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Mechanisms of Strontium Uptake by Laboratory and Brewing Strains of *Saccharomyces cerevisiae*

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Laboratory and brewing strains of *Saccharomyces cerevisiae* were compared for metabolism-independent and -dependent Sr^{2+} uptake. Cell surface adsorption of Sr^{2+} to live cells was greater in the brewing than in the laboratory strain examined. However, uptake levels were greater in denatured (dried and ground) *S. cerevisiae*, and the relative affinities of Sr^{2+} for the two strains were reversed. Results for the brewing *S. cerevisiae* strain were similar whether the organism was obtained fresh from brewery waste or after culturing under the same conditions as for the laboratory strain. Reciprocal Langmuir plots of uptake data for live biomass were not linear, whereas those for denatured biomass were. The more complex Sr^{2+} binding mechanism inferred for live *S. cerevisiae* was underlined by cation displacement experiments. Sr^{2+} adsorption to live cells resulted in release of Mg^{2+}, Ca^{2+}, and H^+, suggesting a combination of ionic and covalent bonding of Sr^{2+}. In contrast, Mg^{2+} was the predominant exchangeable cation on denatured biomass, indicating primarily electrostatic attraction of Sr^{2+}. Incubation of live *S. cerevisiae* in the presence of glucose resulted in a stimulation of Sr^{2+} uptake. Cell fractionation revealed that this increased Sr^{2+} uptake was mostly due to sequestration of Sr^{2+} in the vacuole, although a small increase in cytoplasmic Sr^{2+} was also evident. No stimulation or inhibition of active H^+ efflux resulted from metabolism-dependent Sr^{2+} accumulation. However, a decline in cytoplasmic, and particularly vacuolar, Mg^{2+}, in comparison with that of cells incubated with Sr^{2+} in the absence of glucose, was apparent. This was most marked for the laboratory *S. cerevisiae* strain, which contained higher Mg^{2+} levels than the brewing strain.

Interest in the processes involved in heavy metal uptake by microorganisms has increased considerably in recent years because of concern over the possible transfer of toxic metals from microorganisms through food chains to higher organisms, in addition to the biotechnological potential of microorganisms for metal removal and/or recovery (11, 15, 27). In the latter case, it is acknowledged that for an industry-scale process the cost of producing biomass can be a serious economic disadvantage (10), and consequently the use of waste microbial biomass, e.g., from the brewing industry, would be desirable. However, to date, most studies on microbial heavy metal uptake have examined stock laboratory cultures. The extrapolation of such data to industrially derived microorganisms may be unreliable, particularly in view of the known effects of prior culturing conditions (23) and external physicochemical parameters (16) on metal adsorption. Moreover, the characteristic surface proteins and sugars found in flocculating industrial yeast strains (24) might provide additional metal-binding sites in these organisms. Such differences may also apply to intracellular metal accumulation, as the activity of cellular cation transport mechanisms can be influenced by the history of the cells under investigation (17).

In the present study, strontium uptake in biomass obtained from laboratory and industrial sources was examined. Strontium is a trace element that has no known essential biological role. However, because of the long half-life (~29 years) of the radioisotope ^{90}Sr and its continued discharge as a constituent of radioactive wastewaters from nuclear reactors and in atomic fallout, concern has arisen over the fate of this radionuclide in the environment (19, 29). The divalent cation, Sr^{2+}, displays physicochemical properties similar to those of the abundant and biologically essential cations Ca^{2+} and Mg^{2+} (13), and it has been shown that Sr^{2+} may substitute for one or both of these ions in binding processes at biological cell surfaces as well as in active uptake via divalent cation transport systems. For example, cell wall adsorption of Sr^{2+} resulted in a stoichiometric release of Ca^{2+} and Mg^{2+} from the alga *Vaucheria* sp., indicating binding of these ions to equivalent functional groups at the cell surface (6). Furthermore, a common system is known to mediate active uptake of Sr^{2+}, Ca^{2+}, and Mg^{2+} in the yeast *Saccharomyces cerevisiae*, albeit with a lower affinity for Sr^{2+} than for Ca^{2+} or Mg^{2+} (5).

The purpose of this investigation was to examine the mechanisms of Sr^{2+} uptake and to compare Sr^{2+} uptake capacities in both live and denatured forms of laboratory- and brewery-derived strains of *S. cerevisiae*. Release of cellular Ca^{2+}, Mg^{2+}, and H^+, in response to metabolism-independent and -dependent Sr^{2+} uptake processes, was determined for all biomass types.

MATERIALS AND METHODS

Organisms, media, and growth conditions. A laboratory strain (LS) of *S. cerevisiae*, strain X2180-1B, and a brewing strain (BS) of *S. cerevisiae*, obtained from a local brewery, were routinely maintained on a solid medium comprising (in grams per liter) the following: malt extract, 3.0; yeast extract, 3.0; bacteriological peptone (Oxoid), 5.0; D-glucose, 10.0; and agar (Lab M no. 1), 16.0. For experimental purposes, cultures were grown in a liquid medium comprising (in grams per liter) the following: KH_2PO_4, 2.72; K_2HPO_4, 3.98; $(NH_4)_2SO_4$, 2.0; $MgSO_4 \cdot 7H_2O$, 0.12; $FeSO_4 \cdot 7H_2O$, 0.0022; $ZnSO_4 \cdot 7H_2O$, 0.004; $MnSO_4 \cdot 4H_2O$, 0.004; $CuSO_4 \cdot$

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$5H_2O$, 0.004; D-glucose, 20.0; and yeast extract, 1.0. Cultures were grown at 25°C on an orbital shaker (150 rpm).

Preparation of biomass for experimental use. Cells from the late exponential growth phase (72 h) or obtained freshly as brewery waste were harvested by centrifugation (1,200 × *g*, 10 min) at room temperature and washed three times with distilled, deionized water. The final cell pellet was resuspended in a few milliliters of distilled, deionized water. For use as denatured biomass, final cell suspensions were dried to a constant weight at 55°C and ground by using a mortar and pestle. Particles of less than 125 μm in diameter were selected and used for experiments.

Metabolism-independent Sr^{2+} uptake experiments. Biomass (whole or denatured cells) was added to 100 ml of distilled, deionized water to a final concentration of 0.1 g (dry weight) 100 ml^{-1} in 250-ml Erlenmeyer flasks. The pH of the suspensions was adjusted to 5.5 by using 0.1 M NaOH or HNO_3. pH 5.5 was selected as a pH value high enough to allow the use of the "Good" buffer morpholineethanesulfonic acid (MES), characterized by its low metal-binding properties (12), for metabolism-dependent Sr^{2+} uptake experiments, but low enough to preclude hydrolysis of the alkaline earth metals (3). After preincubation for 1 h at 25°C with rotary shaking (150 rpm), Sr^{2+} (nitrate salt as NO_3^- does not influence the metabolism of *S. cerevisiae*) was added to the suspensions, to the desired final concentration, from appropriate stock solutions. After 5 min of incubation in the presence of Sr^{2+}, biomass was separated by centrifugation (1,200 × *g*, 5 min), and the supernatant was removed and retained for Sr^{2+} analysis. Sr^{2+} uptake values were determined from the difference in final Sr^{2+} concentrations between control flasks without biomass and test flasks.

Displacement of Mg^{2+}, Ca^{2+}, and H^+ by Sr^{2+} in nonmetabolizing biomass. Biomass was added to 100 ml of distilled, deionized water to a final concentration of 0.1 g (dry weight) 100 ml^{-1}. The suspension was adjusted to pH 5.5 by using 0.1 M NaOH or HNO_3. Samples were taken as described above, both immediately prior to and 1 min after the addition of the desired amount of Sr^{2+}; supernatants were retained for Sr^{2+}, Mg^{2+}, and Ca^{2+} analysis. The pH of the suspension was measured continually, by using a W.T.W. E56 precision glass pH electrode, and values at the time of addition of Sr^{2+} and later at equilibrium (30 s to 1 min after Sr^{2+} addition) were used for H^+ displacement calculations.

Proton efflux in metabolizing cells. Live cells were added to 100 ml of distilled, deionized water (previously adjusted to pH 5.5 by using 0.1 M NaOH or HNO_3), to a final concentration of 0.1 g (dry weight) 100 ml^{-1}. The suspension was agitated by using a magnetic stirrer. At the appropriate intervals, glucose (2% [wt/vol]) and Sr^{2+} (to a final concentration of 100 μM) were added to the suspension, and changes in pH were measured continually.

Metabolism-dependent Sr^{2+} uptake experiments. Live cells were added to 100 ml of 10 mM MES buffer, adjusted to pH 5.5 by using 1 M NaOH, to a final concentration of 0.1 g (dry weight) 100 ml^{-1}. Cell suspensions were equilibrated at 25°C with shaking (150 rpm) for 1 h, after which time Sr^{2+} was added to the flasks to a final concentration of 100 μM. When desired, glucose (2% [wt/vol]) was added 5 min prior to the addition of Sr^{2+}. At specified intervals, samples were removed, centrifuged (1,200 × *g*, 5 min), and washed twice with distilled, deionized water. Final cell pellets were analyzed either for compartmentation of intracellular metal ions (as outlined below) or for total cellular ion levels. In the latter case, pellets were digested for 1 h in 0.5 ml of 6 M HNO_3 at 100°C and, after subsequent addition of 3 ml of distilled, deionized water and mixing, cell extracts were centrifuged (1,200 × *g*, 10 min) to remove debris. The supernatants were retained for metal analysis.

Subcellular compartmentation of metal ions. Cell pellets were obtained after 0 and 4 h of incubation with 100 μM Sr^{2+} in the absence and presence of 2% (wt/vol) glucose as described above. The cytoplasmic membrane was permeabilized by resuspending the pellet in 1 ml of 10 mM Tris-MES buffer, pH 6.0, with 0.7 M sorbitol at 25°C. DEAE-dextran (40 μl, 10 mg ml^{-1}) was added to the same buffer, mixed, and incubated for 30 s at 25°C. Cells were separated by centrifugation (8,000 × *g*, 30 s), and the supernatant was removed and retained. The permeabilized cells were then washed three times with 0.7 M sorbitol in 10 mM Tris-MES buffer, pH 6.0, at 0 to 4°C, with incubation for 1 min at each wash. Supernatants were removed and retained after centrifugation at each wash and were combined with that from the initial permeabilization step.

The vacuolar membrane was permeabilized by suspending the pellet in 60% (vol/vol) methanol at 0 to 4°C. After incubation for 30 s, cell fragments were centrifuged (8,000 × *g*, 30 s), and the supernatant was retained and finally combined with those from further washes with 60% (vol/vol) methanol (three times, 1 ml each time) and then 10 mM Tris-MES, pH 6.0 (three times, 1 ml each time). Suspensions were incubated for 1 min at 0 to 4°C for each of these washes. Cytoplasmic and vacuolar extracts were analyzed for Sr^{2+}, Mg^{2+}, and Ca^{2+} as described below.

Metal analysis. Sr^{2+}, Mg^{2+}, and Ca^{2+} were analyzed by using a Perkin-Elmer 3100 atomic absorption spectrophotometer, fitted with a 10-cm single slot burner head, and by using an air-acetylene flame. Metal concentrations were determined by reference to appropriate standard metal solutions.

Dry weights. Dry weights of live cells were determined by using tared foil cups dried to a constant weight at 55°C.

RESULTS

Metabolism-independent Sr^{2+} uptake by *S. cerevisiae*. Adsorption of Sr^{2+} was examined over a range of initial Sr^{2+} concentrations (10 to 2,000 μM) by using live and denatured forms of three types of yeast biomass; these were the LS of *S. cerevisiae* and the BS of *S. cerevisiae*, obtained either fresh from brewery waste (BS yeasts) or after culturing under laboratory conditions (LBS yeasts). Data were plotted as adsorption isotherms, and for all biomass types increased levels of Sr^{2+} uptake (q_e) were observed at increased external Sr^{2+} concentrations (C represents the final Sr^{2+} concentration at equilibrium) (Fig. 1a and c). In live cells, uptake levels were greater in BS than LS yeasts, and at an initial external Sr^{2+} concentration of 1,000 μM, cellular levels of Sr^{2+} were approximately 32 and 20 μmol g (dry weight)$^{-1}$, respectively (Fig. 1a). Sr^{2+} adsorption to LBS yeasts (to approximately 36 μmol g [dry weight]$^{-1}$ at 1,000 μM Sr^{2+}) correlated most closely with the amount observed in BS yeasts. When the data were transformed (Fig. 1b) it was evident that the relationship between reciprocal values for q_e and C was nonlinear and did not fit the Langmuir model (18).

Denatured yeast biomass displayed greater Sr^{2+} uptake capacities than live yeasts, and experiments were conducted at initial Sr^{2+} concentrations of up to 2,000 μM (Fig. 1c). Isotherm plots of the data revealed a curvilinear relationship between Sr^{2+} uptake and final external Sr^{2+} concentration for all the denatured yeast types. Apparent saturation of the biomass Sr^{2+} binding sites occurred at the higher external

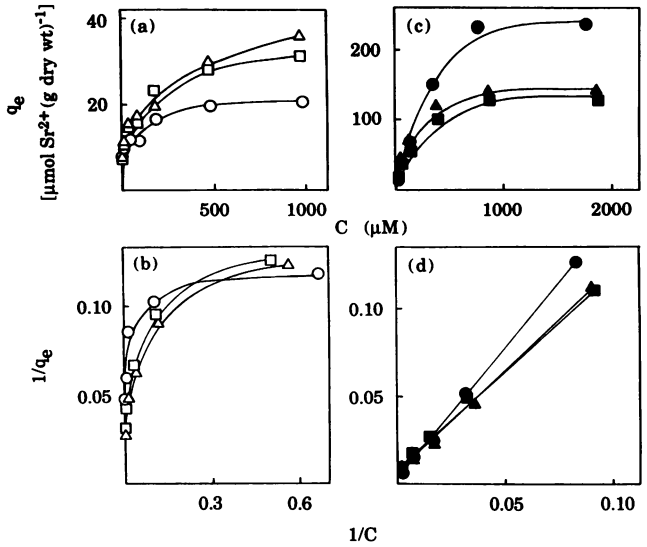

FIG. 1. Langmuir isotherms for metabolism-independent Sr^{2+} uptake by *S. cerevisiae*. Removal of Sr^{2+} was determined after 5 min of incubation of live (open symbols) and denatured (closed symbols) biomass in the presence of the appropriate Sr^{2+} concentration. (a and c) Standard Langmuir isotherms for LS (○ and ●), BS (□ and ■), and LBS (△ and ▲) yeasts. (b and d) The data from panels a and c transformed to reciprocal plots. Typical results from one of three experiments are shown. Differences in uptake values obtained from the different experiments did not exceed 15%.

Sr^{2+} concentrations examined. In contrast to the situation observed in live cells, the greatest Sr^{2+} uptake was observed in LS yeasts. At 2,000 μM Sr^{2+}, levels of uptake were approximately 235, 125, and 140 μmol of Sr^{2+} g (dry weight)$^{-1}$ in LS, BS, and LBS yeasts, respectively (Fig. 1c). Transformation of the data revealed a linear relationship between reciprocal values for q_e and C in the three types of denatured yeast biomass (Fig. 1d). This difference in reciprocal plots for live and dead yeasts was illustrated by the observation that although greater Sr^{2+} uptake by denatured *S. cerevisiae* was apparent at elevated external Sr^{2+} (e.g., 1,000 μM), this situation became less evident at lower Sr^{2+} concentrations, and at 10 μM Sr^{2+}, live biomass types removed approximately 85% of Sr^{2+} from solution, whereas only approximately 50% removal was achieved by denatured yeasts.

Displacement of Mg^{2+}, Ca^{2+}, and H^+ by Sr^{2+} in nonmetabolizing biomass. In order to characterize the nature of metabolism-independent Sr^{2+} uptake in the various biomass types, exchange of Sr^{2+} for the chemically similar cations Mg^{2+} and Ca^{2+}, as well as for the monovalent H^+, was investigated. Preliminary studies indicated that no detectable release of K^+ or Na^+ resulted from Sr^{2+} adsorption in these experiments (results not shown). Relative levels of Mg^{2+} and Ca^{2+} released from cell walls of live yeast, following Sr^{2+} adsorption, were dependent on the yeast strain (Table 1). In LS yeasts, Ca^{2+} was the main exchangeable cation at the cell surface, and at 100 μM Sr^{2+}, relative percentages of released Mg^{2+}, Ca^{2+}, and H^+, of the total exchanged cations, were approximately 19, 60, and 21%, respectively. However, a stoichiometric exchange for Sr^{2+} was not apparent, and the total charge released on Sr^{2+} adsorption was only approximately 45% of that taken up. This was also the case in BS and LBS live yeasts, although

TABLE 1. Displacement of cations by Sr^{2+} in live, nonmetabolizing *S. cerevisiae* cells

Initial Sr^{2+} concn (μM) and cation taken up or released	Amt[a] (μmol g [dry wt]$^{-1}$) of cation taken up or released on Sr^{2+} addition		
	LS	BS	LBS
100			
Sr^{2+}	11.28 ± 0.39	13.92 ± 0.51	14.58 ± 0.82
Mg^{2+}	1.11 ± 0.15	2.82 ± 0.11	3.40 ± 0.40
Ca^{2+}	3.40 ± 0.93	1.32 ± 0.14	2.13 ± 1.34
H^+	1.18 ± 0.15	1.90 ± 0.29	1.53 ± 0.20
1,000			
Sr^{2+}	21.25 ± 2.65	32.64 ± 1.31	35.80 ± 1.22
Mg^{2+}	1.03 ± 0.33	3.84 ± 0.10	3.88 ± 0.43
Ca^{2+}	3.03 ± 1.11	2.46 ± 0.15	3.09 ± 0.74
H^+	1.70 ± 0.10	3.34 ± 0.49	2.52 ± 0.85

[a] Mean values ± standard errors of the means from three replicate determinations are shown.

here, in both cases, Mg^{2+} represented a more important fraction of total exchanged cations than in LS yeast. At 100 μM Sr^{2+}, Mg^{2+} release was approximately 115 and 60% higher than Ca^{2+} release in BS and LBS yeasts, respectively (Table 1). When cation displacement at the cell walls of live cells was examined at 1,000 μM Sr^{2+}, it was evident that increases in cation release relative to the levels observed at 100 μM Sr^{2+} were not proportional to increases in Sr^{2+} adsorption. For example, in LBS yeasts, an approximate 2.5-fold increase in Sr^{2+} uptake, at an external concentration of 1,000 μM Sr^{2+}, resulted in only a 1.3-fold increase in total cation release, and the nonstoichiometry observed at 100 μM Sr^{2+} was amplified (Table 1). Increases in Mg^{2+}, Ca^{2+}, and H^+ release, resulting from an increase in external Sr^{2+} from 100 to 1,000 μM, were evident in live BS and LBS yeasts incubated in the presence of 1,000 μM Sr^{2+}; however, only H^+ release increased in LS yeasts. Levels of Mg^{2+} and Ca^{2+} release remained essentially unchanged at the higher Sr^{2+} concentrations in LS yeasts (Table 1).

The greater Sr^{2+} uptake capacity that resulted from drying and grinding of yeast biomass (Fig. 1) correlated with a greater exchange for Mg^{2+} in denatured yeasts in comparison with that in live yeasts (Table 2). At 1,000 μM Sr^{2+}, levels of Mg^{2+} release in the three types of denatured yeast

TABLE 2. Displacement of cations by Sr^{2+} in denatured, nonmetabolizing *S. cerevisiae*

Initial Sr^{2+} concn (μM) and cation taken up or released	Amt[a] (μmol g [dry wt]$^{-1}$) of cation taken up or released on Sr^{2+} addition		
	LS	BS	LBS
100			
Sr^{2+}	29.25 ± 1.36	31.69 ± 0.62	30.17 ± 0.84
Mg^{2+}	10.80 ± 1.65	10.00 ± 0.54	9.42 ± 0.36
Ca^{2+}	0	0.27 ± 1.82	0
H^+	0.35 ± 0.02	0.14 ± 0.00	0.35 ± 0.03
1,000			
Sr^{2+}	227.3 ± 6.5	142.0 ± 7.9	147.8 ± 2.1
Mg^{2+}	44.30 ± 4.20	21.00 ± 2.69	25.00 ± 2.65
Ca^{2+}	0.57 ± 0.62	0.13 ± 0.05	2.43 ± 0.69
H^+	1.61 ± 0.09	0.83 ± 0.08	1.32 ± 0.09

[a] Mean values ± standard errors of the means from three replicate determinations are shown.

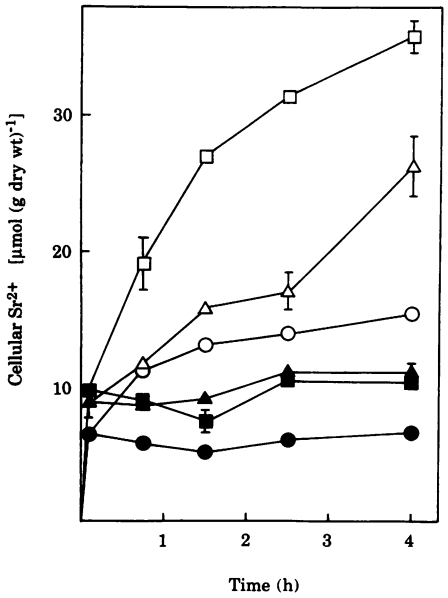

FIG. 2. Influence of glucose on Sr^{2+} uptake by live *S. cerevisiae* cells. Cells were incubated in 10 mM MES buffer with 100 μM Sr^{2+}. The graph shows cellular Sr^{2+} levels in LS (○ and ●), BS (□ and ■), and LBS (△ and ▲) *S. cerevisiae*, incubated in either the presence (open symbols) or the absence (closed symbols) of 2% (wt/vol) glucose. Mean values ± standard errors of the means (where possible) from three replicate determinations are shown.

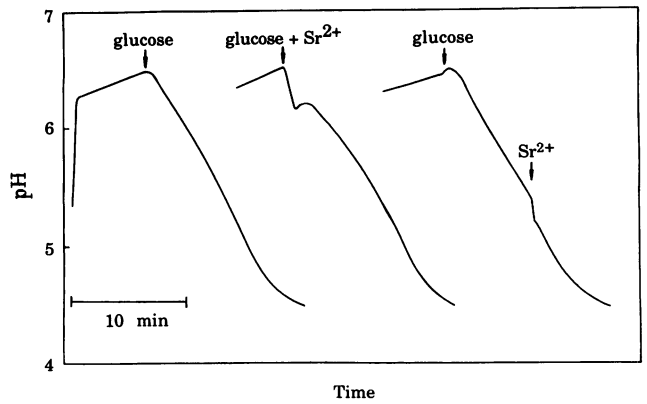

FIG. 3. Influence of glucose and Sr^{2+} on H^+ efflux by live *S. cerevisiae* cells (LS). Glucose (final concentration, 2% [wt/vol]) and Sr^{2+} (final concentration, 100 μM) were added to unbuffered cell suspensions at the indicated intervals. The pH of the suspension was measured continually. Typical results from one of three experiments are shown.

biomass were between 5- and 40-fold greater than the corresponding levels observed in live cells. In contrast, Sr^{2+} displacement of Ca^{2+} and H^+ in denatured biomass was less than that in live cells, and this was particularly evident at 100 μM Sr^{2+}, where Ca^{2+} and H^+ displacement was negligible (Table 2). As with live cells, Sr^{2+} displacement of Mg^{2+}, Ca^{2+}, and H^+ in denatured biomass was nonstoichiometric.

Metabolism-dependent Sr^{2+} uptake. Live cells of LS, BS, and LBS *S. cerevisiae* were incubated in both the absence and presence of 2% (wt/vol) glucose, with 100 μM Sr^{2+}, to examine for active Sr^{2+} uptake. In all cases, the presence of glucose resulted in a stimulation of Sr^{2+} uptake which was attributable to metabolism-dependent intracellular Sr^{2+} accumulation (Fig. 2). For cells incubated in the absence of glucose, Sr^{2+} uptake was complete within 5 min, as no appreciable increase in cellular Sr^{2+} was observed over the subsequent 4 h. Relative levels of Sr^{2+} adsorption to the three live biomass types were consistent with those described above (Fig. 1a), although here measured uptake levels were approximately 30 to 45% lower (Fig. 2). For cells incubated in the presence of glucose, Sr^{2+} uptake continued after 5 min, although, except for LBS yeasts, the rate of active uptake decreased over the 4-h incubation (Fig. 2). As with Sr^{2+} uptake in the absence of glucose, metabolism-dependent Sr^{2+} uptake was greater in BS and LBS yeasts than in LS yeasts, and this difference was larger than that which was due to surface binding only. After 4 h of incubation in the presence of glucose and 100 μM Sr^{2+}, cellular levels of Sr^{2+} in LS, BS, and LBS yeasts were approximately 15, 36, and 26 μmol g (dry weight)$^{-1}$, respectively (Fig. 2).

Displacement of intracellular Mg^{2+}, Ca^{2+}, and H^+ by Sr^{2+} and subcellular compartmentation of divalent cations. Exchange of intracellular H^+ for Sr^{2+} was examined in LS *S. cerevisiae* (Fig. 3). The addition of glucose (2% [wt/vol]), 6 min after the addition of biomass, to unbuffered distilled, deionized water with an original pH of ~5.5 resulted in proton efflux from the cells and a decrease in pH from approximately 6.3 to 4.4 of the external medium over the subsequent 14 min. When Sr^{2+} was added to the suspension, to a final concentration of 100 μM, either at the moment of or 8 min after glucose addition, the subsequent rate of proton efflux and final pH of the suspension were similar to those observed for suspensions incubated in the absence of Sr^{2+} (Fig. 3). The small decrease in external pH that immediately followed Sr^{2+} addition represented cell surface displacement of H^+ by Sr^{2+}, as described previously (Table 1). As with cell surface binding of Sr^{2+}, preliminary studies indicated that no exchange for alkali monovalent cations resulted from active intracellular Sr^{2+} accumulation (results not shown).

After 0 and 4 h of incubation of live LS, BS, and LBS yeasts with 100 μM Sr^{2+} in the absence and presence of glucose, cells were fractionated and cytoplasmic and vacuolar pools were analyzed for Sr^{2+}, Mg^{2+}, and Ca^{2+} (Table 3). In all cases, Sr^{2+} was accumulated within both the cytoplasm and vacuoles of *S. cerevisiae* cells; however, the relative importance of these compartments in intracellular Sr^{2+} localization was dependent on whether uptake was active or passive. In nonmetabolizing yeasts, intracellular Sr^{2+} was predominantly sequestered within the cytoplasm. Although Sr^{2+} uptake in the presence of glucose resulted in elevated levels of the cation in the cytoplasm, increases were more evident in the vacuole; for example, in BS yeasts the increase in vacuolar Sr^{2+}, for cells incubated in the presence rather than the absence of glucose, was an order of magnitude greater than the concomitant increase in cytoplasmic Sr^{2+} (Table 3). Comparison of Mg^{2+} and Ca^{2+} levels for the three biomass types, after 0 and 4 h of incubation, revealed that Sr^{2+} uptake in the absence of glucose resulted in a decline in cytoplasmic levels of both Mg^{2+} and Ca^{2+}, although no effect on vacuolar levels of these ions was discernible. The stimulation of Sr^{2+} uptake by glucose coincided with further loss of Mg^{2+}, but not Ca^{2+}, from the cytoplasm of all three cell types and a decline in vacuolar Mg^{2+}, in comparison with that of cells incubated in the absence of glucose. The decline in vacuolar Mg^{2+} that

TABLE 3. Subcellular distribution of cations in live *S. cerevisiae* cells

Incubation (h) and strain	Presence of glucose (2% [wt/vol])	Intracellular ion levels[a] (μmol g [dry wt]$^{-1}$)					
		Cytoplasm			Vacuole		
		Sr^{2+}	Mg^{2+}	Ca^{2+}	Sr^{2+}	Mg^{2+}	Ca^{2+}
0							
LS	−	0	2.52 ± 0.03	1.39 ± 0.11	0	94.0 ± 0.8	0.82 ± 0.01
LS	+	0	2.34 ± 0.28	1.62 ± 0.38	0	91.2 ± 0.3	0.79 ± 0.10
BS	−	0	6.15 ± 0.37	1.93 ± 0.27	0	38.2 ± 0.2	1.13 ± 0.02
BS	+	0	5.43 ± 0.08	1.64 ± 0.08	0	38.8 ± 0.3	1.09 ± 0.05
LBS	−	0	5.60 ± 0.16	1.19 ± 0.11	0	45.1 ± 0.0	0.67 ± 0.00
LBS	+	0	5.49 ± 0.04	0.98 ± 0.09	0	45.1 ± 0.2	0.69 ± 0.01
4							
LS	−	1.56 ± 0.12	2.06 ± 0.11	1.16 ± 0.09	0.96 ± 0.00	89.2 ± 1.1	0.85 ± 0.05
LS	+	2.28 ± 0.12	1.60 ± 0.06	1.16 ± 0.27	4.81 ± 0.48	81.5 ± 0.8	0.89 ± 0.01
BS	−	2.00 ± 0.12	5.39 ± 0.17	1.22 ± 0.17	1.88 ± 0.00	38.4 ± 0.0	1.11 ± 0.04
BS	+	2.19 ± 0.00	5.16 ± 0.06	1.40 ± 0.35	9.91 ± 0.00	34.6 ± 0.3	1.11 ± 0.09
LBS	−	2.39 ± 0.16	5.07 ± 0.02	0.65 ± 0.06	1.04 ± 0.08	46.3 ± 0.6	0.70 ± 0.08
LBS	+	2.89 ± 0.08	3.47 ± 0.21	0.65 ± 0.06	6.08 ± 0.00	41.0 ± 1.3	0.68 ± 0.12

[a] Mean values ± standard errors of the means from three replicate determinations are shown.

resulted from active Sr^{2+} uptake was small (approximately 8 to 12% loss) in relation to the total vacuolar Mg^{2+} but was comparable to levels of Sr^{2+} accumulated in the vacuole. However, as with cell surface binding of Sr^{2+}, intracellular Sr^{2+} exchange for Mg^{2+} and Ca^{2+} was nonstoichiometric (Table 3). Interestingly, the subcellular compartmentation of Mg^{2+} differed markedly in LS and BS yeasts. Although the total intracellular Mg^{2+} was much greater in LS than in BS or LBS yeasts, cytoplasmic Mg^{2+} was approximately 2.5-fold higher in the latter organisms. In all biomass types Mg^{2+} was predominantly located within the vacuolar compartment; cytoplasmic Mg^{2+} levels were approximately 2.6 and 15.0%, respectively, of those observed in the vacuole of LS and BS *S. cerevisiae* strains (Table 3).

DISCUSSION

The results described here indicate that the mechanisms involved in both passive and active Sr^{2+} uptake processes differ considerably between laboratory and brewing strains of *S. cerevisiae*. Results for BS *S. cerevisiae* were similar whether the organism was used fresh from the brewery or after being cultured in the laboratory under the same conditions as for LS *S. cerevisiae*. The greater passive Sr^{2+} biosorptive capacity of the former organism was, therefore, a result of an inherent physiological or genetic property of the strain rather than a response to external conditions. One difference between LS and BS strains, observable by light microscopy, was the formation of cell aggregates (5 to 15 cells) by the latter; LS yeast was distributed as a roughly homogeneous suspension of single, occasionally budding, cells in solution. Among the criteria for selection of yeast strains for use in industry-scale fermentations is their floc-forming ability. Flocculent yeast cells produce surface proteins termed lectins, and it is known that these proteins are activated by, and have specific binding sites for, Ca^{2+} (24). As Sr^{2+} and Ca^{2+} are analogous alkaline earth metals with similar ionic radii (13), it is likely that Sr^{2+} can also occupy lectin Ca^{2+} binding sites, resulting in higher Sr^{2+} adsorption to floc-forming than non-floc-forming *S. cerevisiae*. A further cause for the present observations may result from extracellular polysaccharide production by the yeasts. Certain polysaccharides have been implicated in the promotion of floc formation by *S. cerevisiae* (14), while the polysaccharides' role in microbial metal uptake is well documented (11).

Sr^{2+} adsorption to live yeasts did not conform to the Langmuir adsorption isotherm. Nonlinear relationships between reciprocal values for q_e and C, like those observed here, are generally considered to indicate multilayer adsorption (30), although it has been suggested that metal adsorption data which do not conform to the Langmuir model are also indicative of complex metal-ligand interactions that would not be accounted for solely by electrostatic attraction (8). In this work, cation displacement data with live *S. cerevisiae* cells support this latter interpretation of the Langmuir model. Replacement of Mg^{2+}, Ca^{2+}, and H^+ by Sr^{2+} suggested that Sr^{2+} was involved in both ionic and covalent bonding to the yeast cell wall. This contrasts to the situation in freshwater algae, in which Sr^{2+} adsorption is characterized by ion-exchange processes involving solely Mg^{2+} and Ca^{2+} (6), although some additional covalent bonding, as determined by H^+ displacement, has been described for marine algae (7). Covalent bonding, resulting in H^+ release, may arise through complex formation of Sr^{2+} with carboxylate, phosphate, or other groups (28). The partially covalent nature of Sr^{2+} adsorption to live yeasts described here was unexpected as, like Mg^{2+} and Ca^{2+}, Sr^{2+} is regarded as a "hard" acid which would characteristically form ionic bonds with ligands (3). However, it should be noted that because of its larger ionic radius, Sr^{2+} is polarized more easily than Mg^{2+} and Ca^{2+}, and covalent bonding of Sr^{2+} is more likely than for Mg^{2+} and Ca^{2+} (16).

The observed nonstoichiometric exchange of Sr^{2+} for Mg^{2+}, Ca^{2+}, and H^+ in live cells suggests that Sr^{2+} was bound to sites not previously occupied by any of the latter ions. However, when Sr^{2+}-loaded cells were washed with distilled, deionized water during sampling in active uptake experiments, measured Sr^{2+} adsorption levels after 5 min were considerably lower than those estimated from external Sr^{2+} levels in passive exchange experiments. These lower values correlated more closely, but not exactly, with stoichiometric cation exchange at the cell surface. It is likely that the Sr^{2+} released by washing of cells represented mobile ions that were taken up into water-filled spaces in the yeast cell wall, the so-called water-extractable fraction (4). In the

present metabolism-independent experiments, the water-filled spaces were presumably devoid of exchangeable Mg^{2+} and Ca^{2+} because of prior washing of cells with distilled, deionized water in preparation for experimental procedures.

The increased biosorptive capacity for Sr^{2+} that resulted from denaturation of *S. cerevisiae* is in agreement with the greater uptake of other heavy metals by dead microbial cell preparations that has been described previously (20). Such phenomena may be due to an increased surface area of ground cell walls as well as to the exposure of intracellular binding sites after denaturation of the biomass. The greater Sr^{2+} uptake described here would certainly favor denatured biomass for the development of a Sr^{2+} removal process, although it should be noted that at the lowest concentration examined (10 μM Sr^{2+}), uptake was higher in live *S. cerevisiae*; one estimate of the Sr^{2+} concentration in a particular radioactive wastewater was of the order of 10 μM (29).

The reversal of relative affinities of the various yeast types for Sr^{2+} that resulted from denaturation of the biomass was unexpected. Clearly, an enhanced metal-binding capacity in live yeasts resulting from any metabolism-dependent production of extracellular material would not be evident in denatured yeasts. Furthermore, possible differential effects of drying and grinding on proteins and other potential Sr^{2+}-binding sites at the cell wall in the different biomass types cannot be discounted.

Further differences in Sr^{2+} adsorption behavior between live and dead cells were evident for the Langmuir models and cation displacement data. The observed linear relationship between reciprocal values of q_e and C in dead cells correlated with Sr^{2+} exchange for Mg^{2+} alone. If it is assumed that Mg^{2+} is adsorbed electrostatically to dead cells, then the simple ionic nature of Sr^{2+} bonding to the biomass that can be inferred from these results suggests that the denaturation process altered or inhibited the cell wall functional groups involved in covalent bonding of Sr^{2+} to live cells in such a way as to prevent their further action in dead cells. Thus, although increased levels of Sr^{2+} were adsorbed to dead biomass, the overall bonding strength was probably weaker. These results may have implications for any postulated Sr^{2+} recovery from loaded biomass.

The stimulation of Sr^{2+} uptake that followed incubation of *S. cerevisiae* with glucose is in agreement with results from earlier studies (9, 22) and is a result of a greater synthesis of membrane transport proteins and/or an increased transplasmalemma proton motive force (17). It is likely that the higher rate of active Sr^{2+} uptake in the BS *S. cerevisiae* was a consequence of the greater surface binding of Sr^{2+} to this organism. Presumably, the increased deposition of Sr^{2+} at the cell surface in BS or LBS yeasts would allow plasma membrane divalent cation transport systems to "see" a higher localized Sr^{2+} concentration. The greatest rate of metabolism-dependent Sr^{2+} uptake that was evident in BS *S. cerevisiae* was probably due to its fresh acquisition from a fermentation process in which conditions would be conducive to a higher metabolic rate.

As with Sr^{2+} adsorption, intracellular Sr^{2+} accumulation by all *S. cerevisiae* strains was concomitant with replacement of appropriate intracellular Mg^{2+} and Ca^{2+} pools. No accurate determination of internal H^+ was possible by the techniques used; however, no stimulation or activation of H^+ efflux from LS yeasts, in response to the onset of active Sr^{2+} influx, was apparent. This differs from evidence presented by Roomans et al. (22) with *S. cerevisiae* Delft II, in which the lag period prior to the commencement of Sr^{2+} influx, 1 to 2 min after the addition of glucose, was similar to that described in a previous study for H^+ efflux (estimated by using measurements of internal pH) (26), and the activity of a Sr^{2+} (Ca^{2+})/H^+ antiport was inferred. Further differences between the present and previous studies relate to the intracellular localization of Sr^{2+} and probable selectivity of cytoplasmic membrane and vacuolar membrane divalent cation transport systems. The localization of cations in yeasts, in the presence of a metabolizable substrate, is primarily determined by active transport in response to electrochemical proton gradients, generated by membrane-bound H^+-ATPases, across the cytoplasmic and vacuolar membranes (17). The greater sequestration of Sr^{2+} in the vacuoles of *S. cerevisiae* cells in the presence of glucose described here, and elsewhere (21), correlated more closely with the localization of Mg^{2+} than Ca^{2+} in the present work. This is interesting, as Ca^{2+} is usually considered the divalent cation with the greater physicochemical similarity to Sr^{2+} (13). Indeed, the Ca^{2+} efflux system on the plasma membrane of *S. cerevisiae* Delft II reported by Theuvenet et al. (25) selectively discriminated against Mn^{2+} (and Mg^{2+}) but catalyzed transport of Sr^{2+} out of the cell, and greater levels of Mn^{2+} and Mg^{2+} were subsequently accumulated into the vacuole of this organism. The apparent lack of, or additional discrimination against, Sr^{2+} of such a system in the *S. cerevisiae* strains examined in this study is of significance, as Ca^{2+} and Sr^{2+} may act as antagonists to the action of Mg^{2+}, e.g., against its function as a cofactor for H^+-ATPases (17); consequently, increased Sr^{2+} toxicity might result in these organisms. Furthermore, in view of the known effects on microbial growth of replacement of cellular K^+ by the monovalent radionuclide Cs^+ (1, 2), it is likely that additional Sr^{2+}-induced growth inhibitory effects may arise from replacement of Mg^{2+}, particularly at high external Sr^{2+} concentrations. In addition to the differences in mechanisms of Sr^{2+} transport from those reported in previous studies, differences in the selective intracellular localization of Mg^{2+} and Ca^{2+} between the two strains of *S. cerevisiae* examined in this study were observed. The greater sequestration of Mg^{2+} in the vacuole, in relation to Ca^{2+}, is in agreement with results from previous reports (17). However, the higher relative level of cytoplasmic Mg^{2+} in the BS yeast, which was accentuated by its lower total intracellular Mg^{2+}, clearly indicates that the affinities and selectivities of transport systems mediating Mg^{2+} fluxes, at the plasmalemma and vacuolar membrane, differ markedly in the LS and BS yeasts examined here. These results are of relevance to uptake of other metals, as in many cases intracellular accumulation is also determined by divalent cation transport systems, and sequestration of metals within vacuoles can represent a cellular detoxification mechanism (11).

From the results presented in this paper, it is clear that marked differences in the mechanisms of both Sr^{2+} adsorption and intracellular Sr^{2+} accumulation exist between the LS and BS yeasts examined. These observations highlight the strong influence that the differential ecophysiology of strains from a single microbial genus may exert on metal uptake characteristics, as well as on the external binding and intracellular distribution of essential ions, such as Mg^{2+} and Ca^{2+}.

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