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Biotransformation of Polycyclic Aromatic Hydrocarbons by Yeasts Isolated from Coastal Sediments

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Yeast abundance in the sediments of 13 coastal sites in Massachusetts was quantified, and the potential of yeast isolates to biotransform polycyclic aromatic hydrocarbons (PAHs) was determined. Plate counts of yeasts varied between 10^2 to 10^7 CFU g (dry weight) of sediment^{-1}. The most abundant genera isolated and identified included *Candida*, *Cryptococcus*, *Rhodotorula*, *Torulopsis*, and *Trichosporon*. More than 50% of the isolates from heavily contaminated sites transformed phenanthrene, as determined by spray-plate screening. The plate counts of phenanthrene-transforming yeasts correlated significantly to the sediment concentrations of phenanthrene. Transformation of [9-^{14}C]phenanthrene and [12-^{14}C]benz[*a*]anthracene by individual isolates varied greatly, ranging from 0.15 to 8.15 μmol of PAH g^{-1} in 120-h incubations. Of the isolated yeasts, *Trichosporon penicillatum* exhibited the greatest capacity for phenanthrene transformation. The ability to transform PAHs appears to be widespread among yeasts in coastal sediments.

The environmental significance of fungal transformation of polycyclic aromatic hydrocarbons (PAHs) is an area of increasing interest (32, 46). Fungal oxidation of PAHs is often a prelude to chemical conjugation, which appears to be a detoxification process, whereas bacteria can oxidize PAHs as an ultimate source of carbon and energy, converting the parent PAH to CO_2 and H_2O (11, 46). The potential for fungi to oxidize PAHs is well known (24, 46). For example, the fungus *Cunninghamella elegans* (9, 12) and the white rot fungus *Phanerochaete chrysosporium* (15, 21, 47) transform phenanthrene. In the presence of glucose, pure cultures of the yeasts *Saccharomyces cerevisiae* and *Candida utilis* oxidize naphthalene and anthracene (23). *Candida guilliermondii*, *Debaromyces hansenii*, *Candida tropicalis*, *Candida maltosa*, and several isolates of *Candida lipolytica* oxidize naphthalene and benzo[*a*]pyrene (10). *S. cerevisiae* also oxidizes benzo[*a*]pyrene (49).

Filamentous fungi and yeast are common in marine environments (1, 19, 26, 29, 33), and a few attempts to isolate PAH-degrading marine fungi have been reported (2, 13, 16, 26). However, the yeast isolates have not been evaluated for their ability to oxidize PAHs in the presence of alternative carbon sources (i.e., cooxidation), yet this may be a significant activity in marine environments. The limited evidence available suggests a minor role for yeasts and fungi in the transformation of PAHs in marine ecosystems. However, because of their common occurrence and observed activity, the potential for yeasts and filamentous fungi to degrade PAHs in marine ecosystems deserves further attention.

Phenanthrene and benz[*a*]anthracene were used as model PAH compounds in this investigation. Both compounds are pollutants in coastal sediment (43), and they are representative of the wide range of physical-chemical properties of PAHs and their susceptibility to biotransformation (38, 48). They have different toxic and mutagenic properties (7, 48) and have been used as model PAHs in several other degradation studies (18, 22, 31, 40). Our major objective was to quantify the presence of yeasts in coastal sediments and to evaluate their potential to biotransform PAHs.

MATERIALS AND METHODS

Sampling sites. Sediment samples were taken from 13 coastal sites in Massachusetts Bay and Buzzards Bay, Massachusetts (see Table 1). All sites were composed of muds consisting of fine silt-clays. Samples were taken at each site with an Eckman dredge (Wildco, Saginaw, Mich.) or sample container (Cole-Palmer, Chicago, Ill.). A composite sample of the top 2 to 3 mm of sediment from each site was placed in a glass bottle and stored on ice during transport to the laboratory. Sampling sites include hydrocarbon-contaminated areas and relatively clean areas. Phenanthrene concentrations for sites 6, 7, and 12 listed in Table 1 were obtained from a government agency report (7a), and concentrations for sites 2, 4, and 13 were reported previously (43). All other phenanthrene concentrations were determined as described below.

Sediment chemical analyses. The organic carbon concentration of sediment was determined on a CHN analyzer (Perkin-Elmer, Norwalk, Conn.). The phenanthrene concentration was determined by extracting 5 g of wet sediment with methanol which was back-extracted with hexane. The methanol-extracted sediment was further extracted with methylene chloride in a Soxhlet extraction apparatus (Kontes, Vineland, N.J.). The hexane and methylene chloride fractions were combined and concentrated. Prior to solvent extraction, an internal standard of [9-^{14}C]phenanthrene or [12-^{14}C]benz[*a*]anthracene was added to the sediments (6).

PAHs were separated and identified by reverse-phase high-performance liquid chromatography (HPLC) and UV-visible spectra (Hewlett-Packard 1040A and a diode-array spectrophotometer fitted with a DynaChem [Sunnyvale, Calif.] high-carbon column [25 cm by 4.6 mm; 5-μm particle size, octadecylsilane, C_{18}]). The mobile phase was a methanol-phosphate buffer gradient (pH 2.7). The phenanthrene concentration was determined by comparing extract HPLC

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peaks with HPLC standard peaks of known concentrations of phenanthrene and correcting for extraction efficiency.

Chemicals. [9-^{14}C]phenanthrene (specific activity, 13.0 mCi mmol^{-1}) was purchased from Amersham Co. (Arlington Heights, Ill.) and purified with Florisil (Fisher Scientific Inc., Fair Lawn, N.J.) by column chromatography to a purity greater than 98% as determined by thin-layer chromatography analysis. [12-^{14}C]benz[*a*]anthracene (specific activity, 49.0 μCi μmol^{-1}) was purified to greater than 98% by liquid-liquid extraction with a 4:1 mixture of 0.15 M KOH–23.38 M dimethyl sulfoxide and hexane.

Yeast enumeration. Four media were compared to select the best medium for enumerating and isolating yeasts from coastal sediments. The media were the following: (i) Sabouraud dextrose agar (SDA; Difco), (ii) *Prototheca* isolation agar (PIA; 37), (iii) yeast extract-malt extract (YM) agar (Difco), and (iv) a dilute organic carbon medium (DM) consisting of 0.1 g of Bacto Peptone, 0.1 g of yeast extract, 0.1 g of $FePO_4$, 18 g of Bacto Agar, and 1 liter of seawater prefiltered through a 0.45-μm-pore-size membrane. The four media were adjusted to pH 4.0 with HCl and amended with streptomycin and tetracycline to final concentrations of 0.03 μg ml^{-1} and 0.02 μg ml^{-1}, respectively. Yeasts were enumerated by standard spread plate methods for yeasts (30, 36). However, by increasing the inoculum size to 1.0 ml, the detection limit of the plate counting procedure was 3.0×10^2 CFU g^{-1}. After the plates were incubated at 22°C for 3 to 5 days, a randomly selected colony of each colony type (on the basis of colony appearance) was picked for microscopic observation. Cells from the selected colony were observed for cell size and morphology consistent with yeasts. Plates with more than 25% of their surface area covered with mold were not used for enumeration, as recommended by Sherry (39).

All reported sediment plate counts of yeasts were performed in triplicate on amended YM agar. Bacteria were enumerated by spread plating on DM with the pH adjusted to 7.2. Detection of PAH degraders among the isolated sediment yeasts was done by using a spray-plate technique. Spray-plates were prepared with 0.5% phenanthrene in acetone as described previously (42). Zones of phenanthrene clearing surrounding the yeast colonies were used as an indicator of presumptive biotransformation.

Identification. Yeast isolates were identified by genus and species by traditional methods of colony appearance, cell morphology, and physiological differences, such as the ability to ferment glucose, assimilate sugars (e.g., cellobiose, galactose, glucose, inositol, lactose, maltose, and sucrose), and grow on nitrate as a sole nitrogen source (30, 36). Identification was confirmed by the use of rapid identification methods based on colorimetric detection of enzymes (API Yeast-Ident) or carbon assimilation (API 20C) (Analytab Products Inc., Plainview, N.Y.).

Biotransformation. Pure-culture biodegradation studies were done as described by Cerniglia and Crow (10). Phenanthrene biotransformation was determined for yeasts and a phenanthrene-degrading bacterium, *Beijerinckia* sp. strain B1 (17, 45). *C. lipolytica* 37-1, a reported PAH biotransformer (10), was also tested for comparison with the isolated yeasts. All experiments were performed by using 25-ml Erlenmeyer flasks with 10 ml of medium incubated in the dark. Flasks were sealed with Teflon-lined serum septa. Each flask contained a CO_2 trap, which consisted of a glass center well containing fluted filter paper saturated with 2.0 N KOH (40).

Cells were grown in saline YM broth with phenanthrene (0.019 mmol 10 ml^{-1}) or benz[*a*]anthracene (0.010 μmol 10 ml^{-1}) for 72 h at 22°C. The cells were concentrated by centrifugation at 8,000 × *g* for 5 min and washed three times with 0.1 M $NH_4H_2PO_4$ solution. For biotransformation, 10^7 cells (determined to be equivalent to 0.1 g [wet weight] of cells) was inoculated into saline YM broth with phenanthrene (0.0190 mmol 10 ml^{-1}) and [9-^{14}C]phenanthrene (0.1 μCi 10 ml^{-1}) or benz[*a*]anthracene (0.010 μmol 10 ml^{-1}) and [12-^{14}C]benz[*a*]anthracene (0.3μCi 10 ml^{-1}) and incubated for 120 h at 22°C. Cell suspensions were examined by microscopy for bacterial contamination. Sterile solutions were tested for viability on nutrient agar plates (Difco).

After incubation, cell suspensions were acidified to pH 3.0 with 0.3 ml of 10% trichloroacetic acid. Any $^{14}CO_2$ evolved was trapped in KOH during incubation and acidification of the cell suspension. The cell suspension was extracted three times with 3 ml of ethyl acetate and three times with 3 ml of methylene chloride. The extraction efficiency for the radiolabeled parent PAH and metabolites was 89% ± 1%. Excess water was removed from the solvent extract with anhydrous sodium sulfate. The solvent was evaporated by passing a stream of N_2 over the sample in a 45°C water bath.

After the residues of dried solvent extracts were dissolved in 60 ml of acetone, the samples were analyzed by thin-layer chromatography to separate polar metabolites and the parent compound. Silica gel plates (A-K6F, fluorescent at 254 nm, 5 by 25 cm, 250-μm layer; Whatman Ltd., Maidstone, England) were developed for 20 min in hexane-toluene (7:3). Radioactive material associated with quenched spots was scraped from the silica plates and counted by liquid scintillation. The mean recovery of radiolabel for both the phenanthrene and benz[*a*]anthracene experiments was calculated at 70% of the total radioactivity added. The standard deviation was ±7%. Recovery was based on the sum of CO_2, solvent extract, aqueous residue, cell residue, and an acetone wash of glassware. Biotransformation was estimated on the basis of radiolabeled polar metabolites formed and $^{14}CO_2$ evolved in pure culture and calculated on the basis of cell dry weight.

Statistical analyses. The Fisher exact test was used to compare all observed zones of phenanthrene clearing by yeasts isolated from each site with phenanthrene contamination at the sample site (44). Data from yeast biotransformation experiments were evaluated by analysis of variance with multiple comparisons against the sterile control by using the Dunnett test (SAS Institute Inc., Cary, N.C.). Significance levels were set at *P* of 0.05 (44).

RESULTS

Sediment analysis. Sampled sediments had organic carbon contents ranging from 0.29 to 9.45% (Table 1). Phenanthrene contamination levels for the sample sites were <2.5 to 63,683 ng g of dry sediment^{-1} (Table 1).

Enumeration of total yeast populations. Of the four media used (SDA, YM agar, PIA, and DM), the YM agar gave counts greater than or equal to those of the other media in six of eight sediments tested. Therefore, YM agar was used for the remainder of the experiment to enumerate and isolate estuarine yeasts. Yeast populations from $<10^2$ to 10^7 CFU g [dry weight] of sediment^{-1} were enumerated from 13 different coastal sediments on YM agar (Table 1). Yeasts were isolated from all sampling sites. However, two samples (West Polpis Harbor, north and south) had densities below levels that could be accurately enumerated by the method used. For comparison, bacterial counts from the same sample sites were several orders of magnitude higher than the

TABLE 1. Location, chemical characteristics, and yeast counts of sediment sampling sites along the Massachusetts coastline

Site no.	Massachusetts site of isolation	Longitude (East)	Latitude (North)	Yeast counts (CFU/g [wet wt])		Phn (ng/g)[a]	TOC[b] (%)
				Total[c]	Phn+[d]		
1	Fort Point Channel, Boston Harbor	71°03'00"	42°20'66"	2.5×10^3	5.8×10^2	18,987, 63,683[e]	4.74
2	Island End River, Chelsea	71°03'25"	42°23'50"	2.8×10^5	1.5×10^5	49,094[e]	4.00
3	Little Pond, Falmouth	70°35'30"	41°32'30"	8.1×10^2	$<10^2$	ND[f]	9.45
4	Neponset River, Dorchester	71°02'30"	42°18'10"	3.1×10^3	1.33×10^3	1,110[e]	3.50
5	Quaise Marsh, Nantucket	70°02'30"	41°17'50"	4.1×10^7	$<10^2$	<2.5	8.28
6	Savin Hill Cove, Dorchester	71°03'10"	42°18'45"	1.2×10^3	6.5×10^2	0.71[g]	3.44
7	Gull Point, Germantown, Quincy	70°57'35"	42°15'02"	4.5×10^2	$<10^2$	0[g]	0.29
8	Spectacle Island, Boston Harbor	70°59'50"	42°19'50"	1.4×10^3	2.4×10^2	<2.5, 0.94[g]	2.43
9	Station R, Buzzards Bay	70°53'34"	41°29'27"	1.8×10^3	ND[f]	516	1.71
10	West Polpis-North, Nantucket	70°01'10"	41°18'25"	$<10^2$	$<10^2$	<2.5	4.13
11	West Polpis-South, Nantucket	70°01'20"	41°17'25"	$<10^2$	$<10^2$	<2.5	4.07
12	Wollaston Beach, Quincy	71°00'10"	42°16'30"	4.7×10^3	$<10^2$	0.50[g]	3.72
13	World's End, Hingham	70°53'05"	42°16'20"	3.0×10^3	$<10^2$	665[g]	0.99

[a] Phn, phenanthrene concentration (ng/g of dry sediment).
[b] TOC, total organic carbon.
[c] Plate counts were performed in triplicate.
[d] Phn+, yeast CFU that displayed zones of phenanthrene clearing on overlay plates.
[e] Phenanthrene concentration from listed values in Shiaris and Jambard-Sweet (43).
[f] ND, not determined.
[g] Phenanthrene concentration from listed values in reference 7a.

yeast counts. Bacterial counts ranged from 10^6 to 10^9 CFU g [dry weight] of sediment^{-1} (data not shown).

Identification. Of 31 isolates, 21 were identified to the level of genus and species. Ten isolates with cell morphology and cell size indicative of yeasts gave variable or inconclusive results and could not be identified. *Candida* and *Rhodotorula* were the most frequently isolated genera. Other genera isolated were *Cryptococcus*, *Torulopsis*, and *Trichosporon* (Tables 2 to 4). The yeast-like alga *Prototheca* sp. was also isolated.

Enumeration of presumptive phenanthrene degraders. By using the spray-plate screening technique, the frequency of phenanthrene clearance zones per CFU of yeasts varied with site (Table 1). Sites with phenanthrene concentrations of >1,000 ng g [dry weight] of sediment^{-1} were defined as heavily contaminated. Yeast isolates from three of three heavily contaminated sites displayed phenanthrene clearance zones. In contrast, yeasts isolated from six of eight sites with low phenanthrene contamination did not have phenanthrene clearance zones. A statistically significant difference ($P = 0.045$) was observed between the frequency of yeasts with phenanthrene clearance zones isolated from contaminated sites and the frequency of yeasts with phenanthrene clearance zones isolated from noncontaminated sites.

TABLE 2. Ability of yeast isolates that form clearing zones on phenanthrene-sprayed plates to transform [9-^{14}C]phenanthrene

Yeast species	Phenanthrene transformed (μmol g^{-1})	Isolate code identification
Candida krusei	0.93 (0.48)[b]	10
Candida lipolytica	0.32 (0.10)	CL
Candida lusitaniae	0.56 (0.06)	2
Candida parapsilosis	0.19 (0.02)	16
Candida rugosa	0.28 (0.03)	1
Candida zeylanoides	3.77 (0.29)[b,c]	19
Cryptococcus albicans	0.26 (0.01)	21
Rhodotorula glutinis	0.73 (0.13)	12
Rhodotorula minuta	0.38 (0.04)	23
Rhodotorula rubra	1.17 (0.01)[b]	29
Torulopsis glabrata	1.84 (1.13)[b]	28
Trichosporon penicillatum	8.15 (3.58)[b,c]	3
Cryptococcus sp.	0.23 (0.13)	14
Rhodotorula sp.	0.15 (0.09)	24

[a] Values are the means (standard deviations) of triplicate samples measuring formation of polar ^{14}C-metabolites and evolved $^{14}CO_2$ during a 120-h incubation.
[b] Statistically significant ($P < 0.05$) difference from sterile control in formation of polar metabolites.
[c] Statistically significant ($P < 0.05$) difference from sterile control in formation of $^{14}CO_2$.

Biotransformation. Of 31 yeast isolates presumptively determined to biotransform phenanthrene as indicated by the spray-plate screening method, 13 yeasts were tested to confirm transformation of [9-^{14}C]phenanthrene. These 13 yeasts included representatives of 7 genera and 11 species (Table 2). Phenanthrene biotransformation by the yeast isolates (0.15 to 8.15 μmol g^{-1} in 120 h) indicated a wide range of activity. Of the 13 yeast isolates tested, 5 transformed [9-^{14}C]phenanthrene (Table 2). Isolates were recorded as confirmed transformers of phenanthrene if they showed a statistically significant difference from the sterile control ($P < 0.05$) in formation of polar metabolites or evolution of $^{14}CO_2$. On the basis of metabolite formation and evolution of $^{14}CO_2$, five phenanthrene-transforming yeasts were confirmed, namely, *Candida krusei*, *Candida zeylanoides*, *Trichosporon penicillatum*, *Torulopsis glabrata*, and *Rhodotorula rubra*.

Phenanthrene biotransformation by the reported PAH-degrading culture *C. lipolytica* 37-1 was 0.32 μmol g^{-1}, which was not significantly different ($P > 0.05$) from that of the sterile control. However, phenanthrene transformation for the reported phenanthrene-degrading bacterium *Beijerinckia* sp. strain B1 was greater (1.2×10^2 μmol g^{-1}) than that of any yeast isolates.

Isolates with less-pronounced phenanthrene clearing on agar plates and slow phenanthrene biotransformation were not scored as phenanthrene-biotransforming yeasts. Isolates did not always retain the ability to form zones of clearing on subsequent platings. Initially, *C. krusei* and *Rhodotorula glutinis* displayed zones of phenanthrene clearing, but on subsequent platings phenanthrene clearing was not ex-

TABLE 3. Cell morphology and colony appearance of yeast isolates

Isolate identification code	Description[a]
1	2.5- to 7.5-μm spheroid cells, budding (YE); white colony (YM), pseudomycelium (CM)
2	2.5- to 5.0-μm cells, budding, pseudomycelium, white colony (CM)
3	7.5-μm cells, binary fission (YE); white-dry colony, true mycelium, arthrospores (CM)
10	2.5-μm spheroid cells, multipolar budding (YE); white mucoidal colony, pseudomycelium (CM)
12	5.0-μm irregular spheroid cells, budding (YE); yellow carotenoid colony (SDA), no pseudomycelium (CM)
13	5.0-μm spheroid cells, budding (YE); yellow carotenoid moist colony, multipolar budding, no pseudomycelium (CM)
14	5.0-μm spheroid cells, budding (YE); white moist colony (SDA), no fermentation
16	2.5- to 7.5-μm spheroid cells, budding (YE); white colony, pseudomycelium (CM); does not assimilate cellobiose, assimilates raffinose
19	2.5- to 7.5-μm spheroid cells, budding (YE); white colony (YM), pseudomycelium (CM)
21	2.5- to 7.5-μm cells, budding, no spores, no pellicle (YE); white, shiny, slightly mucoid colony (CM, SDA); no pseudomycelium, subsurface growth (CM)
24	5.0-μm spheroid cells, budding (YE); pink moist colony (SDA)
28	2.5- by 2.5- to 7.5-μm ovoid cells, budding (YE); white colony, no pseudomycelium (CM)
29	5.0-μm spheroid cells, budding (YE); red moist colony (SDA); budding, no pseudomycelium (CM)
CL	2.5- to 7.5-μm elongated cells, budding, binary fission, no pellicle, vacuoles, (YE); white mucoid colony, abundant pseudomycelium (CM); white folded or wrinkled colony (YM, SDA)

[a] YE, yeast extract broth; CM, cornmeal agar.

pressed. *C. krusei* still maintained the ability to produce polar metabolites from phenanthrene. Conversely, *R. glutinis* also lost the ability to transform radiolabeled phenanthrene.

Benz[*a*]anthracene transformation by yeast isolates was slow (0.13 to 0.29 μmol g^{-1} in 120 h) when compared with phenanthrene transformation. Limited oxidation of [12-^{14}C]benz[*a*]anthracene to polar metabolites was observed, but $^{14}CO_2$ evolution was not detected. The two yeast isolates tested, *C. krusei* and *Rhodotorula minuta*, biotransformed benz[*a*]anthracene at 0.29 and 0.24 μmol g^{-1}, respectively, which was significantly different statistically ($P < 0.05$) from the amount biotransformed by the sterile control. Transformation of benz[*a*]anthracene (0.13 μmol g^{-1}) by the laboratory culture of *C. lipolytica* 37-1 was not significantly higher than that by the sterile control.

DISCUSSION

Yeasts are often isolated from waters enriched in nutrients, while yeast populations in unpolluted waters are low (19). The presence of yeasts in marine waters is well documented (27, 36), and elevated yeast densities have been observed at nutrient-rich haloclines in estuaries (33). In

TABLE 4. Carbon assimilation of yeast isolates[a]

Carbon source	Growth of isolate:													
	1	2	3	10[b]	12	13	14	16	19	21	24	28	29	CL
None	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	−	−	+	−	−	+	−	+	+	−	+	±	−	+
2-Keto-D-gluconate	−	+	−	−	+	+	+	±	+	+	+	−	−	−
L-Arabinose	−	−	−	−	−	+	−	+	−	+	+	−	+	−
Xylose	−	−	+	−	−	−	+	+	−	+	+	−	+	−
Adonitol	−	+	−	−	+	−	−	+	±	±	+	−	−	−
Xylitol	−	−	−	−	±	−	−	−	−	−	−	−	−	−
Galactose	−	−	+	−	−	−	−	+	±	+	±	−	−	−
Inositol	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Sorbitol	−	+	−	−	+	−	+	+	−	+	+	−	−	−
methyl-D-Glucoside	−	+	−	−	+	−	−	+	−	+	−	−	−	−
N-Acetyl-D-glucosamine	−	−	−	+	−	−	+	+	+	+	−	−	−	−
Cellobiose	−	+	−	−	+	−	−	−	−	±	−	−	−	−
Lactose	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Maltose	−	+	−	−	+	−	±	+	−	+	+	−	+	−
Sucrose	−	+	−	−	+	+	+	+	−	+	+	−	+	−
Trehalose	−	+	−	−	+	−	−	+	+	+	+	+	+	−
Melezitose	−	+	−	−	+	+	+	+	−	+	+	−	+	−
Raffinose	−	−	−	−	±	−	+	−	−	+	+	−	+	−

[a] Results from API 20C method with incubation for 72 h are growth (+) and no growth (−). Isolates 21, 24, and 28 were identified on the basis of API Yeast-Ident results (data not shown).
[b] Isolate 10 showed no growth at 42°C.

coastal sediments, typical yeast densities fall between 10^2 and 10^4 yeast CFU g^{-1} (2, 16). Therefore, the majority of sites on the Massachusetts Bay coastline had yeast populations within the densities expected for marine sediment. The exceptions were elevated yeast counts at the Island End River and Quaise Marsh sites (2.8×10^5 and 4.1×10^7 yeast CFU g of sediment^{-1}, respectively) and the low counts at the two West Polpis Harbor sites ($<10^2$ yeast CFU g of sediment^{-1}). The observed variant yeast counts did not correlate with sediment total organic carbon as might be expected (Table 1), but the reported yeast counts are not a thorough analysis of the spatial distribution of yeasts in these sediments. Rather, the counts are average yeast abundances in the sediments based on composite sampling of each site.

Candida and *Rhodotorula* were the most frequently isolated yeast genera from Massachusetts coastal sediments. These findings are similar to those of Hagler et al. (19), who reported that *Candida* and *Rhodotorula* were the most frequently isolated genera from a polluted estuary. Norkrans (33) also isolated *Candida* and *Rhodotorula*, among other genera, from a Swedish estuary. Yeast flora in near-shore waters can be strongly influenced by pollution from terrestrial sources, including sewage. Yeasts found in aquatic environments are generally asporogenous and oxidative or weakly fermentative (36). Therefore, the yeasts isolated from the aerobic zone in Massachusetts coastal sediments were similar to those expected in overlying waters.

The correlation of hydrocarbon-degrading yeast densities with the level of hydrocarbon exposure has been demonstrated previously (1). This trend was also significant in Massachusetts coastal sediments. As with exposure to other hydrocarbons (3), chronic exposure of yeasts to phenanthrene in coastal sediments appears to select for yeasts or enhance the capacity of yeast populations to biotransform phenanthrene.

The potential for yeasts to metabolize aromatic compounds is well established (8, 13). Recent evidence by Wright and Ratledge (50) shows that some isolates possess more than one pathway to degrade single-aromatic ring compounds. Yeast isolates that oxidize PAH in the presence of an alternate carbon source also have been described (10, 23, 34). Here we have demonstrated that a variety of marine isolates can also metabolize PAHs. Of 13 yeasts that cleared phenanthrene upon primary plating, 5 were quantitatively confirmed as phenanthrene transformers by radioisotope and chromatographic methods. In a previous report (42), all of the bacteria that formed clear zones in the phenanthrene overlay also transformed radiolabeled phenanthrene, but this high correlation was not observed with yeasts. The reasons are uncertain, but the apparent loss of phenanthrene transformation capacity by some yeasts may be accounted for by changes in the induction of yeast cytochrome P-450 or altered bioavailability of phenanthrene in the different media.

T. penicillatum displayed especially rapid phenanthrene biotransformation (8.15 μmol g^{-1} in 120 h) in comparison with the other yeast isolates. A *Trichosporon* sp. has been previously implicated in hydrocarbon degradation. Ahearn et al. (2) reported that marine *Trichosporon* isolates are able to utilize hexadecane and kerosene. The repeated isolation and independent demonstration of the *Trichosporon* sp. as a hydrocarbon-degrading yeast suggests that it may be a useful model for examining PAH degradation by yeasts and for potential use in bioremediation technologies.

The larger PAHs, in this case benz[*a*]anthracene, are not as amenable to metabolism by yeasts as the smaller, more-water-soluble PAHs. Also, rapid screening techniques, such as the spray-plate method, cannot be used. However, we determined that benz[*a*]anthracene was biotransformed by two isolates, *C. krusei* and *R. minuta*. Biotransformation of either phenanthrene or benz[*a*]anthracene by yeasts has not, to our knowledge, been previously reported. Thus, the list of PAHs that can be oxidized by yeasts and the group of yeast genera and species identified as PAH transformers are expanded (10, 23). Any attempt to compare degradation between fungi and bacteria can be confusing. Degradation by yeasts and fungi generally is reported per dry weight of biomass (28), whereas bacterial degradation is generally reported per cell (45). If we compare biotransformation on a biomass basis, yeast degradation was less effective than bacterial degradation. However, when compared on a per-cell basis, biotransformation among yeasts and bacteria was equivalent. Yeasts displayed phenanthrene biotransformation of 8.04×10^{-10} to 10.80×10^{-12} μmol cell^{-1}; the bacterium *Beijerinckia* sp. strain B1 demonstrated biotransformation of 3.6×10^{-11} μmol cell^{-1}. Considering the disparity of cell size between bacteria and yeasts, calculation on a biomass rather than a per-cell basis seems the more correct means of comparison for environmental significance (5, 36).

The ability of yeasts to transform PAHs appears to be widespread in marine environments. Since PAH-transforming populations are well adapted to their environments (4, 14, 20, 25, 40, 41), isolates from diverse environments may have different degradative potentials. In general, yeasts are less susceptible to changes in pH and salinity than bacteria (36). Therefore, yeasts may have an advantage in fluctuating conditions caused by terrestrial run-off, atmospheric fallout, oil spills, sewage discharge, or offshore drilling. However, since the biomass of yeasts is orders of magnitude lower than that of bacteria in marine sediments, the potential of yeasts to be major agents of PAH transformation in this habitat appears to be lower than the potential of the bacterial degraders. Although yeasts may not be the primary transformers of phenanthrene in coastal sediments, the study of yeasts and filamentous fungi from marine ecosystems could lead to new systems for use in innovative biotechnologies (35).

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