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Wall Synthesis by *Pediococcus cerevisiae* and by a Substrain That Requires Methicillin for Growth

B. J. WILKINSON[1] AND P. J. WHITE
Department of Microbiology, The University, Sheffield, S10 2TN England

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Pediococcus cerevisiae and *P. cerevisiae* CRD synthesized peptidoglycan when incubated in a wall synthesis solution inadequate to allow growth.

Pediococcus cerevisiae CRD is a substrain of *P. cerevisiae* ATCC 8081 that requires a penicillin (preferably methicillin) for growth in a partly defined medium. Several characteristics of its physiology and wall have been described (6, 7). Because the primary site of action of penicillins is thought to be inhibition of peptidoglycan biosynthesis (5, 8), this process was investigated in both parent and strain CRD organisms in the hope of elucidating the role of the drug in the metabolism of the methicillin-requiring organisms. For this purpose, a simple solution was devised that allowed synthesis of walls but did not allow growth, owing to the exacting nutritional requirements of these organisms.

Parent organisms (strain 8081) were grown in a medium (PYGAC) consisting of peptone (2%), yeast extract (1%), glucose (2%), and sodium acetate $3H_2O$ (2%), pH 6.5. Strain CRD was grown in PYG medium (PYGAC medium with acetate omitted) (pH 6.5) or in PYGAC medium plus methicillin (100 μg/ml) pH 6.5. Both strains were harvested in the exponential phase of growth and washed in 0.05 M phosphate buffer (pH 6.5) and distilled water. Washed organisms were suspended at a density of 0.3 to 0.4 mg (dry wt) of bacteria per ml of wall synthesis solution (solution W; pH 6.5), which consisted of (grams/liter): KH_2PO_4, 13.6; glucose, 1.8; $(NH_4)_2SO_4$, 1; $MgSO_4 \cdot 7H_2O$, 0.2; L-lysine, 0.2; L-aspartate, 0.2; L-glutamate, 0.2; and L-alanine, 0.4. The optical density at 610 nm (OD_{610}) of the suspension was measured at intervals during incubation at 37 C; samples (5 ml) were taken at intervals for isolation of peptidoglycan and for measurement of protein. Peptidoglycan was isolated by the method of Park and Hancock (4) with the addition of water washes after the hot trichloroacetic acid and trypsin (100 μg/ml) treatments. Peptidoglycan preparations were hydrolyzed in sealed tubes at 105 C with 6 N HCl for 18 h (amino acid release) or with 4 N HCl for 4 h (for amino sugar release). Hexosamines were measured by the method of Levvy and McAllan (2), ninhydrin-positive substances were measured by the method of Jacobs (1), and protein was measured by the method of Lowry et al. (3).

When exponential-phase parent or strain CRD organisms were incubated in solution W, the turbidity of the suspension increased (Fig. 1 and 2). The most rapid turbidity increase of parent organisms occurred in the first hour (20% increase), after which the turbidity increased more slowly to a final value of 35% after 4 h. Strain CRD showed a slower, roughly linear rise of turbidity for longer than parent strain. Omission of $(NH_4)_2SO_4$ or all four amino acids or glucose decreased the rate of turbidity rise and the final level achieved (Fig. 1 and 2). Omission of glutamate, lysine, or alanine singly from solution W had marked effects, whereas omission of magnesium sulfate had generally smaller effects. However, omission of aspartate did not much lower the turbidity increment. Several compounds were added to solution W; L-cystine, L-methionine, sodium acetate, cytosine, glycerol, uracil, and guanine all showed no effect on the turbidity increment.

"Cell wall" antibiotics such as benzyl penicillin, cycloserine, bacitracin, cloxacillin, and ceporin all diminished the turbidity increment, whereas chloramphenicol, streptomycin and aminopterin did not. The turbidity increment of parent organisms was quite susceptible to methicillin (250 μg/ml gave substantial inhibition), whereas even very high concentrations of methicillin (3 mg/ml) caused little inhibition of

[1] Present address: Department of Biochemistry, University of Cambridge, Cambridge, England.

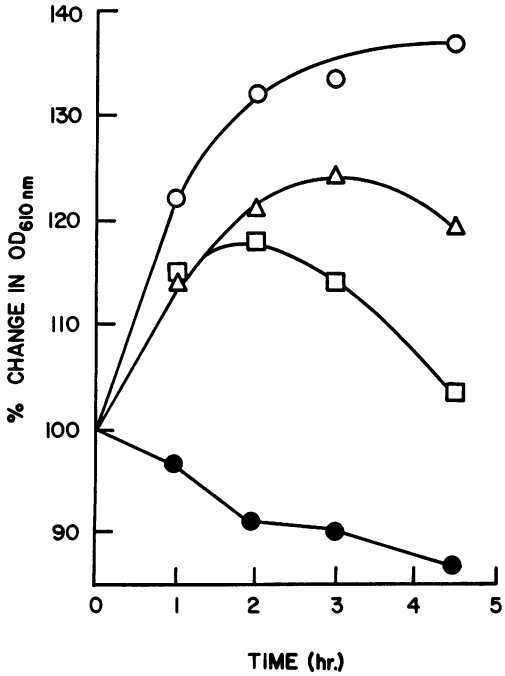

FIG. 1. *Effects of omitting components of solution W on the change in turbidity of a suspension of parent organisms. Organisms were grown in PYGAC medium and harvested, washed, and resuspended in solution W (60 ml in a 100-ml conical flask) at a density of 0.3 to 0.4 mg (dry wt)/ml. The turbidity of the suspension was measured after dilution (1 ml plus 5 ml of water) at 610 nm at various times during incubation of the organisms standing at 37 C. Symbols: ○, complete solution W; △, omission of L-amino acids; □, omission of $(NH_4)_2SO_4$; ●, omission of glucose.*

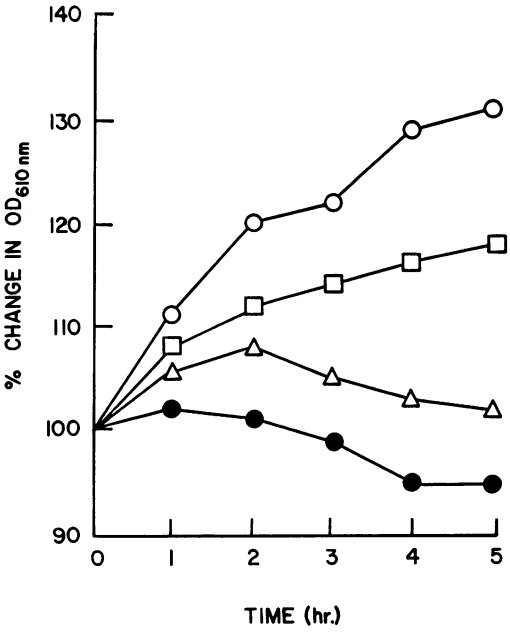

FIG. 2. *Effects of omitting components of solution W on the change in turbidity of a suspension of CRD organisms. Organisms were grown in PYG medium; further experimental procedure was as in Fig. 1. Symbols: ○, complete solution W; △, omission of L-amino acids; □, omission of $(NH_4)_2SO_4$; ●, omission of glucose.*

the turbidity increment in strain CRD; the turbidity increment shown by this strain was not stimulated by methicillin. Strain CRD made wall (increased in turbidity) in the absence of methicillin for at least 4 h whether the organisms had been grown in the presence (PYGAC + methicillin) or absence (PYG) of the drug.

To confirm that organisms which showed a rise in turbidity on incubation in solution W were really synthesizing peptidoglycan, samples of organisms were analyzed chemically at intervals for mucopeptide and protein. With both strains, there was a marked increase of bound hexosamine and of ninhydrin-positive material in the peptidoglycan isolated from the organisms present in a constant sample volume (Table 1). The ratio of hexosamine to total ninhydrin-positive material in the peptidoglycan was 0.3:1.0, i.e., approximately two molecules of hexosamine per seven ninhydrin-posi-

TABLE 1. *Synthesis of mucopeptide by Pediococcus cerevisiae 8081 and CRD*[a]

Strain	(i) Hexosamine[b]	(ii) Ninhydrin-positive material[b]	(i)/(ii)	Turbidity (OD_{610})	Protein[c]
8081					
0 h	0.180	0.60	0.33	0.267	0.54
5 h	0.520	1.72	0.30	0.370	0.64
	(288)[d]	(286)[d]		(139)[d]	(120)[d]
CRD					
0 h	0.290	1.1	0.26	0.237	0.55
5 h	0.540	1.76	0.31	0.290	0.61
	(186)[d]	(160)[d]		(120)[d]	(110)[d]

[a] Organisms were harvested from PYGAC (8081) or PYG (CRD) washed and suspended in solution W (0.3 to 0.4 mg [dry wt] of organisms per ml). The suspensions were incubated at 37 C and assayed for protein. Peptidoglycan was isolated by solvent extraction, hydrolyzed, and assayed for hexosamine and ninhydrin-positive material.

[b] Micromoles per milligram (dry weight) of organisms at zero time.

[c] Milligrams per milligram (dry weight) of organisms at zero time.

[d] Percent of initial value.

tive molecules. This is consistent with the peptidoglycan being made up of repeating units of two molecules of hexosamine, two of alanine, one of glutamate, one of lysine, and one of aspartate. Protein content did not increase markedly. The composition of the peptidoglycan of exponential-phase parent organisms was determined and found to contain the following components (expressed as molar ratios): glutamic acid, 1.0; alanine, 1.8; aspartic acid, 0.67; lysine, 0.88; muramic acid, 0.56; and glucosamine, 0.74. The peptidoglycan of exponential phase CRD organisms was composed of Glu, 1.0; Ala, 1.8; Asp, 0.65; Lys, 0.91; muramic acid, 0.66; and glucosamine, 0.79. The composition of the peptidoglycan isolated from either organism after 4 h of incubation in solution W was very similar to that of exponential-phase organisms. Cell walls prepared from organisms incubated for 4 h in solution W seemed to be somewhat more susceptible to lysozyme than exponential-phase walls.

Thus, peptidoglycan synthesis can occur under these conditions of incubation with parent and strain CRD organisms, and the wall formed under these conditions appears to be similar to that formed during normal growth.

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