as 25 to 50% of the area of the mucus layer on the epithelium of the pieces of mucosa from small and large intestines of both SPF and GF mice.

Growing and labeling of *T. pintolopesii* 108-1 with [^{3}H]methionine. *T. pintolopesii* 108-1 grew well in flasks of medium containing 1 μCi of [^{3}H]methionine per ml. Depending upon the experiment, from 78 to 95% of radioactivity detectable in the cultures was incorporated into a fraction precipitable with trichloroacetic acid. Assuming all label in that fraction was incorporated into yeast cells, we calculated that each disintegration per minute represented 46.2 to 70.4 (Table 2) yeast cells, depending upon the experiment.

Assay of radiolabeled *T. pintolopesii* 108-1 adherence. Epithelial tissues from six areas of the gastrointestinal tracts of GF mice were exposed to *T. pintolopesii* 108-1 labeled with [^{3}H]methionine. The tissues were then treated as described in Materials and Methods for assay of the levels of radioactivity associated with them. The experiments were repeated twice; essentially identical results were obtained each time. Data presented (Table 2) are derived from an experiment in which each disintegration per minute of radioactivity represented 70.4 yeast cells on the average. High levels of radioactivity were found to be associated with all tissues exposed to such labeled yeast suspensions. The radioactivity could not be accounted for as label leaking from yeast cells. We concluded, therefore, that the labeled yeast cells adhered essentially equally well to tissues from all areas of the tract (Table 2).

TABLE 2. *Level of radioactivity associated with, and number of cells of T. pintolopesii adhering to, mucosal epithelial surfaces removed from various areas of the mouse gastrointestinal tract and exposed to the yeast cells in vitro*[a]

Epithelium[b]	dpm/mg (wet wt) of tissue[c]	Unadjusted no. of yeast cells ($\times 10^{-6}$)[d]	Adjusted no. of yeast cells ($\times 10^{-6}$)[e]
Sec.	65.8 (48.7–91.0)	4.6 (3.4–6.4)	3.9
NS	75.9 (31.3–125.0)	5.3 (2.2–8.8)	5.0
US	67.7 (24.2–145.0)	4.8 (1.7–10.2)	4.4
LS	67.8 (57.8–100.0)	4.8 (3.6–7.0)	4.3
Cec.	67.6 (27.6–144.0)	4.8 (1.9–10.1)	4.2
Col.	61.9 (36.3–85.1)	4.4 (2.6–6.0)	3.8

[a] As assessed with liquid scintillation assay.

[b] See footnote *c* of Table 1 for identification of abbreviations.

[c] Calculated without adjustment for leaking of label from the yeast cells from the following formula: (disintegrations per minute/vial) · (mean percent incorporation)/(milligram, wet weight, of tissue/vial) (see Materials and Methods).

[d] Arithmetical average of number of yeast cells per gram (wet weight) of mucosal tissue calculated without adjustment for possible leaking of radioactive label from yeast cells; range in parentheses (five mice per group). Values were calculated by multiplying disintegrations per minute/milligram of tissue by 70.4 yeast cells/disintegrations per minute and by 10^3 to adjust the value to number of yeast cells per gram of tissue (wet weight). The number of yeast cells/disintegrations per minute (70.4) was estimated from the following formula: cells/disintegrations per minute = (mean number of cells per milliliter)/(mean trichloroacetic acid-precipitable disintegrations per minute per milliliter) (see Materials and Methods).

[e] Arithmetical average of number of yeast cells per gram (dry weight) of mucosal tissue adjusted for possible leaking of radioactive label from the yeast cells (see Materials and Methods).

DISCUSSION

In conventional mice and rats colonized naturally, *T. pintolopesii* forms layers visible microscopically only on the gastric secreting epithelium (11). Likewise, as demonstrated in this study, in SPF mice possessing an indigenous microbiota normally free of it, the yeast forms layers only on the secreting epithelium. By contrast, in monoassociated gnotobiotic (i.e., ex-GF) mice, it forms layers on both the secreting and nonsecreting surfaces in the stomach. These observations confirm earlier ones (7) indicating that in vivo the organism is not specific in colonizing a particular gastric epithelial surface. At no time, however, has it ever been observed to form layers in vivo on surfaces in the small or large intestines. By contrast, in vitro in the assays that we use, the organism adheres not only to gastric epithelia but also to epithelia taken from various parts of the small and large bowels. Thus, although restricted to colonizing gastric surfaces in vivo, the yeast may be able to adhere

to any gastrointestinal epithelium.

Evidence for that hypothesis must be interpreted with care, however, when derived from experiments in which the tissues are exposed to the microbial cells in vitro. Microbial surfaces are well known to be influenced in molecular structure by the composition of the media in which the organisms are grown (13). Moreover, the surfaces of the microbial cells and also the tissues may be altered by environmental conditions such as hydrogen ion concentration in buffer solutions, such as the PBS used in our assay (3, 5). Thus, microbial cells grown in vitro may well adhere to tissues in vitro by mechanisms that differ from those by which they adhere to the surfaces in vivo.

We did not try to duplicate in vitro the environmental conditions prevailing on the epithelial surfaces in vivo. We do not know how to do so; the conditions prevailing on the surfaces in vivo are not known. Nevertheless, we believe that our findings support the hypothesis that the yeast has the capacity to adhere to any epithelial surface in the mouse gastrointestinal tract. In vivo, the organism colonizes the gastric secreting epithelium in animals with a layer of lactobacilli on the nonsecreting epithelium and both surfaces in animals without such a bacterial layer. We are uncertain whether or not the organism actually adheres to epithelial cells in the secreting gastric mucosa. A theoretical case can be made that it colonizes mucin on the surface and does not adhere to any cells in the habitat (9). Nevertheless, in analogy with the lactobacilli that adhere to keratinizing epithelium (2, 8, 13), the yeast undoubtedly adhere to that surface when they colonize it in the living animal (8, 12). Keratinized epithelium differs considerably in architecture and secretions from the secreting gastric epithelium. Thus, if the organism adheres to the secreting epithelium in colonizing it (as we believe it does), then it lacks considerably in specificity when it adheres to the keratinized surface. If it lacks specificity in adhering to gastric surfaces, then it may well be sufficiently nonspecific to adhere to any gastrointestinal surface, as indicated by our findings with the assays for adherence in vitro.

Depending upon the area in the murine gastrointestinal tract, the epithelium consists of keratinized squamous cells or of columnar, secreting cells. In the latter case, the microvillous membranes of the epithelial cells are covered ordinarily by a glycocalyx and mucous layer (D. C. Savage, *In* G. Bitton and K. C. Marshall, ed., *Absorption of Microorganisms to Surfaces,* in press). Cells of *T. pintolopesii* may have the capacity to adhere to macromolecules in any of these structures in vivo as well as in vitro. In vivo, however, ecological factors other than the capacity of the microbe to adhere to a particular structure may influence whether or not it can remain and multiply on (i.e., colonize) a surface. For example, its ability to grow well at low pH's is undoubtedly an important factor in its colonization of gastric surfaces (1). Thus, it may colonize gastric epithelial surfaces rather than intestinal surfaces because nutritional and environmental factors, rather than a capacity to adhere to a particular surface, circumscribe which habitat it can colonize.

As with gastric pH, some of those nutritional and environmental factors derive from host function (6). Other factors derive, however, from activities of members of the indigenous microbiota other than *T. pintolopesii* (6). That hypothesis derives from the observation that the yeast forms layers on both the secreting and nonsecreting gastric surfaces in monoassociated gnotobiotic mice. As has been noted, the organisms will also colonize the nonsecreting epithelium of the stomachs of conventional mice treated orally with antibacterial drugs (7). The pertinent effect of the drugs in such animals is to remove the lactic acid bacteria that normally colonize the nonsecreting gastric epithelium (10, 12). When the lactobacilli disappear, the yeast normally present only on the secreting surface can then form layers on the nonsecreting surface. When treatment with the antibiotic is discontinued and the mice are recolonized with lactobacilli, the yeast can no longer be found in layers on the nonsecreting surface (7).

T. pintolopesii cannot colonize the nonsecreting surface in mice with lactobacilli colonizing it, undoubtedly because the bacteria compete successfully with it for available nutrients or sites of attachment on the surface or produce compounds that are toxic for it (6). When the lactic acid bacteria are removed by drugs, then the yeast can colonize the nonsecreting epithelium, presumably because it finds the nutritional and environmental conditions prevailing in the habitat suitable to it (1) and possibly because it can adhere to any epithelial surface in the murine gastrointestinal canal.

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LITERATURE CITED

1. **Artwohl, J. E., and D. C. Savage.** 1979. Determinants in microbial colonization of the murine gastrointestinal tract: pH, temperature, and energy-yielding metabolism of *Torulopsis pintolopesii*. Appl. Environ. Microbiol. 37:697-703.

2. **Fuller, R.** 1973. Ecological studies on the *Lactobacillus* flora associated with the crop epithelium of the fowl. J. Appl. Bacteriol. **36**:131-139.
3. **Gibbons, R. J., and J. Van Houte.** 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. **29**:19-44.
4. **Jones, G. W., and J. M. Rutter.** 1972. Role of the K88 antigen in the pathogenesis of neonatal diarrhea caused by *Escherichia coli* in piglets. Infect. Immun. **6**:918-927.
5. **Marshall, K. C.** 1976. Interfaces in microbial ecology, p. 1-156. Harvard University Press, Cambridge, Mass.
6. **Savage, D. C.** 1972. Survival on mucosal epithelia, epithelial penetration, and growth in tissues of pathogenic bacteria, p. 25-57. *In* H. Smith and J. H. Pearce (ed.), Microbial pathogenicity in man and animals. Cambridge University Press, Cambridge, England.
7. **Savage, D. C.** 1969. Microbial interference between indigenous yeast and lactobacilli in the rodent stomach. J. Bacteriol. **98**:1278-1283.
8. **Savage, D. C.** 1977. Microbial ecology of the gastrointestinal tract. Annu. Rev. Microbiol. **31**:107-133.
9. **Savage, D. C.** 1978. Factors involved in colonization of gut epithelial surfaces. Am. J. Clin. Nutr. **31**:S131-S135.
10. **Savage, D. C., and R. V. H. Blumershine.** 1974. Surface-surface associations in microbial communities populating epithelial habitats in the murine gastrointestinal ecosystem: scanning electron microscopy. Infect. Immun. **10**:240-250.
11. **Savage, D. C., and R. J. Dubos.** 1967. Localization of indigenous yeast in the murine stomach. J. Bacteriol. **94**:1811-1816.
12. **Savage, D. C., R. Dubos, and R. W. Schaedler.** 1968. The gastrointestinal epithelium and its autochthonous bacterial flora. J. Exp. Med. **127**:67-76.
13. **Smith, H.** 1977. Microbial surfaces in relation to pathogenicity. Bacteriol. Rev. **41**:475-500.
14. **Suegara, N., M. Morotomi, T. Watanabe, Y. Kawai, and M. Mutai.** 1975. Behavior of microflora in the rat stomach: adhesion of lactobacilli to the keratinized epithelial cells of the rat stomach in vitro. Infect. Immun. **12**:173-179.