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Differences in Adhesion of *Candida albicans* 3153A Cells Exhibiting Switch Phenotypes to Buccal Epithelium and Stratum Corneum

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Cells of the laboratory strain 3153A of *Candida albicans* can be stimulated to undergo high-frequency phenotypic switching by a low dose of UV. We have compared the adhesive properties of cells exhibiting the basic original smooth (o-smooth) phenotype and three switch phenotypes (star, irregular wrinkle, and revertant smooth) to buccal epithelium and stratum corneum. The generalized hierarchy of adhesion is as follows: o-smooth > irregular wrinkle > revertant smooth > star. This is the inverse of the hierarchy of the proportions of elongate hyphae formed by these phenotypes in culture. These results suggest that the differences in adhesion between o-smooth and the three switch phenotypes of strain 3153A reflect, at least in part, the level of interference due to the formation of elongate hyphae, which tend to cause clumping in suspension. No major differences in the levels of adhesion of cells of the different phenotypes between buccal epithelium and stratum corneum were observed. Results which demonstrate that buccal epithelium induces germination (hypha formation) by conditioning the medium are also presented.

Candida albicans lives in the natural floras of healthy individuals as a commensal, causing no apparent damage and inducing no apparent inflammation in the surrounding tissue. However, under a number of predisposing conditions, *C. albicans* multiplies and penetrates the host tissue, causing inflammation and tissue destruction (6, 21). In immunocompromised individuals, the infection can be systemic and disseminated and, in many cases, can lead to death (7). An initial step in the infection of oral, vaginal, and gastrointestinal mucosa is the attachment of the organism to the epithelial surface (17, 21). Attached cells in turn form hyphae which penetrate tissue (3, 10, 12). Therefore, the adhesive properties of *C. albicans* play a role in pathogenesis, and many studies have documented adhesive differences between strains (9, 11, 16, 18, 23). In addition, adhesive differences between the bud and hyphal forms of growth have been demonstrated (3, 14), and in at least one strain, WO-1, adhesive properties vary as a result of high-frequency phenotypic switching (13).

Most strains of *C. albicans* are capable of switching between a number of general phenotypes which can be discriminated by colony morphology (22, 24, 29, 31). Switching occurs spontaneously and, in some cases, quite frequently (e.g., once in every 100 cell divisions) (24, 29, 30). Switching is reversible and can affect most physiological and morphological characteristics of a cell, including many of the putative virulence factors (20, 28–30). Although the general characteristics of switching are similar in different strains, the phenotypes in their switching repertoires can vary dramatically. Changes in adhesion due to switching were first observed in the white-opaque phase transition in strain WO-1 (13). Cells in the white phase showed greater adherence than cells in the opaque phase to buccal epithelium, but cells in the opaque phase exhibited a higher level of cohesion (13). White and opaque cells have also been demonstrated to differ dramatically in cellular morphology (1, 2, 4, 25). In particular, opaque cells exhibit unique cell wall pimples (1, 2) and are larger and longer than white cells (25). Although the white-opaque transition has been characterized in more detail than any other switching system, the opaque phenotype, in particular, has been considered unusual, since the opaque colony morphology and the unique opaque cell morphology are not standard components of the switching repertoire of the majority of other strains of *C. albicans* (29). We have, therefore, examined the adhesive properties of the variant phenotypes in a more mainstream switching system, that of strain 3153A (24), in order to assess whether changes in adhesion are associated with switching in *C. albicans*. Our results demonstrate that there are dramatic differences in the levels of adhesion to both buccal epithelium and stratum corneum between cells in the basic original smooth (o-smooth) phenotype and cells in at least two switch phenotypes of strain 3153A and that this difference may be due to the number of hyphae these cells form when they enter stationary phase. Our results also demonstrate that buccal epithelium induces hypha formation by conditioning the supporting medium.

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MATERIALS AND METHODS

Maintenance of stock cultures and isolation of switch phenotypes. Cells from a storage slant of strain 3153A were plated on agar containing the nutrient composition of Lee's medium (15) supplemented with 70 μg of arginine per ml and 0.1 mM $ZnSO_4$ (modified Lee's medium [5]) and incubated for 9 days at 25°C. More than 99.9% of the colonies exhibited the o-smooth colony morphology (24). Cells from a representative smooth colony were grown to mid-log phase in liquid modified Lee's medium and were then treated with a low dose of UV irradiation, according to the method of Morrow et al. (19), which killed 8% of the cell population. Irradiated cells were plated on agar containing modified Lee's medium at approximately 55 CFU per plate (100-by-15-mm standard agar plates).

Star, irregular wrinkle, and revertant smooth (r-smooth) colonies were isolated and grown in liquid modified Lee's medium at 25°C for 9 days and stored in liquid nitrogen. Switching frequencies were determined by plating cells from each of the switch phenotypes on agar containing modified Lee's medium at a density of 50 to 100 cells per plate. Colony phenotypes were scored after 7 to 9 days at 25°C.

Adhesion assay. Porcine buccal mucosa was obtained from a local slaughterhouse. Connective tissue and muscle were removed with a scalpel, and the remaining buccal mucosa was cut into 15-mm-diameter discs and attached to the bottom of 16-mm-diameter wells of cell culture clusters (Costar, Cambridge, Mass.) with Prisma Universal Bond (L.D. Caulk Co.). Stratum corneum was obtained by separating porcine epidermis after incubation at 65°C for 1 min. The separated epidermis was placed in a 1% (wt/vol) solution of trypsin (Sigma Chemical Co., St. Louis, Mo.) in phosphate-buffered saline (PBS; 0.02 M phosphate buffer, 0.15 M NaCl [pH 7.0]) for 24 h at 4°C, after which the tissue was washed twice in distilled water and placed in a fresh 1% trypsin solution for 2 h at 37°C. The tissue was washed once more, and the stratum corneum layer was recovered for use in the assays. Intact stratum corneum was cut into 15-mm-diameter discs and attached to wells of cell culture clusters as described for buccal epithelium.

To evaluate the adhesion of yeast cells to epithelial surfaces, yeast cells were transferred to 50 ml of modified Lee's medium containing 100 μCi of [C^{14}]glucose and incubated at 25°C in a gyratory water bath for 24 h. Cells treated in this manner were in early stationary phase. Cells were pelleted, washed twice in PBS, and resuspended in PBS to a final concentration of 10^7 cells per ml. One-milliliter samples were inoculated into the wells containing buccal epithelium or stratum corneum tissue discs. The cultures were rotated in a gyratory incubator at 37°C at 30 rpm. The wells were then washed three times with 10 ml of PBS, and each tissue sample was placed in 1 ml of TS-2 tissue solubilizer (Research Products International Corp., Mount Prospect, Ill.) for 24 h. Nine milliliters of Ria-Solve II counting cocktail (Research Products International Corp.) was added to each digested tissue sample, and radioactivity was measured with a Beckman LS-7800 liquid scintillation counter. The level of radioactivity (counts per minute) was used directly to assess the incubation time for maximum adhesion. To compare the relative levels of adhesion between o-smooth and switch phenotypes, counts per minute was converted to number of cells (cells per milliliter) from standard curves generated in each experiment for each of the switch phenotypes.

In each experiment, assays for each phenotype were repeated five times. In addition, one tissue disc from each experimental group was homogenized and portions of the nonadherent yeast cells in the supernatant and adherent yeast cells in the homogenate were plated onto agar containing modified Lee's medium to verify that the adherent and nonadherent candidal populations exhibited the original or variant colony morphologies. Each time the cells from an irregular wrinkle colony were plated, mass conversion of more than 50% of the population to an r-smooth phenotype occurred (23a).

Statistical methods. The number of adherent organisms was converted to the logarithm (base 10) of cells per tissue. Means and standard errors were calculated for the five replicates performed for each experimental group. Statistical differences in binding between the different phenotype-epithelium combinations were analyzed by one-way analysis of variance with posttests using Tukey-Kramer multiple comparison. Student's *t* tests were used to compare the adhesion between tissue types for each of the phenotypes.

Scanning electron microscopy. Epithelial discs were fixed in 0.5% glutaraldehyde in cacodylate buffer at 4°C overnight. The tissue was then dehydrated by using a series of 25, 50, 75, and 100% ethanol for 30 min each. Following critical-point drying with a Balzers CPD 030, samples were sputter-coated with gold-palladium in a Balzers SCD 040. The samples were then examined with an Amray 1820 scanning electron microscope.

Carbon analysis. Buccal epithelium and stratum corneum discs were incubated with 1 ml of PBS in the wells of cell culture clusters in the absence of yeast cells for 3 h at 37°C. Supernatants were serially diluted, and 5-μl samples of each dilution were applied on silica gel thin-layer chromatography plates (Alltech Assoc., Deerfield, Ill.). The plates were then air dried, sprayed with 50% sulfuric acid, charred at 220°C, and analyzed with a Bio-Rad model 620 densitometer. Standards consisted of serial dilutions of bovine serum albumin. A control consisted of PBS incubated with no tissue.

Analysis of phenotypes of cells in suspension and adhering to tissue. To assess the phenotype of cells in suspension, cells were inoculated into a hemocytometer, and more than 200 cells were analyzed for the proportion of mature mother cells, buds (up to three-fourths the diameter of mother cells), and hyphae. Hyphae had to be at least half a cell diameter long and were scored only once regardless of length and regardless of whether they were attached to a mother cell. The proportions by phenotype of cells adhering to tissue were scored by scanning electron microscopy. Eight randomly chosen, independent fields were scored for each test sample.

RESULTS

Switching in strain 3153A. When cells from storage cultures of strain 3153A were plated on agar containing modified Lee's medium and incubated for 7 to 9 days at 25°C, the resulting colonies exhibited the basic o-smooth colony morphology (Fig. 1A) (24). When cells from o-smooth colonies were in turn plated, no variant colony morphologies were observed in 5,000 colonies, indicating a switching frequency of less than 2×10^{-4}. However, cells treated with a low dose of UV irradiation which killed 8% of the cell population formed variant colony morphologies (in this case irregular wrinkle, star, and ring) at a stimulated frequency of 10^{-2}, as previously reported (24). An irregular wrinkle colony (Fig. 1B) was picked from the irradiated culture, and the cells from the colony were clonally plated. From this clonal plating, a star colony (Fig. 1C) and an r-smooth colony were in turn picked. Cells of these three switch phenotypes (irregular wrinkle, star, and r-smooth) switched to smooth or other variant colony morphologies (e.g., star switched to irregular wrinkle and smooth) at frequencies equal to or greater than 10^{-3}. An example of a switch from star to irregular wrinkle is shown in Fig. 1D. Although r-smooth colonies emanating from the other switch phenotypes appeared superficially similar to o-smooth colonies (Fig. 1A), they exhibited a rougher surface and more irregular edge and switched to other phenotypes at frequencies at least two orders of magnitude higher than the basic o-smooth phenotype, as previously reported (24).

Adherence of cells with switch phenotypes to buccal epithelium and stratum corneum. To measure the time for maximum adherence under the assay conditions employed, 10^7 radioactive o-smooth, star, irregular wrinkle, or r-smooth cells obtained from early-stationary-phase cultures were incubated for increasing lengths of time with 15-mm discs of buccal epithelium or stratum corneum and the log counts per minute was plotted as a function of incubation time at 37°C. Adherence to both buccal epithelium (Fig. 2A) and stratum corneum (Fig. 2B) saturated after 3 h for the basic o-smooth phenotype as

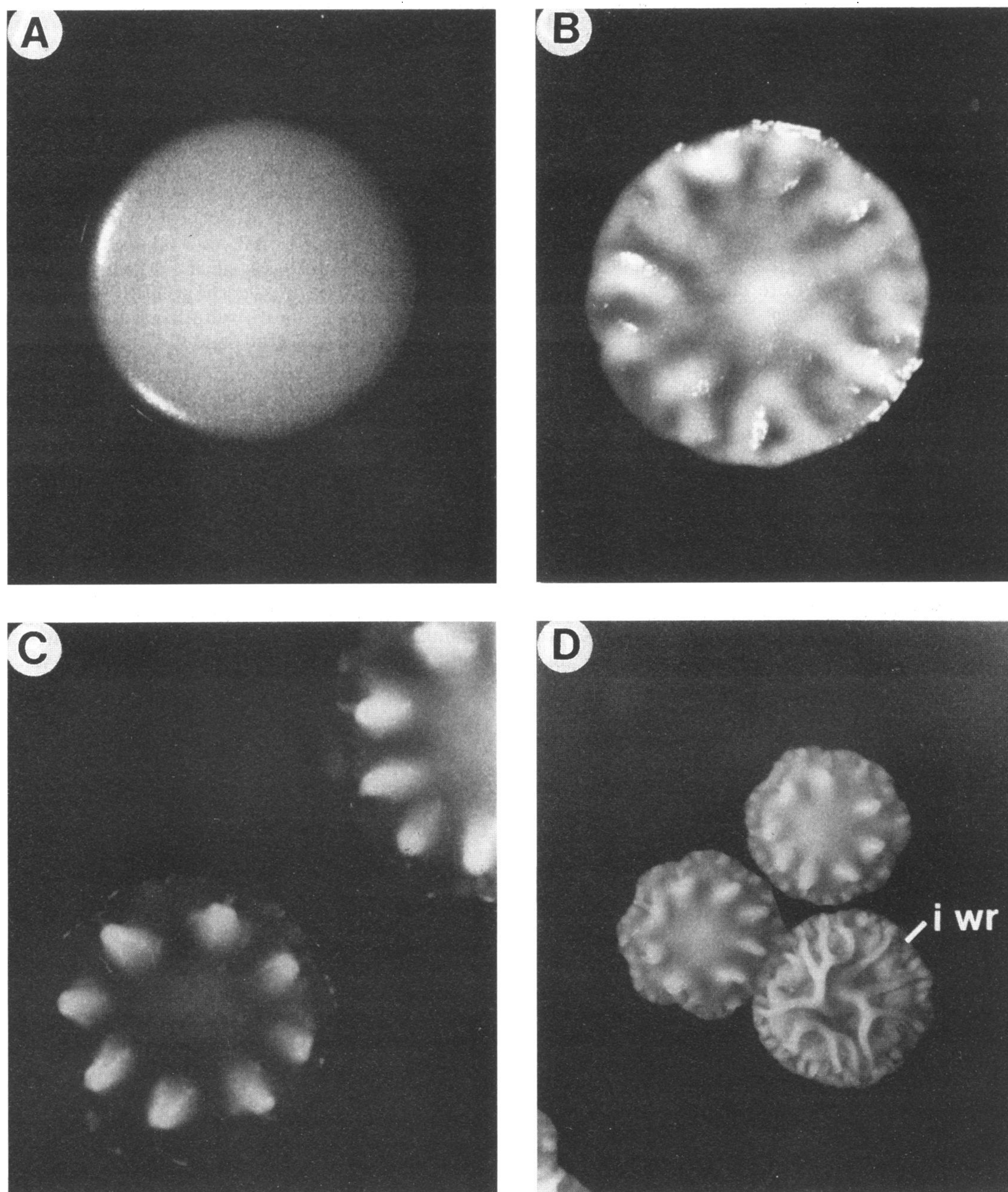

FIG. 1. Examples of colony morphologies in the switching repertoire of *C. albicans* 3153A. (A) O-smooth colony, (B) irregular wrinkle colony, (C) star colonies, (D) switch from star to irregular wrinkle (i wr). In the culture for D, cells from a single star colony were plated and roughly 99% of the colonies exhibited the parent star phenotype.

well as the three variant phenotypes. Therefore, the incubation time in subsequent adherence assays was 3 h. Since the assay we are using does not discriminate between *Candida* cells adhering to tissue and *Candida* cells adhering to other cells contacting tissue, our definition of adhesion includes both groups.

In comparing the adhesion properties of o-smooth, star, irregular wrinkle, and r-smooth cells to buccal epithelium and stratum corneum, counts per minute per cell for each of the phenotypes was determined in each experiment in order to convert counts per minute per tissue disc to cells per tissue disc. The results of two independent experiments were similar (Fig. 3). In both cases, the standard errors of the mean (Fig. 3, error bars) between independent assays were low. The adhesion of o-smooth cells to buccal epithelium was significantly greater than adhesion of star or r-smooth cells (Table 1) but not significantly greater than the adhesion of irregular wrinkle cells (Table 1). In the two independent experiments, the levels of adhesion of star cells were 27 and 25% those of o-smooth and irregular wrinkle, respectively, and the levels of adhesion of r-smooth were 53 and 54% those of o-smooth and irregular wrinkle, respectively. In the two independent experiments, the adhesion of star cells was 50 and 46% that of r-smooth. Therefore, the hierarchy of adhesion to buccal epithelium was

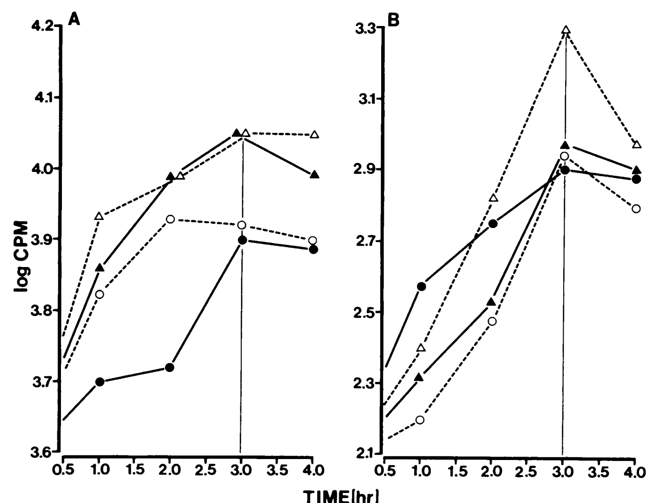

FIG. 2. Adhesion to buccal epithelium (A) and stratum corneum (B) as a function of time. In each assay, 10^7 radioactive cells were incubated with tissue discs under the conditions described in Materials and Methods. The log counts per minute were then plotted as a function of time. Δ, o-smooth; ○, star; ▲, irregular-wrinkle; ●, r-smooth. Note that for all phenotypes with both tissues, saturation occurred after 3 h.

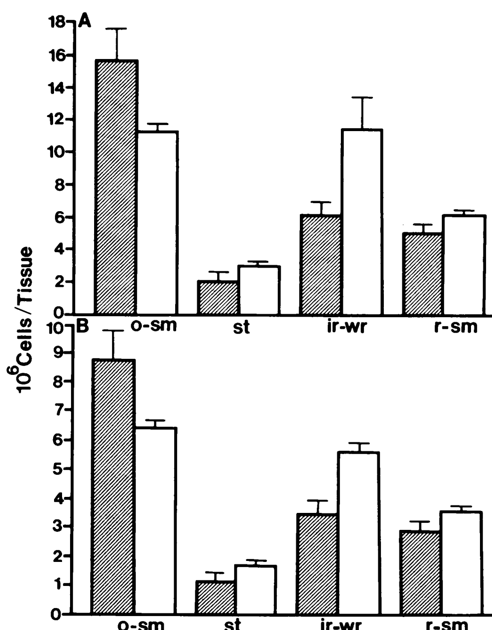

FIG. 3. A comparison of levels of adhesion between the basic o-smooth phenotype cells (o-sm) and the variant phenotype star (st), irregular wrinkle (ir-wr), and r-smooth (r-sm) cells. Adhesion assays were run for 3 h, as described in Materials and Methods. Counts per minute per tissue was converted to cells per tissue from standard curves generated in each experiment for each phenotype. A and B represent independent experiments. Hatched columns represent adhesion to stratum corneum. Open columns represent adhesion to buccal epithelium. Standard errors of the means for five measurements are presented as bars.

as follows: o-smooth ≅ irregular wrinkle > r-smooth > star. This relative hierarchy held true in both experiments, even though the total numbers of cells per tissue varied, on average, between the experiments.

The adhesion of o-smooth cells to stratum corneum was significantly greater than adhesion of cells of the three tested switch phenotypes (Fig. 3; Table 1). In the two independent experiments, the levels of adhesion of star cells to stratum corneum were 13 and 14% that of o-smooth cells, the levels of adhesion of irregular wrinkle cells were 39 and 40% that of o-smooth cells, and the levels of adhesion of r-smooth cells were 32 and 32% that of o-smooth cells. The levels of adhesion of irregular wrinkle and revertant smooth cells to stratum corneum were not significantly different, but the levels of adhesion of both to stratum corneum were significantly greater than that of star. The hierarchy of adhesion to stratum corneum was, therefore, as follows: o-smooth > irregular wrinkle ≅ r-smooth > star. The hierarchies for buccal epithelium and stratum corneum were, therefore, similar, and this is evident in both of the independent experiments (Fig. 3). There was, however, one difference. The adhesion of irregular wrinkle cells to buccal epithelium was significantly greater than adhesion to stratum corneum (Table 2).

The phenotypes of o-smooth, star, irregular wrinkle, and r-smooth cells in suspension and when adhering to tissue. In contrast to the basic o-smooth phenotype, several of the switch phenotypes of strain 3153A formed hyphae and pseudohyphae in colony domes on agar (27, 29), and in the case of the star phenotype (originally referred to as variant M10), it was originally demonstrated that cells formed hyphae in modified Lee's medium when they entered stationary phase at 25°C (5). We therefore examined the phenotypes of o-smooth and variant cells incubated with the two types of tissue. Since, under the conditions of the adhesion assay, cells were incubated with anchored tissue in buffered salt solution, we first measured the proportions of mature budding cells, buds, and hyphae in buffer. In quantitating proportions, we counted the three cell types as "compartments," not taking into consideration whether they were attached to a mother or daughter cell. At the time cells were harvested for adhesion assays, o-smooth cultures contained less than 1% hyphal compartments and irregular wrinkle cultures contained approximately 4% hyphal compartments. In contrast, star cultures contained approximately 49% hyphal compartments and r-smooth cultures con-

TABLE 1. Differences between switch phenotypes in adhesion of cells by one-way analysis of variance with posttest Tukey-Kramer multiple comparison

Tissue[a]	Phenotype comparison	P[b]	Significance[c]
BE	O-smooth vs. star	<0.001	S, S
	O-smooth vs. irregular wrinkle	>0.05, >0.05	NS, NS
	O-smooth vs. r-smooth	<0.01, <0.01	S, S
	Star vs. irregular wrinkle	<0.001, <0.001	S, S
	Star vs. r-smooth	<0.001, <0.001	S, S
	Irregular wrinkle vs. r-smooth	<0.001, <0.001	S, S
SC	O-smooth vs. star	<0.001, <0.001	S, S
	O-smooth vs. irregular wrinkle	<0.01, <0.01	S, S
	O-smooth vs. r-smooth	<0.01, <0.01	S, S
	Star vs. irregular wrinkle	<0.001, <0.001	S, S
	Star vs. r-smooth	<0.01, <0.01	S, S
	Irregular wrinkle vs. r-smooth	>0.05, >0.05	NS, NS

[a] BE, buccal epithelium; SC, stratum corneum.
[b] P values for two independent experiments are provided.
[c] S, statistically significant; NS, not statistically significant.

TABLE 2. Differences between buccal epithelium and stratum corneum in adhesion of cells by Student's t test

Phenotype	P^a	Significance[b]
O-smooth	<0.05, <0.06	S, NS
Star	<0.09, <0.10	NS, NS
Irregular wrinkle	<0.02, <0.02	S, S
R-smooth	<0.20, <0.10	NS, NS

[a] P values for two independent experiments are provided.
[b] S, statistically significant; NS, not statistically significant.

tained approximately 40% hyphal compartments. These proportions remained stable after 3 h of incubation in buffer solution (Table 3).

After o-smooth cells were incubated with buccal epithelium for 3 h, however, 38% of the adhering cell compartments and 50% of the suspended cell compartments were short hyphae (Table 3). When o-smooth cells were incubated with stratum corneum, 15% of the adhering cell compartments and 7% of the suspended cell compartments were short hyphae (Table 3). In both cases, the level of hyphal compartments contrasted markedly with the low levels in control cultures in buffer alone (i.e., without tissue). It should be emphasized that hyphae formed by o-smooth cells were short and, in most cases, would be scored as germ tubes (Fig. 4A and B).

Similar results were obtained with irregular wrinkle cultures. For incubation with buccal epithelium, 44% of the adhering cell compartments and 43% of the suspended cell compartments were scored as short hyphae. Again, this was in sharp contrast to the 4% level of short hyphal compartments in control cultures of irregular wrinkle cells in buffer alone (Table 3). As in the case of o-smooth cells, incubation with stratum corneum also stimulated hypha formation, but to a far lower degree than did buccal epithelium incubation (Table 3). Again, the hyphae formed by irregular wrinkle cells were relatively short and, in most cases, would be scored as germ tubes (Fig. 5A and B).

Since the proportion of yeast-phase, or budding-cell, compartments was close to the proportion of hyphal compartments in star and r-smooth cultures in buffer, these cultures appear to have reached a near-maximum level of hypha formation prior to incubation with tissues. Indeed, in both cases the levels of hyphal compartments were approximately the same for cells (i) adhering to buccal epithelium, (ii) in suspension in buccal epithelium cultures, (iii) adhering to stratum corneum, (iv) in suspension in stratum corneum cultures, and (v) in buffer alone (Table 3). In the case of star cell cultures, the proportion of hyphal compartments ranged between 43 and 54%, and in the case of r-smooth cultures, the proportion ranged between 40 and 48%. The hyphae in star (Fig. 4C and D) and r-smooth (Fig. 5C and D) cultures were quite elongate, contrasting markedly with the short hyphae, or germ tubes, of o-smooth (Fig. 4A and B) and irregular wrinkle (Fig. 5A and B) cultures. In addition, there was significant clumping of cells in the suspended cultures of star and r-smooth cells.

Hyphal induction by buccal epithelium and stratum corneum. Incubation with buccal epithelium stimulated an increase in hyphal compartments in o-smooth cultures from less than 1 (the level in buffer) to 38% in the adhering population and 50% in the suspended population (Table 3); stratum corneum stimulated an increase from less than 1 (the level in buffer) to 15% in the adhering population and 7% in the suspended population (Table 3). Incubation with buccal epithelium also stimulated an increase in hyphal compartments in irregular wrinkle cultures from 4 (in buffer) to 44% in the adhering population and 43% in the suspended population (Table 3); stratum corneum stimulated an increase from 4 (in buffer) to 8% in the adhering population and 20% in the suspended population (Table 3). Since the strong induction by buccal epithelium and the weaker induction by stratum corneum of hypha formation occurred in the suspended portion of the culture as well as the adhering portion of o-smooth and irregular wrinkle cells, we hypothesized that the medium must be conditioned. To test this possibility, buccal epithelium and stratum corneum were incubated in buffer for 3 h under assay conditions in the absence of *C. albicans*. The supernatants (i.e., without tissue) were then transferred to new chambers, inoculated with o-smooth and irregular wrinkle cells, and incubated for an additional 3 h. The supernatant from the buccal epithelium culture induced 42% hyphal compartments in the o-smooth population and 43% hyphal compartments in the irregular wrinkle population (Table 3). The supernatant from the stratum corneum induced 4% hyphal compartments in the o-smooth population but induced only negligible levels of hyphal compartments in the irregular wrinkle population (Table 3). These results demonstrate that at least in the case of the buccal epithelium, the induction of hyphal compartments is due to conditioning of the medium. Since pH can have a profound effect on the bud-to-hypha transition in nutrient medium at 37°C (8, 26), we measured the pH of the medium before and after incubation for every switch phenotype and each of the two tissues. In all cases, the pH before incubation was 7.0 and after incubation was between 6.85 and 6.95. Since the transition pH for hyphal induction in nutrient medium is

TABLE 3. Distribution by phenotype of cell compartments in adhesion cultures, in conditioned medium, or in buffer

Cell phenotype	Growth conditions[a]	Compartments (%)[b]					
		Adhering to tissue			In supernatant		
		Y	B	H	Y	B	H
O-smooth	BE	48	14	38	48	2	50
	SC	74	11	15	93	0	7
	BE conditioned				53	5	42
	SC conditioned				84	12	4
	BU				77	23	0
Star	BE	46	0	54	50	0	50
	SC	48	2	50	57	0	43
	BE conditioned				50	3	47
	SC conditioned				50	1	49
	BU				50	1	49
Irregular wrinkle	BE	52	4	44	54	3	43
	SC	68	24	8	80	0	20
	BE conditioned				57	3	40
	SC conditioned				73	27	0
	BU				87	9	4
R-smooth	BE	48	6	46	50	2	48
	SC	47	8	45	50	10	40
	BE conditioned				53	2	45
	SC conditioned				52	5	43
	BU				50	10	40

[a] Cells were incubated for 3 h under all conditions. BE, buccal epithelium; SC, stratum corneum; BE conditioned, buffer conditioned for 3 h by buccal epithelium; SC conditioned, buffer conditioned for 3 h by stratum corneum; BU, buffer.
[b] Y, mature cells in budding yeast phase; B, bud attached to mother cell and exhibiting less than 66% of the mother cell's volume; H, germ tubes or hyphae emanating from a mother cell in budding yeast form. Approximately 400 cells were scored under each set of conditions.

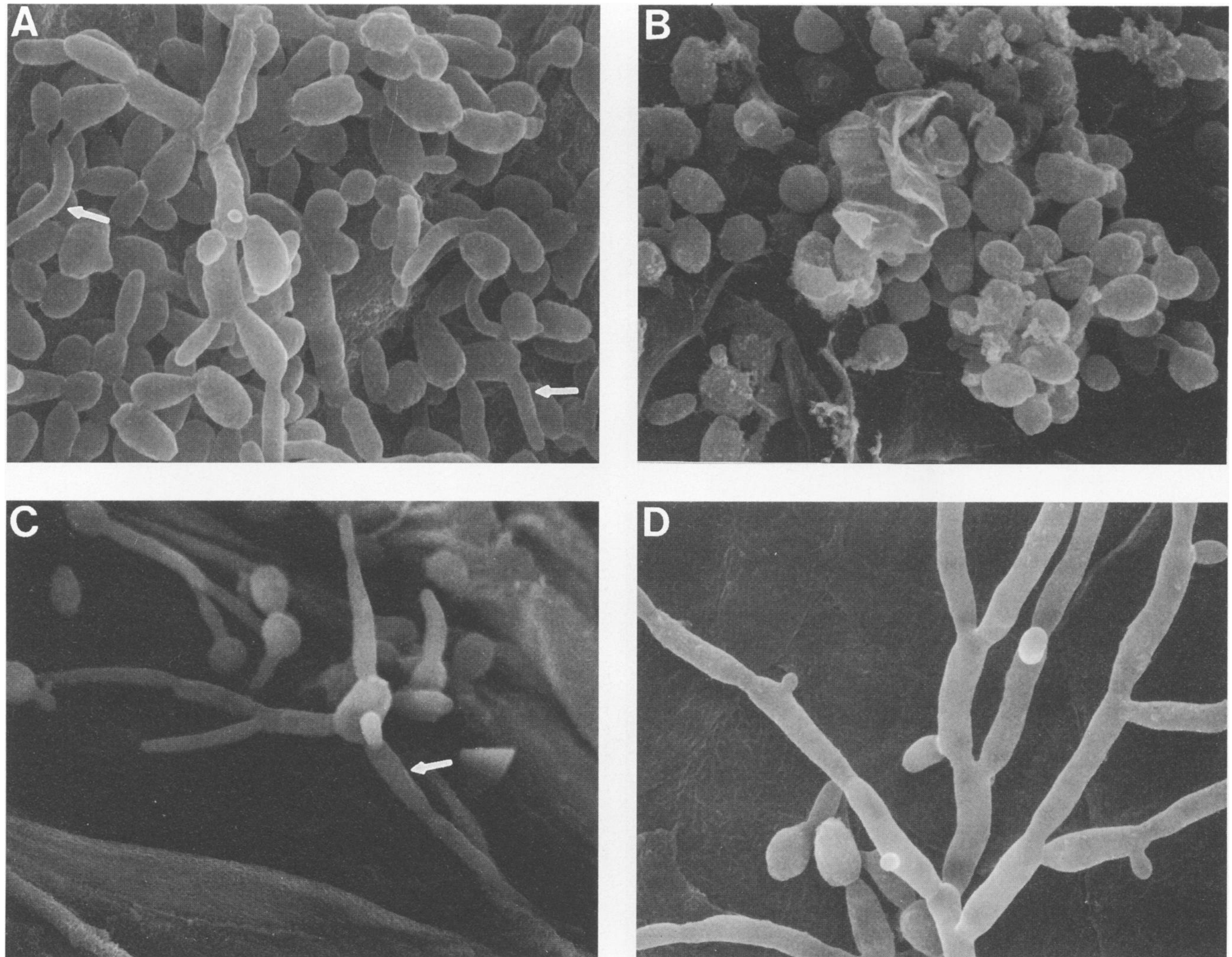

FIG. 4. Cells of o-smooth phenotype adhering to buccal epithelium (A) and stratum corneum (B) and of star phenotype adhering to buccal epithelium (C) and stratum corneum (D) after 3 h of incubation. The arrows in A point to elongate germ tubes, and the arrow in C points to a long hypha.

6.5 (i.e., a pH below 6.5 is conducive for bud formation and above 6.5 is conducive for hypha formation at 37°C [8]), we can conclude that the pH of the supporting medium is conducive but not sufficient for the induction of hyphae in buccal epithelium cultures.

The induction of germ tube formation may depend not only upon high temperature and pH but also upon a carbon source to support germ tube growth. We therefore tested whether buccal epithelium and stratum corneum released organic molecules into the incubation medium under the incubation conditions employed. Discs of buccal epithelium and stratum corneum were incubated with phosphate buffer lacking *C. albicans* for 3 h under adhesion assay conditions. Conditioned medium was serially diluted, plated on silica gel, charred, and scanned. Measurements were compared with serial dilutions of bovine serum albumin standards. Medium conditioned by buccal epithelium contained an average of 14 mg of charrable carbon per ml. Medium conditioned by stratum corneum and unconditioned phosphate buffer solution contained no detectable charrable carbon.

DISCUSSION

In order for *C. albicans* cells to penetrate tissue, they must first adhere to epithelium. Adhering cells then extend hyphae into the tissue, and the hyphae grow, branch, and release budding cells. It has been reasonably well documented that hyphae are more adhesive than yeast phase cells (3, 14) and that variations in adhesiveness exist between strains (see reference 21 for a review). In addition, it has been demonstrated that high-frequency phenotypic switching can result in reversible changes in adhesiveness. Kennedy et al. (13) found that in the white-opaque phase transition in *C. albicans* WO-1 (25, 29), cells expressing the white budding phenotype were more adhesive to buccal epithelium than cells expressing the opaque budding phenotype. However, the opaque budding cell exhibits a unique morphology (2, 4, 25, 29) and the white-opaque transition is found in less than 5% of *C. albicans* strains (29). To test whether changes in adhesiveness represent a general characteristic of switching in *C. albicans*, we compared the adhesive characteristics of the switch phenotypes of a more common switching system, that of laboratory strain 3153A

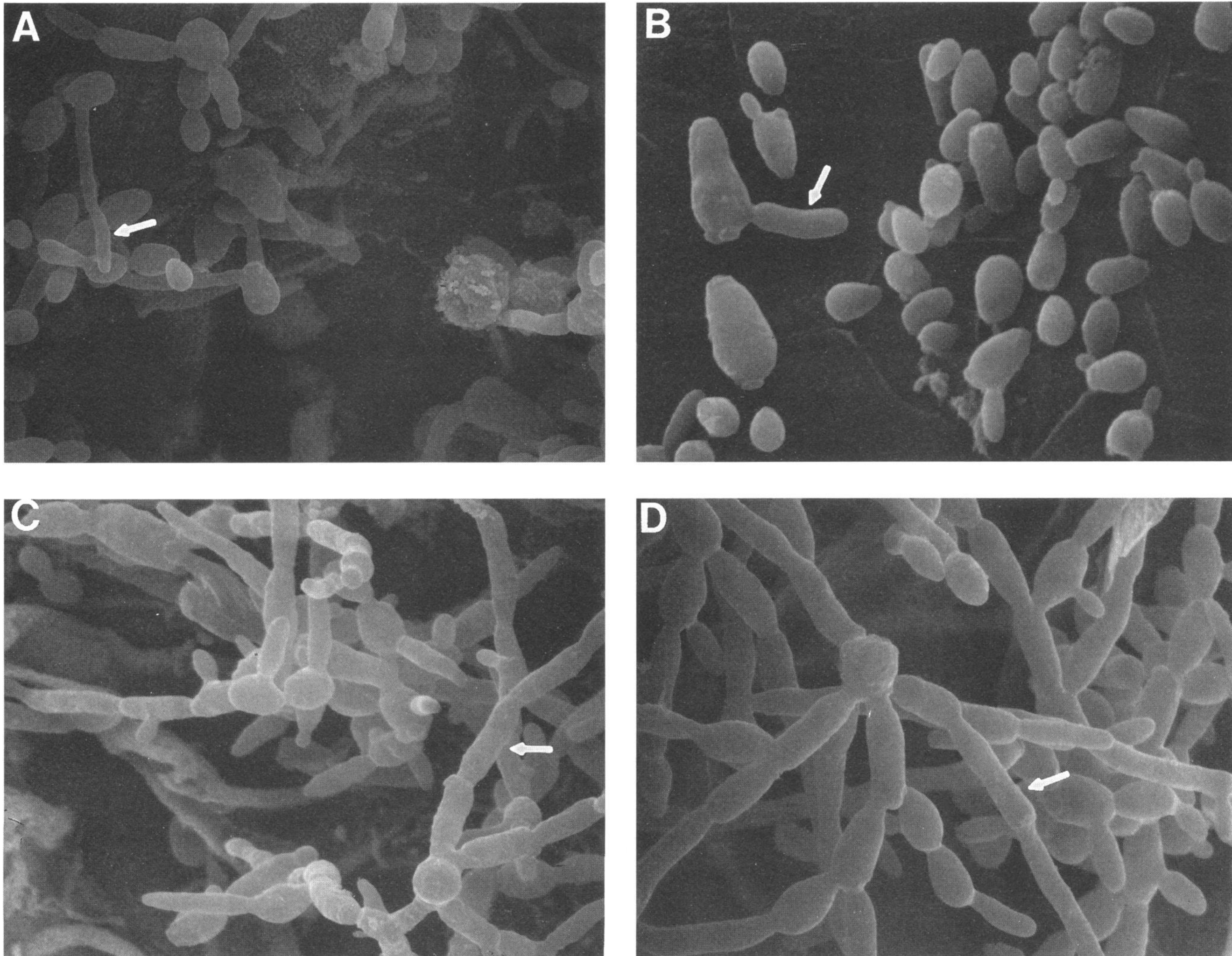

FIG. 5. Cells of irregular wrinkle phenotypes adhering to buccal epithelium (A) and stratum corneum (B) and r-smooth cells adhering to buccal epithelium (C) and stratum corneum (D) after 3 h of incubation. The arrows point to an elongate germ tube in A, a wide germ tube in B, and quite long pseudohyphae in C and D.

(24). We compared the basic o-smooth phenotype with three of the seven variant phenotypes in the 3153A switching repertoire, and we have compared cells just after they had entered stationary phase, since it was previously demonstrated that star cells express a variant phenotype in liquid culture when entering stationary phase (5). We have found that, although there is no significant difference between o-smooth and irregular wrinkle cells in their properties of adhesion to buccal epithelium, there are significant differences between o-smooth and either star or r-smooth cells. The levels of adherence of star and r-smooth cells to buccal epithelium are approximately 26 and 54% that of o-smooth adherence, respectively. The hierarchy of adherence to buccal epithelium was determined as follows: o-smooth ≅ irregular wrinkle > r-smooth > star. The hierarchy of adherence to stratum corneum was similar but not identical: o-smooth > irregular wrinkle ≅ r-smooth > star. If the adherence properties to the tissues are pooled, the following more generalized hierarchy results: o-smooth > irregular wrinkle > r-smooth > star. This generalized hierarchy of adherence inversely follows the hierarchy of hypha formation by these cells at stationary phase, which is as follows: o-smooth (0%) < irregular wrinkle (4%) < r-smooth (40%) < star (49%). This may be the key to understanding the differences in adhesion between o-smooth and the three tested phenotypes. The majority of star and r-smooth cells formed hyphae in early stationary phase, and incubation with buccal epithelium or stratum corneum, therefore, had little further effect on the proportion of hyphae in the incubation medium. The hyphae were long in these cultures, and clumping was evident among suspended cells. These cultures on average exhibited the lowest levels of adhesion to buccal epithelium and stratum corneum. In contrast, the majority of o-smooth and irregular wrinkle cells retained the budding phenotype in early stationary phase, and incubation with buccal epithelium, and to a lesser extent stratum corneum, induced germ tube formation. However, these germ tubes were far shorter than the hyphae already present in stationary-phase star and r-smooth cultures. Since o-smooth and irregular wrinkle cells exhibited the highest levels of adhesion, we can tentatively conclude that the presence of mature hyphae at the onset of the adhesion assay may interfere with adhesion. This does not rule out the possibility that qualitative differences in the cell wall surfaces

also play a role. Changes in surface antigens have been demonstrated in the white-opaque phase transition in strain WO-1 (1, 2, 4). Opaque cells contain one or more opaque-specific surface antigens not present on the surface of white cells, and a 14.5-kDa opaque-specific antigen has been localized to the unique opaque cell wall pimple (2).

We have also found that buccal epithelium induces germ tube formation in o-smooth cell cultures, which contain less than 0.5% hyphae in early stationary phase, and in irregular wrinkle cell cultures, which contain roughly 5% hyphae in early stationary phase. Medium first conditioned by buccal epithelium also induced germ tube formation in both o-smooth and irregular wrinkle cultures after the removal of tissue, indicating that buccal epithelium releases one or more components involved in induction. Since incubation conditions included a temperature of 37°C and pH of 7.0, all that was required for hypha induction may have been a carbon source (8, 26). Charring experiments demonstrated that buccal epithelium released carbon-containing molecules into the incubation medium. We therefore do not believe that buccal epithelium releases a specific hypha-inducing molecule but rather that it provides a carbon source for daughter cell growth under conditions conducive to the hyphal phenotype. We are now in the process of characterizing the molecules released by buccal epithelium which support germ tube formation in o-smooth and irregular wrinkle cell cultures.

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