Control of Dimorphism in *Candida albicans* by Zinc: Effect on Cell Morphology and Composition

By H. YAMAGUCHI

*Department of Microbiology, Faculty of Medicine, University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan*

(Received 3 April 1974; Revised 7 October 1974)

Zinc participates in the morphogenesis of a wide range of micro-organisms. In several dimorphic fungi such as *Histoplasma capsulatum* (Pine & Peacock, 1958), *Mucor rouxii* (Bartnicki-Garcia & Nickerson, 1962) and *Candida albicans* (Widra, 1964), zinc completely reverses the filamentous or mycelial phase of growth. Fungal cultures in which the morphology is influenced by a single factor can be useful for the study of metabolic processes underlying the dimorphic phase conversion and in this paper the changes in some macromolecular constituents of *C. albicans*, associated with its morphological transformation, are described with respect to the concentration of zinc added to the defined basal medium.

METHODS

Candida albicans strain 6713, which forms extensive filaments under some nutritional conditions (Yamaguchi, 1974*a*) was grown on a medium depleted of trace metals and containing (per l): glucose, 8 g; $(\text{NH}_4)_2\text{SO}_4$, 8 g; KH_2PO_4 , 3.6 g; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 1.2 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g; D-biotin, 0.1 μg . In zinc-replete medium $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ was added at 9 μM . Zinc-deficient medium was unsupplemented. Medium was inoculated (per ml) with about 10 μg dry wt of *C. albicans* taken from the surface of a 2-day-old culture grown at 37 °C on Sabouraud's glucose agar slants, washed three times with double-distilled water, and finally resuspended in water.

Growth conditions and methods for assay of dry weight, the proportion of yeastlike-phase (Y) cells to filamentous-phase (F) cells during growth, and the content of DNA, RNA and protein were as previously described (Yamaguchi, 1974*a*). DNA phosphorus and RNA phosphorus were calculated by using the atomic extinction coefficient for phosphorus proposed by Logan, Mannell & Rossiter (1952). Insoluble inorganic polyphosphate in the hot 1 M-HClO₄ extract of the yeast was determined by adsorbing the nucleic acids on charcoal according to the method of Harold (1960), and the residual phosphorus, all of which was acid-labile, was taken as insoluble inorganic polyphosphate. After incinerating with conc. H₂SO₄ plus conc. HNO₃, the resulting orthophosphate was determined by the method of Fiske & SubbaRow (1925).

RESULTS AND DISCUSSION

The yield of *C. albicans* after 24 h growth was maximal with 3 μM -Zn²⁺; zinc above 10⁻⁵ M was inhibitory to growth (Fig. 1). Zinc-deficient cultures consisted almost entirely of F cells but the proportion of Y cells increased with increasing concentrations of zinc, reaching a maximal level at 9 μM -Zn²⁺ (Fig. 1). Observations with the light microscope agreed with these results, which were obtained using a mechanical filtration method.

Fig. 1. Effect of different concentrations of zinc added to medium on total growth and morphology of *Candida albicans*. ●, Dry wt of yeast; ○, proportion of Y cells.

Table 1. Effect of zinc on the yield and concentrations of macromolecular constituents of *Candida albicans* during exponential and post-exponential growth

Age of culture (h)	Yield (g dry wt/l)	Content (per mg cell dry wt)			
		Protein (μg)	DNA (μg P)	RNA (μg P)	Polyphosphate (μg P)
Zn ²⁺ added at 9 × 10 ⁻⁶ M					
4	0.12	292	0.32	2.84	12.8
8	0.59	290	0.17	3.00	12.3
12	1.92	312	0.18	3.00	12.1
18	3.01	376	0.16	2.62	—
24	3.13	405	0.19	2.53	12.0
No addition of Zn ²⁺					
4	0.11	273	0.35	3.60	16.0
8	0.51	306	0.27	2.43	19.1
12	0.89	300	0.22	1.67	20.2
18	1.75	309	0.21	0.79	—
24	2.08	314	0.23	0.69	20.4

For the first 6 h of growth, zinc-deficient and zinc-replete cultures grew at the same rate with a doubling time of about 1.5 h. Subsequently, zinc-replete cultures still grew exponentially for about 5 h before slowing down, whereas the growth rate of zinc-deficient cultures dropped to about half but growth continued almost linearly at this rate for another 15 h. Growth of the deficient cultures over the latter period consisted essentially of filament elongation. It continued for at least 24 h after the yeast had reached its maximum dry weight.

Changes in content of several macromolecules were examined in both zinc-replete and zinc-deficient cultures (Table 1). There was little difference between the two cultures in the protein and DNA contents throughout the growth phase. The concentrations of RNA and inorganic polyphosphate did not change significantly when there was an adequate concentration of zinc, but when zinc was deficient the RNA content fell to a third and the content of inorganic polyphosphate doubled during the post-exponential phase of growth. The

inhibition of RNA synthesis and the accumulation of polyphosphate in the zinc-deficient cultures were apparent before inhibition of growth was observed.

The present data suggest that zinc has a primary function in metabolism of RNA in *C. albicans*, rather than protein or DNA. Zinc may be required for the synthesis of RNA or in preventing its degradation. Whatever the mechanism of action may be, the complete cessation of net RNA synthesis at the transitory stage from Y growth to F growth, under a certain degree of zinc deficiency, may imply that subsequent filamentous elongation takes place without net ribosomal formation. There is therefore a significant difference in the metabolic processes involved in mycelial elongation (F growth) and simple cell division (Y growth). Low RNA concentrations and a high incidence of F forms also occur when *C. albicans* is grown with an insufficient supply of yeast extract (Yamaguchi, 1974*a*) or biotin (Yamaguchi, 1974*b*).

Polyphosphate probably functions as store of 'high-energy' phosphate, as it may participate in the reversible synthesis of ADP and ATP (Yoshida & Yamataka, 1953). The accumulation of polyphosphate in zinc-deficient cultures of *C. albicans*, therefore, suggests that cessation of both net RNA synthesis and growth is not due to a failure of energy-yielding metabolism. There may be an antagonism between polyphosphate and nucleic acid metabolism, each competing for ATP, as has been postulated by Smith, Wilkinson & Duguid (1954) and Mudd, Yoshida & Koike (1958). The physiological role of such metabolism of phosphorous compounds in the F growth is being investigated.

I am grateful to Professor K. Iwata for valuable advice and to Miss Akiko Sawanobori for excellent technical assistance.

REFERENCES

- BARTNICKI-GARCIA, S. & NICKERSON, W. J. (1962). Nutrition, growth, and morphogenesis of *Mucor rouxii*. *Journal of Bacteriology* **84**, 841-858.
- FISKE, C. H. & SUBBAROW, Y. (1925). The colorimetric determination of phosphorus. *Journal of Biological Chemistry* **66**, 375-400.
- HAROLD, F. M. (1960). Accumulation of inorganic polyphosphate in mutants of *Neurospora crassa*. *Biochimica et biophysica acta* **45**, 172-188.
- LOGAN, J. E., MANNELL, W. A. & ROSSITER, R. J. (1952). A note on the determination of deoxypentose nucleic acid and pentose nucleic acid in tissue from the nervous system by ultraviolet absorption. *Biochemical Journal* **51**, 480-482.
- MUDD, S., YOSHIDA, A. & KOIKE, M. (1958). Polyphosphate as accumulator of phosphorus and energy. *Journal of Bacteriology* **75**, 224-235.
- PINE, L. & PEACOCK, C. L. (1958). Studies on the growth of *Histoplasma capsulatum*. IV. Factors influencing conversion of the mycelial phase to the yeast phase. *Journal of Bacteriology* **75**, 167-174.
- SMITH, I. W., WILKINSON, J. F. & DUGUID, J. P. (1954). Volutin production in *Aerobacter aerogenes* due to nutrient imbalance. *Journal of Bacteriology* **68**, 450-463.
- WIDRA, A. (1964). Phosphate directed Y-M variation in *Candida albicans*. *Mycopathologia et mycologia applicata* **23**, 197-202.
- YAMAGUCHI, H. (1974*a*). Dimorphism in *Candida albicans*. I. Morphology-dependent changes in cellular content of macromolecules and respiratory activity. *Journal of General and Applied Microbiology* **20**, 87-99.
- YAMAGUCHI, H. (1974*b*). Mycelial development and chemical alteration of *Candida albicans* from biotin insufficiency. *Sabouraudia* (in the Press).
- YOSHIDA, A. & YAMATAKA, A. (1953). On the metaphosphate of the yeast. *Journal of Biochemistry* **40**, 85-94.

The insert enclosed in Volume 86, Part 1, January (Fig. 1. Matching matrix of the 98 test cultures based on 180 pooled characters) should be inserted in Volume 85, Part 2, December, facing p. 293.

Index of Authors

- ATTWOOD, M. M., *see* HARDER, W., MATIN, A. & ATTWOOD, M. M. Studies on the physiological significance of the lack of a pyruvate dehydrogenase complex of *Hyphomicrobium* sp. 319
- AUDHYA, T. K. & RUSSELL, D. W. Enniatin production by *Fusarium sambucinum*: primary, secondary, and unitary metabolism 327
- BAKER, D. A. & PARK, R. W. A. Changes in morphology and cell wall structure that occur during growth of *Vibrio* sp. NCTC4716 in batch culture 12
- BARNETT, J. A., BASCOMB, S. & GOWER, J. C. A maximal predictive classification of Klebsiellae and of the yeasts 93
- BASCOMB, S., *see* BARNETT, J. A., BASCOMB, S. & GOWER, J. C. A maximal predictive classification of Klebsiellae and of the yeasts 93
- BEEVER, R. E. Regulation of 2-phosphoenolpyruvate carboxykinase and isocitrate lyase syntheses in *Neurospora crassa* 197
- BHATTACHARJEE, J. K., *see* KURTZ, M. Biosynthesis of lysine in *Rhodotorula glutinis*: role of pipercolic acid 103
- BRILES, E. B. & TOMASZ, A. Physiological studies on the pneumococcal Forssman antigen: a choline-containing lipoteichoic acid 267
- BROWN, A. D. Microbial water relations. Effects of solute concentration on the respiratory activity of sugar-tolerant and non-tolerant yeasts 241
- BROWN, C. M. & DILWORTH, M. J. Ammonia assimilation by *Rhizobium* cultures and bacteroids 39
- BUHAGIAR, R. W. M. *Torulopsis bacarum*, *Torulopsis pustula* and *Torulopsis multis-gemmis* sp. nov. three new yeasts from soft fruit 1
- BU'LOCK, J. D., *see* MILLONIG, G., DE ROSA, M., GAMBACORTA, A. & BU'LOCK, J. D. Ultrastructure of an extremely thermophilic acidophilic micro-organism 165
- BU'LOCK, J. D., *see* DE ROSA, M., GAMBACORTA, A. & BU'LOCK, J. D. Extremely thermophilic acidophilic bacteria convergent with *Sulfolobus acidocaldarius* 156
- BUTCHER, D. N., *see* PHILLIPS, R. Attempts to induce tumours with nucleic acid preparations from *Agrobacterium tumefaciens* 311
- CATEN, C. E., *see* MERRICK, M. J. The inheritance of penicillin titre in wild-type isolates of *Aspergillus nidulans* 283
- CHILD, J. J., *see* SIETSMAN, J. H., CHILD, J. J., NESBITT, L. R. & HASKINS, R. H. Chemistry and ultrastructure of the hyphal walls of *Pythium acaethicum* 29
- COETZEE, J. N. Chromosome transfer in *Proteus mirabilis* mediated by a hybrid plasmid 133
- COLEMAN, G. & STORMONTH, D. A. Stimulation of the differential rate of exoenzyme formation in *Bacillus amyloliquefaciens* by streptolydigin, an inhibitor of RNA chain elongation 194
- COLES, R. S., JUN. The effect of coenzyme leakage and replacement on the growth and metabolism of two fusobacteria 147
- COPLAND, H. J. R., *see* MOSELEY, B. E. B. Involvement of a recombination repair function in disciplined cell division of *Micrococcus radiodurans* 343
- DATTA, N., *see* HEDGES, R. W., RODRIGUEZ-LEMOINE, V. & DATTA, N. R factors from *Serratia marcescens* 88
- DATTA, N., *see* RODRIGUEZ-LEMOINE, V., JACOB, A. E., HEDGES, R. W. & DATTA, N. Thermosensitive production of their transfer systems by group S plasmids 111
- DILWORTH, M. J., *see* BROWN, C. M. Ammonia assimilation by *Rhizobium* cultures and bacteroids 39
- EBRINGER, L., *see* ŠMARDÁ, J., EBRINGER, L. & MACH, J. The effect of colicin E2 on the flagellate *Euglena gracilis* 363
- EYLAN, E., *see* LINDENBAUM, I., EYLAN, E. & RAANANI, E. Enhancement of growth of *Leptospira icterohaemorrhagiae* by tissue cell cultures 358
- FOX, R. F. & McCLAIN, D. E. Enzyme electrophoretograms in the analysis of taxon relatedness of *Micrococcus cryophilus*, *Branhamella catarhalis* and atypical neisserias 210
- GAMBACORTA, A., *see* MILLONIG, G., DE ROSA, M., GAMBACORTA, A. & BU'LOCK, J. D. Ultrastructure of an extremely thermophilic acidophilic micro-organism 165
- GAMBACORTA, A., *see* DE ROSA, M., GAMBACORTA, A. & BU'LOCK, J. D. Extremely thermophilic acidophilic bacteria convergent with *Sulfolobus acidocaldarius* 156
- GIBBONS, R. A., JONES, G. W. & SELLWOOD, R. An attempt to identify the intestinal receptor for the K88 adhesin by means of a haemagglutination inhibition test using glycoproteins and fractions from sow colostrum 228
- GOODELL, E. W. & SCHWARZ, U. Sphere-rod morphogenesis of *Escherichia coli* 201
- GOWER, J. C., *see* BARNETT, J. A., BASCOMB, S. & GOWER, J. C. A maximal predictive classification of Klebsiellae and of the yeasts 93
- GUEST, J. R., *see* WATTS, D. J. Studies on the vitamin nutrition of the cellular slime mould *Dictyostelium discoideum* 333
- GÜNTHER, T., RICHTER, L. & SCHMALBECK, J. Phospholipids of *Escherichia coli* in magnesium deficiency 191

- HARDER, W., MATIN, A. & ATTWOOD, M. M. Studies on the physiological significance of the lack of pyruvate dehydrogenase complex in *Hyphomicrobium* sp. 319
- HASKINS, R. H., *see* SIETSMAN, J. H., CHILD, J. J., NESBITT, L. R. & HASKINS, R. H. Chemistry and ultrastructure of the hyphal walls of *Pythium acanthicum* 29
- HEDGES, R. W., RODRIGUEZ-LEMOINE, V. & DATTA, N. R factors from *Serratia marcescens* 88
- HEDGES, R. W., *see* RODRIGUEZ-LEMOINE, V., JACOB, A. E., HEDGES, R. W. & DATTA, N. Thermosensitive production of their transfer systems by group S plasmids 111
- HOLLINGDALE, M. R. Isolation of lipopolysaccharide from the walls of *Micropolyspora faeni*: chemical composition and serological reactivity 250
- HOLMGREN, J. & LÖNNROTH, I. Oligomeric structure of cholera toxin: characteristics of the H and L subunits 49
- JACOB, A. E., *see* RODRIGUEZ-LEMOINE, V., JACOB, A. E., HEDGES, R. W. & DATTA, N. Thermosensitive production of their transfer systems by group S plasmids 111
- JARVIS, A. W., LAWRENCE, R. C. & PRITCHARD, G. G. Glucose repression of enterotoxins A, B and C and other extracellular proteins in staphylococci in batch and continuous culture 75
- JONES, G. W., *see* GIBBONS, R. A., JONES, G. W. & SELLWOOD, R. An attempt to identify the intestinal receptor for the K88 adhesin by means of a haemagglutination inhibition test using glycoproteins and fractions from sow colostrum 228
- KIDBY, D. K., *see* MURRAY, A. D. Sub-cellular location of mercury in yeast grown in the presence of mercuric chloride 66
- KINGHORN, J. R. & PATEMAN, J. A. Mutations which affect amino acid transport in *Aspergillus nidulans* 174
- KINGHORN, J. R. & PATEMAN, J. A. The structural gene for NADP L-glutamate dehydrogenase in *Aspergillus nidulans* 294
- KIYOMIYA, A., *see* OHTA, K., KIYOMIYA, A., KOYAMA, N. & NOSOH, Y. The basis of the alkalophilic property of a species of *Bacillus* 259
- KOYAMA, N., *see* OHTA, K., KIYOMIYA, A., KOYAMA, N. & NOSOH, Y. The basis of the alkalophilic property of a species of *Bacillus* 259
- KURTZ, M. & BHATTACHARJEE, J. K. Biosynthesis of lysine in *Rhodotorula glutinis*: role of pipercolic acid 103
- LAWN, A. M. & MEYNELL, E. Extrusion of sex pili by rapidly washed R⁺ *Escherichia coli* 188
- LAWRENCE, R. C., *see* JARVIS, A. W., LAWRENCE, R. C. & PRITCHARD, G. G. Glucose repression of enterotoxins A, B and C and other extracellular proteins on staphylococci in batch and continuous culture 75
- LINDENBAUM, I., EYLAN, E. & RAANANI, E. Enhancement of growth of *Leptospira icterohaemorrhagiae* by tissue cell cultures 358
- LÖNNROTH, I., *see* HOLMGREN, J. Oligomeric structure of cholera toxin: characteristics of the H and L subunits 49
- LOUGHLIN, R. E. Polarity of the *cysJIIH* operon of *Salmonella typhimurium* 275
- MCCARTHY, D. H. & RAWLE, C. T. The rapid serological diagnosis of fish furunculosis caused by 'smooth' and 'rough' strains of *Aeromonas salmonicida* 185
- MCCLAINE, D. E., *see* FOX, R. F. Enzyme electrophoretograms in the analysis of taxon relatedness of *Micrococcus cryophilus*, *Branhamella catarrhalis* and atypical neisserias 210
- MCCORMICK, D. B., *see* SHIH, J. C. H., ROZO, M. L., WRIGHT, L. D. & MCCORMICK, D. B. Characterization of the growth of *Pseudomonas putida* LP on lipoate and its analogues: transport, oxidation, sulphur source and enzyme induction 217
- MACH, J., *see* ŠMARDÁ, J., EBRINGER, L. & MACH, J. The effect of colicin E2 on the flagellate *Euglena gracilis* 363
- MARCHANT, R., *see* PATTON, A. M. The effect of cytochalasin B on hyphal morphogenesis in *Polyporus biennis* 301
- MARRIOTT, M. S. Isolation and chemical characterization of plasma membranes from the yeast and mycelial forms of *Candida albicans* 115
- MATIN, A., *see* HARDER, W., MATIN, A. & ATTWOOD, M. M. Studies on the physiological significance of the lack of a pyruvate dehydrogenase complex in *Hyphomicrobium* sp. 319
- MERRICK, M. J. & CATEN, C. E. The inheritance of penicillin titre in wild-type isolates of *Aspergillus nidulans* 283
- MEYNELL, E., *see* LAWN, A. M. Extrusion of sex pili by rapidly washed R⁺ *Escherichia coli* 188
- MILLONIG, G., DE ROSA, M., GAMBACORTA, A. & BU'LOCK, J. D. Ultrastructure of an extremely thermophilic acidophilic micro-organism 165
- MONTI-BRAGADIN, C., SAMER, L., ROTTINI, G. D. & PANI, B. The compatibility of Hly factor, a transmissible element which controls α -haemolysin production in *Escherichia coli* 367
- MOSELEY, B. E. B. & COPLAND, H. J. R. Involvement of a recombination repair function in disciplined cell division of *Micrococcus radiodurans* 343
- MURRAY, A. D. & KIDBY, D. K. Sub-cellular location of mercury in yeast grown in the presence of mercuric chloride 66
- NESBITT, L. R., *see* SIETSMAN, J. H., CHILD, J. J., NESBITT, L. R. & HASKINS, R. H. Chemistry and ultrastructure of the hyphal walls of *Pythium acanthicum* 29
- NOSOH, Y., *see* OHTA, K., KIYOMIYA, A., KOYAMA, N. & NOSOH, Y. The basis of the alkalophilic property of a species of *Bacillus* 259
- OHTA, K., KIYOMIYA, A., KOYAMA, N. & NOSOH, Y. The basis of the alkalophilic property of a species of *Bacillus* 259

- PANI, B., *see* MONTI-BRAGADIN, C., SAMER, L., ROTTINI, G. D. & PANI, B. The compatibility of Hly factor, a transmissible element which controls α -haemolysin production in *Escherichia coli* 367
- PARK, R. W. A., *see* BAKER, D. A. Changes in morphology and cell wall structure that occur during growth of *Vibrio* sp. NCTC4716 in batch culture 12
- PATEMAN, J. A., *see* KINGHORN, J. R. Mutations which affect amino acid transport in *Aspergillus nidulans* 174
- PATEMAN, J. A., *see* KINGHORN, J. R. The structural gene for NADP L-glutamate dehydrogenase in *Aspergillus nidulans* 294
- PATTON, A. M. & MARCHANT, R. The effect of cytochalasin B on hyphal morphogenesis in *Polyporus biennis* 301
- PHILLIPS, R. & BUTCHER, D. N. Attempts to induce tumours with nucleic acid preparations from *Agrobacterium tumefaciens* 311
- PRITCHARD, G. G., *see* JARVIS, A. W., LAWRENCE, R. C. & PRITCHARD, G. G. Glucose repression of enterotoxins A, B and C and other extracellular proteins in staphylococci in batch and continuous culture 75
- RAANANI, E., *see* LINDENBAUM, I., EYLAN, E. & RAANANI, E. Enhancement of growth of *Leptospira icterohaemorrhagiae* by tissue cell cultures 358
- RAWLE, C. T., *see* MCCARTHY, D. H. The rapid serological diagnosis of fish furunculosis caused by 'smooth' and 'rough' strains of *Aeromonas salmonicida* 185
- RICHTER, L., *see* GÜNTHER, T., RICHTER, L. & SCHMALBECK, J. Phospholipids of *Escherichia coli* in magnesium deficiency 191
- RODRIGUEZ-LEMOINE, V., *see* HEDGES, R. W., RODRIGUEZ-LEMOINE, V. & DATTA, N. R. factors from *Serratia marcescens* 88
- RODRIGUEZ-LEMOINE, V., JACOB, A. E., HEDGES, R. W. & DATTA, N. Thermosensitive production of their transfer systems by group S plasmids 111
- DE ROSA, M., GAMBACORTA, A. & BU'LOCK, J. D. Extremely thermophilic acidophilic bacteria convergent with *Sulfolobus acidocaldarius* 156
- DE ROSA, M., *see* MILLONIG, G., DE ROSA, M., GAMBACORTA, A. & BU'LOCK, J. D. Ultrastructure of an extremely thermophilic acidophilic micro-organism 165
- ROTTINI, G. D., *see* MONTI-BRAGADIN, C., SAMER, L., ROTTINI, G. D. & PANI, B. The compatibility of Hly factor, a transmissible element which controls α -haemolysin production in *Escherichia coli* 367
- ROZO, M. L., *see* SHIH, J. C. H., ROZO, M. L., WRIGHT, L. D. & MCCORMICK, D. B. Characterization of the growth of *Pseudomonas putida* LP on lipoate and its analogues: transport, oxidation, sulphur source and enzyme induction 217
- RUSSELL, D. W., *see* AUDHYA, T. K. Enniatin production by *Fusarium sambucinum*: primary, secondary, and unitary metabolism 327
- SAMER, L., *see* MONTI-BRAGADIN, C., SAMER, L., ROTTINI, G. D. & PANI, B. The compatibility of Hly factor, a transmissible element which controls α -haemolysin production in *Escherichia coli* 367
- SCHMALBECK, J., *see* GÜNTHER, T., RICHTER, L. & SCHMALBECK, J. Phospholipids of *Escherichia coli* in magnesium deficiency 191
- SCHWARTZ, U., *see* GOODELL, E. W. Sphere-rod morphogenesis of *Escherichia coli* 201
- SELLWOOD, R., *see* GIBBONS, R. A., JONES, G. W. & SELLWOOD, R. An attempt to identify the intestinal receptor for the K88 adhesin by means of a haemagglutination inhibition test using glycoproteins and fractions from sow colostrum 228
- SHIH, J. C. H., ROZO, M. L., WRIGHT, L. D. & MCCORMICK, D. B. Characterization of the growth of *Pseudomonas putida* LP on lipoate and its analogues: transport, oxidation, sulphur source and enzyme induction 217
- SIETSMA, J. H., CHILD, J. J., NESBITT, L. R. & HASKINS, R. H. Chemistry and ultrastructure of the hyphal walls of *Pythium acanthicum* 29
- ŠMARDÁ, J., EBRINGER, L. & MACH, J. The effect of colicin E2 on the flagellate *Euglena gracilis* 363
- STORMONTH, D. A., *see* COLEMAN, G. Stimulation of the differential rate of exoenzyme formation in *Bacillus amyloliquefaciens* by streptolydigin, an inhibitor of RNA chain elongation 194
- TOMASZ, A., *see* BRILES, E. B. Physiological studies on the pneumococcal Forssman antigen: a choline-containing lipoteichoic acid 267
- WATTS, D. J. & GUEST, J. R. Studies on the vitamin nutrition of the cellular slime mould *Dictyosporium discoideum* 333
- WRIGHT, L. D., *see* SHIH, J. C. H., ROZO, M. L., WRIGHT, L. D. & MCCORMICK, D. B. Characterization of the growth of *Pseudomonas putida* LP on lipoate and its analogues: transport, oxidation, sulphur source and enzyme induction 217
- YAMAGUCHI, H. Control of dimorphism in *Candida albicans* by zinc: effect on cell morphology and composition 370

Index of Subjects

- Aeromonas salmonicida*, serological examination of 'smooth' and 'rough' strains (McCarthy & Rowle) 185
- Agrobacterium tumefaciens* and tumour formation (Phillips & Butcher) 311
- Alkalophile, *Bacillus* species of (Ohta, Kiyomiya, Koyama & Nosoh) 259
- Amino acid transport in *Aspergillus nidulans* (Kinghorn & Pateman) 174
- Ammonia assimilation by *Rhizobium* and nodules (Brown & Dilworth) 39
- Antibiotic production by *Fusarium sambucinum* (Audhya & Russell) 327
- Antigen, Forssman, of pneumococcus (Briles & Tomasz) 267
- Aspergillus nidulans*, inheritance of penicillin titre (Merrick & Caten) 283
- Aspergillus nidulans*, mutations affecting amino acid transport in (Kinghorn & Pateman) 174
- Aspergillus nidulans*, structural gene for NADP L-glutamate dehydrogenase (Kinghorn & Pateman) 294
- Bacillus*, alkalophilic species (Ohta, Kiyomiya, Koyama & Nosoh) 259
- Bacillus amyloliquefaciens*, effect of streptolydigin on exoenzyme formation (Coleman & Stormonth) 194
- Bisnor-lipoic acid, bacterial metabolism (Shih, Rozo, Wright & McCormick) 217
- Branhamella catarrhalis*, enzyme electrophoretograms in taxonomy of (Fox & McClain) 210
- Candida albicans*, effect of zinc on composition and dimorphism (Yamaguchi) 370
- Candida albicans*, plasma membranes from yeast and mycelial forms (Marriott) 115
- Carbon sources for *Hyphomicrobium* sp. (Harder, Matin & Attwood) 319
- Cell division in *Micrococcus radiodurans* (Moseley & Copland) 343
- Chlorophyll synthesis in *Euglena gracilis*, inhibition by colicin E2 (Šmarda, Ebringer & Mach) 363
- Cholera toxin, H and L subunits of (Holmgren & Lönnroth) 49
- Chromosome transfer in *Proteus mirabilis* (Coetzee) 133
- Clamp connexions, effect of cytochalasin B on (Patton & Marchant) 301
- Classification, maximal predictive, for yeasts and Klebsiellae (Barnett, Bascomb & Gower) 93
- Coenzymes of fusobacteria (Coles) 147
- Colicin E2, effect on *Euglena gracilis* (Šmarda, Ebringer & Mach) 363
- Compatibility of Hly factor (Monti-Bragadin, Samer, Rottini & Pani) 367
- Conjugation in *Proteus mirabilis* (Coetzee) 133
- CysJIIH* operon of *Salmonella typhimurium*, polarity of (Loughlin) 275
- Cytochalasin B, effect of *Polyporus biennis* (Patton & Marchant) 301
- Dictyostelium discoideum*, vitamin nutrition (Watts & Guest) 333
- Dimorphism in *Candida albicans*, effect of zinc (Yamaguchi) 370
- Enniatin production by *Fusarium sambucinum* (Audhya & Russell) 327
- Enterotoxins, staphylococcal, effect of glucose and growth rate on (Jarvis, Lawrence & Pritchard) 75
- Escherichia coli*, compatibility of Hly factor (Monti-Bragadin, Samer, Rottini & Pani) 367
- Escherichia coli*, extrusion of sex pili by (Lawn & Meynell) 188
- Escherichia coli* K88 receptor, identification by haemagglutination inhibition test (Gibbons, Jones & Sellwood) 228
- Escherichia coli*, magnesium deficiency and phospholipid content in (Günther, Richter & Schmalbeck) 191
- Escherichia coli*, sphere-rod morphogenesis of (Goodell & Schwarz) 201
- Euglena gracilis*, effect of colicin E2 on (Šmarda, Ebringer & Mach) 363
- Exoenzyme formation in *Bacillus amyloliquefaciens* (Coleman & Stormonth) 194
- Forssman antigen of pneumococcus, physiology (Briles & Tomasz) 267
- Furunculosis and rapid identification of *Aeromonas salmonicida* (McCarthy & Rowle) 185
- Fusarium sambucinum*, enniatin production by (Audhya & Russell) 327
- Fusobacterium*, coenzyme leakage from (Coles) 147
- Genetics of penicillin titre in *Aspergillus nidulans* (Merrick & Caten) 283
- L-Glutamate dehydrogenase, NADP, in *Aspergillus nidulans*, structural gene for (Kinghorn & Pateman) 294
- Glutamine synthetase and glutamate synthase in *Rhizobium* (Brown & Dilworth) 39
- Glucose, repression of extracellular proteins in staphylococci by (Jarvis, Lawrence & Pritchard) 75
- Growth of *Leptospira icterohaemorrhagiae*, enhancement by tissue cell cultures (Lindenbaum, Eylan & Raanani) 358
- Haemagglutination by K88 adhesin and glycoprotein inhibition (Gibbons, Jones & Sellwood) 228
- Heterokaryon incompatibility, relationship to penicillin yield in *Aspergillus nidulans* (Merrick & Caten) 283
- Hly factor, compatibility of (Monti-Bragadin, Samer, Rottini & Pani) 367

- Hyphae of *Polyporus biennis*, effect of cytochalasin B on morphogenesis of (Patton & Marchant) 301
- Hyphae of *Pythium acanthicum*, composition of walls (Sietsma, Child, Nesbitt & Haskins) 29
- Hyphomicrobium* sp., pyruvate dehydrogenase deficiency in (Harder, Matin & Attwood) 319
- Isocitrate lyase, regulation of synthesis in *Neurospora crassa* (Beever) 197
- K88 antigen, inhibition of haemagglutination by sow colostrum (Gibbons, Jones & Sellwood) 228
- Klebsiellae, a maximal predictive classification of (Barnett, Bascomb & Gower) 93
- Leakage of coenzymes from fusobacteria (Coles) 147
- Leptospira icterohaemorrhagiae*, growth enhancement by tissue cell cultures (Lindenbaum, Eylan & Raanani) 358
- Lipoic acid, metabolism by *Pseudomonas putida* LP (Shih, Rozo, Wright & McCormick) 217
- Lipopolysaccharide from walls of *Micropolyspora faeni*, composition and serological reactivity (Hollingdale) 250
- Lipoteichoic acid of pneumococcus, physiology of (Briles & Tomasz) 267
- Lysine biosynthesis in *Rhodotorula glutinis*, role of pipercolic acid in (Kurtz & Bhattacharjee) 103
- Magnesium deficiency and phospholipid composition in *Escherichia coli* (Günther, Richter & Schmalbeck) 191
- Membranes, plasma, from yeast and mycelial forms of *Candida albicans* (Marriott) 115
- Mercury, sub-cellular location in yeast (Murray & Kidby) 66
- Metabolism and enniatin production by *Fusarium sambucinum* (Audhya & Russell) 327
- Micrococcus cryophilus*, enzyme electrophoretograms in taxonomy of (Fox & McClain) 210
- Micrococcus radiodurans*, recombination repair and cell division in (Moseley & Copland) 343
- Micropolyspora faeni*, lipopolysaccharide from walls (Hollingdale) 250
- Morphogenesis in *Vibrio* NCTC4716 (Baker & Park) 12
- Mutations affecting amino acid transfer in *Aspergillus nidulans* (Kingham & Pateman) 174
- Neisserias, atypical, enzyme electrophoretograms in taxonomy of (Fox & McClain) 210
- Neurospora crassa*, 2-phosphoenolpyruvate carboxykinase and isocitrate lyase synthesis (Beever) 197
- Nodules, ammonia assimilation in (Brown & Dilworth) 39
- Nucleic acid from *Agrobacterium tumefaciens*, and tumour induction (Phillips & Butcher) 311
- Penicillin FL-1060, formation of spheres of *Escherichia coli* with (Goodell & Schwarz) 201
- Penicillin, inheritance of titre in *Aspergillus nidulans* (Merrick & Caten) 283
- Peptidoglycan in walls of *Vibrio* NCTC4716 (Baker & Park) 12
- 2-Phosphoenolpyruvate carboxykinase, regulation of synthesis in *Neurospora crassa* (Beever) 197
- Phospholipids in magnesium-deficient *Escherichia coli* (Günther, Richter & Schmalbeck) 191
- Pili, sex, extrusion by rapidly washed R⁺ *Escherichia coli* (Lawn & Meynell) 188
- Pipercolic acid, role in lysine biosynthesis in *Rhodotorula glutinis* (Kurtz & Bhattacharjee) 103
- Plasma membranes from yeast and mycelial forms of *Candida albicans* (Marriott) 115
- Plasmids from *Serratia marcescens* (Hedges, Rodriguez-Lemoine & Datta) 88
- Plasmids, group S, thermosensitive transfer system (Rodriguez-Lemoine, Jacob, Hedges & Datta) 111
- Plasmids in *Proteus mirabilis* (Coetzee) 133
- Pneumococcus, Forssman antigen of (Briles & Tomasz) 267
- Polarity of the *cysJIIH* operon (Loughlin) 275
- Polyporus biennis*, cytochalasin B and hyphal morphogenesis (Patton & Marchant) 301
- Proteins of staphylococci, extracellular, effect of glucose and growth rate on (Jarvis, Lawrence & Pritchard) 75
- Proteus mirabilis*, chromosome transfer in (Coetzee) 133
- Pseudomonas putida* LP, metabolism of lipoate and analogues (Shih, Rozo, Wright & McCormick) 217
- Pyruvate dehydrogenase deficiency in *Hyphomicrobium* sp. (Harder, Matin & Attwood) 319
- Pythium acanthicum*, hyphal wall composition (Sietsma, Child, Nesbitt & Haskins) 29
- Radiation and cell division in *Micrococcus radiodurans* (Moseley & Copland) 343
- Recombination and cell division in *Micrococcus radiodurans* (Moseley & Copland) 343
- Respiration apparatus in sugar-tolerant and non-tolerant yeasts (Brown) 241
- R factors from *Serratia marcescens* (Hedges, Rodriguez-Lemoine & Datta) 88
- Rhizobium, ammonia assimilation by (Brown & Dilworth) 39
- Rhodotorula glutinis*, lysine biosynthesis in (Kurtz & Bhattacharjee) 103
- Salmonella typhimurium*, polarity of the *cysJIIH* operon (Loughlin) 275
- Serology, rapid examination of 'smooth' and 'rough' strains of *Aeromonas salmonicida* (McCarthy & Rowle) 185
- Serratia marcescens*, plasmids from (Hedges, Rodriguez-Lemoine & Datta) 88
- Spheroplasts of *Escherichia coli*, morphogenetic capacity of (Goodell & Schwarz) 201
- S plasmids, thermosensitive transfer system (Rodriguez-Lemoine, Jacob, Hedges & Datta) 111
- Staphylococci, glucose repression of extracellular proteins of (Jarvis, Lawrence & Pritchard) 75
- Streptolydigin, stimulation of exoenzyme formation in *Bacillus amyloliquefaciens* by (Coleman & Stormonth) 194

- Sulfolobus acidocaldarius*, acidophilic thermophiles convergent with (de Rosa, Gambacorta & Bu'Lock) 156
- Taxonomy of *Micrococcus cryophilus*, *Branhamella catarrhalis* and atypical neisserias (Fox & McClain) 210
- Temperature, effect on transfer of group S plasmids (Rodriguez-Lemoine, Jacob, Hedges & Datta) 111
- Tetranor-lipoic acid, bacterial metabolism (Shih, Roza, Wright & McCormick) 217
- Thermophile, acidophilic, ultrastructure of (Millonig, de Rosa, Gambacorta & Bu'Lock) 165
- Thermophiles, acidophilic, convergent with *Sulfolobus acidocaldarius* (de Rosa, Gambacorta & Bu'Lock) 156
- Tissue cultures, effect on leptospires (Lindenbaum, Eylan & Raanani) 358
- Torulopsis*, new species from fruit (Buhagiar) 1
- Toxin, cholera, H and L subunits of (Holmgren & Lönnroth) 49
- Transfer of group S plasmids, thermosensitivity of (Rodriguez-Lemoine, Jacob, Hedges & Datta) 111
- Transport of amino acids in *Aspergillus nidulans* (Kingham & Pateman) 174
- Tumour induction by *Agrobacterium tumefaciens* (Phillips & Butcher) 311
- Ultrastructure of thermophilic acidophilic microorganisms (Millonig, de Rosa, Gambacorta & Bu'Lock) 165
- Vibrio* NCTC4716, morphological changes in (Baker & Park) 12
- Vitamin nutrition of *Dictyostelium discoideum* (Watts & Guest) 333
- Walls, hyphal, composition in *Pythium acanthicum* (Sietsma, Child, Nesbitt & Haskins) 29
- Walls of *Vibrio* NCTC4716, changes in peptidoglycan layer (Baker & Park) 12
- Water relations of sugar-tolerant yeasts (Brown) 241
- Yeasts, a maximal predictive classification of (Barnett, Bascomb & Gower) 93
- Yeasts, new species of *Torulopsis* from fruit (Buhagiar) 1
- Yeasts, sugar-tolerant and non-tolerant, respiratory apparatus in (Brown) 241
- Yeast, subcellular location of mercury in (Murray & Kidby) 66
- Zinc, effect on composition and dimorphism of *Candida albicans* (Yamaguchi) 370