REFERENCES

Abbas, H.K., Mirocha, C.J. & Shier, W.T. (1984) Mycotoxins produced from fungi isolated from foodstuffs and soil: comparison of toxicity in fibroblasts and rat feeding tests. Environmental Microbiology. **48**, 654-661.

Abedi, Z.H. & Scott, P.M. (1969) Detection of toxicity of aflatoxins, sterigmatocystin and other fungal toxins by lethal action on zebra fish larvae. Journal of the Association of Official Analytical Chemists. **52**, 963-969.

Adak, G.K., Corry, J.E.L. & Moss, M.O. (1987a) Use of impedimetry to detect trichothecene mycotoxins. 2. Limits of sensitivity of four organisms to T-2 toxin and the effect of solvent and test medium. International Journal of Food Microbiology. **5**, 15-27.

Adak, G.K., Corry, J.E.L. & Moss, M.O. (1987b) Use of impedimetry to detect trichothecene mycotoxins. 1. Screen for susceptible microorganisms. International Journal of Food Microbiology. **5**, 1-13.

Alexander, K.T.W., Mitropoulos, K.A. & Gibbons, G.F. (1974) A possible role for cytochrome P450 during the synthesis of zymosterol from lanosterol by *Saccharomyces cerevisiae*. Biochemical and Biophysical Research Communications. **60**, 460-467.

Allcroft, R., Carnaghan, R.B.A., Sargeant, K. & O'Kelly, J. (1961) Toxic factor in Brazilian groundnut meal. Veterinary Records. **73**, 428-429.

Anema, P.J. (1964) Purification and some properties of β-galactosidase of *Bacillus subtilis*. Biochimica et Biophysica Acta. **89**, 495-506.

Angsubhakorn, S. (1991) Mycotoxins and human health risks. An overview. In Mycotoxin Prevention and Control. Eds. Semple, R.L., Frio, A.S., Hicks, P.A. & Lazare, J.V. 25-32.

Aoyama, Y., Yoshida, Y., Sonoda, Y., & Sato, R. (1987) Journal of Biological Chemistry. **262**, 1239-1243.

Aoyama, Y., Yoshida, Y., & Sato, R. (1984) Yeast cytochrome P-450 catalysing lanosterol 14α-demethylation. II. Lanosterol metabolism by purified $P450_{14DM}$ and by intact microsomes. Journal of Biological Chemistry. **259**, 1661-1666.

Asp, N.G. (1971) Human small-intestinal β-galactosidases. Biochemical Journal. **121**, 299-308.

Auffray, Y. & Boutibonnes, P. (1987) Genotoxic activity of some mycotoxins using the SOS chromotest. Mycopathologia. **100**, 49-53.

Azari, M.R. & Wiseman, A. (1982) Studies on the procedure to measure accurately the binding properties of benzo(a)pyrene to cytochrome P450/448. Biochemical Society Transactions. **10**, 133-134.

Babich, H. & Barenfreud, E. (1991) Cytotoxicity of T-2 toxin and its metabolites determined with the neutral red cell viability assay. Applied and Envirinmental Microbiology. **57**, 2101-2103.

Bachmann, M., Luthy, J. & Schlatter, C. (1979) Toxigenicity and mutagenicity of moulds of the *Aspergillus glaicus* group. Identification of physicon and three related anthroquinones as main toxic constituents from *Aspergillus chevalieri*. Journal of Agricultural and Food Chemistry. **27**, 1342-1347.

Barbacid, M. & Vazquez, D. (1974) Binding of (acetyl-^{14}C) trichodermin to the peptidyl transferase centre of eukaryotic ribosomes. European Journal of Biochemistry. **44**, 437-444.

Bartholomew, R.M. & Ryan, D.S. (1980) Lack of mutagenicity of some phytoestrogens in the *Salmonella*/mammalian microsome assay. Mutation Research. **78**, 317-321.

Baxter, J.A., Chia, L-S., Hsieh, D.W. & Datta, S.K. (1987) Survey of sensitivity of 22 strains of yeasts to T-2 toxin in relation to growth on glucose and glycerol media. Bulletin of Environmental Contamination and Toxicology. **39**, 86-91.

Baxter, C.S., Wey, H.E., & Burg, W.R. (1981) A prospective analysis of the potential risk associated with inhalation of aflatoxin-contaminated dusts. Food and Cosmetic Toxicology. **19**, 765-769.

Beasley, V.R., Bunner, D.L., Poppenga, R.H. (1989) Conclusions and future directions for research. In Trichtohecene Mycotoxins: Pathophysiological Effects. Ed. Beasley, V.R. Vol 2. 170-188.

Becci, P.J., Hess, F.G., Johnson, W.G., Gallo, M.A., Babish, J.G., Dailey, R.E. & Parent, R.A. (1981) Applied Toxicology. **1**, 256-

Beckwith, J. (1978) in The Operon. Eds. Miller, J.H. & Reznilsoff, W.S. Cold Spring Harbour Laboratory, Cold Spring Harbour, NY. 11-30.

Beeton, S. & Bull, A.T. (1989) Biotransformation and detoxification of T-2 toxin by soil and freshwater bacteria. Applied and Environmental Microbiology. **55**, 190-197.

Betina, V. (1989) Mycotoxins as secondary metabolites. In Mycotoxins. Bioactive Molecules. Vol. 9. Elsevier.

Bezuidenhout, S.C., Gelderblom, W.C.A., Gorst-Allman, C.P., Horak, R.M., Marasas, W.F.O., Spiteller, G. & Vleggaar, R. (1988) Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. Journal of the Chemical Society, Chemical Communication. 743-745.

Bhat, R.V., Beedu, S.R., Ramakrishna, YY. & Munshi, K.L. (1989) Outbreak of trichothecene mycotoxicosis associated with consumption of mould-damaged wheat products in Kashmir Valley, India. Lancet, 7, 35-37.

Bijl, J.,Rosseau, D.M., Dive, D. & Peteghem, C.H. (1988) Potentials of a sychronised culture of *Tetrahymena pyriformis* for toxicity studies of mycotoxins. Journal of the Association of Official Analytical Chemists. **71**, 282-285.

Bligh, H,F,J. (1988) Comparative and molecular analysis of cytochrome P450 in yeast. PhD Thesis. University of Sheffield.

Blount, W.P. (1961) Turkey "X" disease. Journal of the British Turkey Federation. **9**, 52-61.

Boguslawski, G. (1985) Effects of polymyxin B sulphate and polymyxin B nonapeptide on growth and permeability of the yeast *Saccaromyces cerevisiae*. Molecular and General Genetics. **199**, 401-405.

Boutibonnes, P., Malherbe, C., Auffray, Y., Kogbo, W. & Marais, C. (1983) Mycotoxin sensitivity to *Bacillus thuringiensis*. IRCS Medicine, Science and Biochemistry: Pharmacology, **11**, 430-431.

Bradburn, N. (1993) The analysis of toxic metabolites of *Alternatia, Aspergillus* and *Fusarium* species and their presence in animal feeds. PhD Thesis. University of Portsmouth.

Bradburn, N. Coker, R.D. & Blunden, G. (1994) The aetiology of turkey 'X' disease. Phytochemistry. **35**, 817.

Broce, D., Grodner, R.M., Killebrew, R.L. & Bonner, F.L. (1970) Ochratoxins A and B: Confirmation by microbiological assay using *Bacillus ceres mycoides*. Journal of the Association of Offical Analytical Chemists. **53**, 616-619.

Buckelew, A.R., Chakravarti, A., Burge, W.R., Thomas, V.M. & Ikawa, M. (1972) Effect of mycotoxins and coumarins on the growth of *Bacillus megaterium* from spores. Journal of Agricultural and Food Chemistry. **20**, 431-433.

Buckle, A.E. & Sanders, M.F. (1990) An appraisal of bioassay methods for the detection of mycotoxins. A review. Letters in Applied Microbiology. **10**. 155-160.

Bul, J., Dive, D. & Van Peteghem, C. (1981) Comparison of some bioassay methods for mycotoxin studies. Environmental Pollution (Series A). **26**, 173-182.

Bullerman, L.B. (1986) Mycotoxins and food safety. A scientific status summary by the Institute of Food Technologists' expert panel on food safety and nutrition. Food Technology (May). 59-66.

Bullerman, L.B., Schroeder, L & Park, K-Y. (1984) Formation and control of mycotoxins in food. Jouranl of Food Protection. **47**, 637-646.

Burditt, S.J., Hagler, W.M. & Hamilton, P.B. (1984) Feed refusal due to ochratoxicoses in turkeys. Food Technology (May). 59-66.

Burmeister, H.R. & Hesseltine, C.W. (1970) Biological assays for two mycotoxins produced by *Fusarium tricinctum*. Applied Microbiology. **20**, 437-440.

Burmeister, H.R. & Hesseltime, C.W. (1966) Survey of the sensitivity of microorganisms to aflatoxin. Applied Microbiology. **14**, 403-406.

Busby, W.F. & Wogan, G.W. (1984) Aflatoxins. In Chemical Carcinogens. **2**, ACS Monogragh. Ed. Searle, C.E. 182-229.

Butler, H.T., Schuette, S.A., Pacholec, F. & Poole, C.F. (1983) Characterisation of a scanning densitometer for high performance thin-layer chromatography. Journal of Chromatography. **261**, 55-63.

Callen, D.F., Wolf, C.R. & Philpot, R.M. (1980) Cytochrome P-450 mediated genetic activity and cytotoxic of seven halogenated aliphatic hydrocarbons in *Saccharomyces cerevisiae*. Mutation Research. **77**, 55-63.

Callen, D.F. & Philpot, R.M. (1977) Cytochrome P-450 and the activation of promutagens in *Saccharomyces cerevisiae*. Mutation Research. **45**, 309-324.

Cannon, M., Jimenez, A. & Vazquez, D. (1976) Compitition between trichodermin and several other sisquiterpene antibiotics for binding to their receptor site(s) on eukaryotic ribosomes. Biochemical Journal. **156**, 289-294.

Carnaghan, R.B.A., Hartley, R.D. & O'Kelly, J. (1963) Toxicity and fluorescent properties of the aflatoxins. Nature. **200**, 1101.

Carrasco, L. & Vasquez, D. (1973) Differences in eukaryotic ribosomes detected by the selective action of an antibiotic. Biochimica et Biophysica Acta. **319**, 209-215.

Carrasco, L. & Vazquez, D. (1972) Survey of inhibitors of different steps of protein synthesis by mammalian ribosomes. Journal of Antibiotics. **25**, 732-737.

Carrasco, L., Barbacid, M. & Vazquez, D. (1973) The trichodermin group of antibiotics, inhibitors of peptide bond formation of eukaryotic ribosmes. Biochimica et Biophysica Acta. **312**, 368-376,

Carter, C.J. & Cannon, M. (1977) Structural requirements for the inhibitory action of 12,13-epoxytrichothecenes on protein synthesis in eukaryotes. Biochemical Journal. **166**, 399-409.

Cawood, M.E., Gelderblom, W.C.A., Vleggaar, R., Behrend, Y., Thiel, P.G. & Marasas, W.F.O. (1991) Isolation of the fumonisin mycotoxins: a quantitative approach. Journal of Agricultural and Food Chemistry. **39**, 1958-1962.

Chen, K-C, Houng, J-R & Ling, A.C. (1985) Product inhibitin of the enzymatic hydrolysis of lactose. Enzyme and Microbial Technology. **7**, 510-514.

Chen, S.C.G., Wei, R.D. & Hsieh, D.P.H. (1981) Purification and some properties of chicken liver aflatoxin B_1 reductase. Food and Cosmetic Toxicology. **19**, 19-24.

Chi, M.S. & Mirocha, C.J. (1978) Necrotic oral lesions in chickens fed diacetoxyscirpenol, T-2 toxin and croticin. Poultry Science. **57**, 807-808.

Chihara, S., Tobita, T., Yahata, M., Ito, A. & Koyama, Y. (1973) Enzymatic degredation of colistin. Isolation and identification of α-N-acyl-αγ-diaminobutyric acid and colistin nonapetide. Agricultural and Biological Chemistry. **37**, 2455-2463.

Chu, F.S. & Li, G.Y. (1994) Simultaneous occurence of fumonisin B_1 and other mycotoxins in mouldy corn collected from the Peoples Republic of China in regions with high incidence of esophagal cancer. Applied and Environmental Microbiology. **60**, 847-852.

Ciegler, A., Beckwith, A.C. & Jackson, L.K. (1976) Teratogenicity of patulin and patulin adducts formed with cysteine. Applied and Environmental Microbiology. **31**, 644-649.

Citti, J.E., Sandine, W.E. & Elliker, P.R. (1965) β-galactosidase of *Streptococcus lactis*. Journal of Bacteriology. **89**, 937-942.

Clements, M.L. (1968a) Note on a microbiological assay for aflatoxin B_1: a rapid confirmatory test by effects on growth of *Bacillus megaterium*. Journal of the Association of Offical Anlaytical Chemists. **51**, 611-612.

Clements, M.L. (1968b) Rapid confirmatory test for aflatoxin B_1 using *Bacillus megaterium* Journal of Association of Offical Anlaytical Chemists. **51**, 1192-1194.

Cohen, R.B., Tsou, K.C., Rutenberg, S.H. & Seligman, A.M. (1958) The colorimetic estimation and histochemical demonstration of β-D-galactosidase. Journal of Biological Chemistry. **195**, 239-249.

Coker, R.D. (1984) High performance liquid chromatography and other chemical quantification methods used in the analysis of mycotoxins in food. In Analysis of Food Contaminants. Ed. Gilbert, J. Elsevier Applied Science Publishers. 207-263.

Cole, R.J. & Cox, R.H. (1981) Handbook of toxic fungal metabolites. New York, Academic Press.

Comp, P.C. & Lester, G. (1971) Properties of extracellular β-galactosidase secreted by *Neurospora crassa*. Journal of Bacteriology. **107**, 162-167.

Connolly, P. & Corry, J.E.L. (1990) Effect of polymyxin B nonapeptide and polymyxin B sulphate on trichothecene mycotoxin sensitivity of yeast using a conductimetric instrument. International Journal of Food Microbiology. **10**, 73-90.

Corley, R.H., Swanson, S.P. & Buck, W.B. (1985) Glucuronide conjugates of T-2 toxin and metabolites in swine bile and urine. Journal of Agricultural and Food Chemistry. **33**, 1085-1089.

Coulombe, R.A. (1993) Symposium: biolocial action of mycotoxins. Jouranl of Dairy Science. **76**, 880-891.

Coulombe, R.A., Shelton, D.W., Sinnhuber, R.O. & Nixon, J.E. (1982) Comparative mutagenicity of aflatoxins using a *Salmonella*/trout hepatic enzyme activation system. Carcinogenesis. **3**, 1261-1264.

Craven, G.R., Steers, E. Jr. & Anfinsen, C.B. (1965) Purification, composition and molecular weight of β-galactosidase from *Escherichia coli*, K12. Journal of Biological Chemistry. **240**, 2468-2477.

Creppy, E.E., Röschenthaler, R. & Dirheimer, G. (1984) Inhibition of protein synthesis in mice by ochratoxin A and its prevention by phenylalanine. Food and Chemical Toxicology. **22**, 883-888.

Creppy, E.E., Nern, D., Steyn, P.S., Vleggaar, R., Röschenthaler, R. & Dirheimer, G. (1983) Comparative study on the effect of ochratoxin A analogues on yeast aminoacyl-tRNA synthetases and on the growth and protein synthesis of hepatoma cells. Toxicology Letters. **19**, 217-221.

Croy, R.G. & Wogan, G.N. (1981) Temporal patterns of covalent DNA adducts in rat liver after single and multiple doses of aflatoxin B_1. Cancer Research. **41**, 197-202

Cundliffe, E. & Davis, J.E. (1977) Inhibition of initiation, elongation and termination of eukaryotic protein synthesis be trichothecene fungal toxins. Antimicrobial Agents in Chemotheroapy. **11**, 491-499.

Cundliffe, E., Cannon, M., Davies, J. (1974) Mechanism of inhibition of eukaryotic protein synthesis by trichothecene fungal toxins. Procedings of the National Academy of Sciences. USA. **71**, 30-34.

Curtis, R.F., Coxon, D.T. & Levett, G. (1974) Toxicity of fatty acids in assays for mycotoxins using the brine shrimp (*Artemia salina*). Food and Cosmetic Toxicology. **12**, 233-235.

D'Aquino, M., Bejar, S. & Bollini, E. (1986) *Bacillus subtilis* rec assay for quantification of aflatoxins. Jouranl of Food Protection. **49**, 974-976.

Dalvi, R.R. & Salunkhe, D.K. (1990) Mycotoxins in foods and feeds, their potential health hazards and possible control: an overview. Journal of Maharashtra Agricultural Univeristy. **15**, 36-40.

Davis, R. (1964) Lactose utilisation and hydrolysis in *Saccharomyces fragilis*. Journal of General Microbiology. **37**, 81-98.

Davis, A. (1956) Some factors affecting lactase formation and activity in *Saccharomyces fragilis*. Journal of General Microbiology. **14**, 425-439.

De Waart, J., van Aken, F. & Pouw, H. (1972) Detection of orally toxic microbial metabolites in foods with bioassay systems. 222, 96-114.

Degan, G.H. & Neumann, H.G. (1978) The major metabolite of aflatoxin B_1 in the rat is a glutathione conjugate. Chemical and Biological Interactions. **22**, 239-255.

Dell, M.P.K. (1993) The development of methods for the quality control of foodstuffs contaminated with mycotoxins. PhD Thesis. University of Greenwich.

Detroy, R.W. & Hesseltine, C.W. (1970) Aflatoxicol: structure of a new transformation product of aflatoxin B_1. Canadian Journal of Biochemistry. **48**, 830-832.

Dickson, R.C. & Barr, K. (1983) Characterisation of lactose transport in *Kluyveromyces lactis*. Journal of Bacteriology. **154**, 1245-1251.

Dickson, R.C., Dickson, L.R. & Markin, J.S. (1979) Purification and properties of an inducible β-galactosidase from the yeast *Kluyveromyces lactis* Journal of Bacteriology. **137**, 51-61.

Dickson R.C. & Markin, J.S. (1980) Physiological studies of β-galactosidase induction in *Kluyveromyces lactis* Journal of Bacteriology. **142**, 777-785.

Dive, D., Moreau, S. & Canan, M. (1978) Use of cilliate protozoan for fungal toxins studies. Bulletin of Environmental Contamination and Toxicology. 19, 489-495.

Dorner, J.W., Cole, R.J. & Diener, L. (1984) The relationship of *Aspergillus flavus* and *Aspergillus parasiticus* with reference to production of aflatoxins and cyclopiazonic acid. Mycopathologia, **87**, 13-15.

Dorner, J.W., Cole, R.J., Lomax, L.G., Gosser, H.S. & Diener, U.L. (1983) Cyclopiazonic acid production by *Aspergillus flavus* and its effect on broiler chickens. Applied and Environmental Microbiology. **46**, 698-703.

Draughon, F.A. & Churchville, D.C. (1985) Effects of pesticides on zearalenone production in culture and in corn plants. Phytopathology, **75**, 553-556.

Dunn, J.J., Lee, L.S. & Ciegler, A. (1982) Mutagenicity and toxicity of aflatoxin precursors. Environmental Mutagens. **4**, 19-26.

Eaton, D.L., Ramsdell, H.S. & Neal, G.E. (1994) Biotransformation of aflatoxins.. In The Toxicicology of Aflatoxins. Human Health, Veterinary and Agricultural Significance. Ed. Eaton, D.L. & Groopman, J.D. Academic Press. 45-72.

Eaton, D.L. & Ramsdell, H.S. (1992) Species and diet related differences in aflatoxin biotransformation. In. Handbook of Applied Mycology: Mycotoxins in Ecological Systems. Ed. Bhatnager, D., Lillehoj, E.B. & Arora, D.K. 157-182. Marcel Dekker.

Eaton, D.L., Monroe, D.H., Bellamy, G. & Kalman, D.A. (1988) Identification of a novel dihydroxy metabolite of aflatoxin B_1 produced *in vitro* and *in vivo* in rats and mice. Chemical Research and Toxicology. **1**, 108-114.

Edington, M.E., Hodkinson, J. & Seftel, H.C. (1972) Disease patterns in a South African rural Bantu population. South African Medical Journal. **46**, 968-976.

Ellison, R.A. & Kotsonis, F.N. (1974) *In vitro* metabolism of T-2 toxin. Applied Microbiology. **27**, 423-424.

Eppley, R.M. (1974) Sensitivity of brine shrimps (*Artemia salina*) to trichothecenes. Journal of the Association of Offical Analytical Chemists. **57**, 618-620.

Eppley, R.M. & Bailey, W.J. (1973) 12,13-epoxy-delta-trichothecenes as the probable mycotoxins responsible for stachybotryotoxicosis. Science. **181**, 758-760

Essigmann, J.M., Green, C.L., Croy, R.G., Fowler, K.W., Buchi, G. & Wogan, G.N. (1983) Interactions of aflatoxin B_1 and alkylating agents with DNA: structural and functional studies. Cold Spring Harbour Symposium. Quantitative Biology. **47**, 327-337.

Essigmann, J.M., Croy, R.G., Bennett, R.A. & Wogan, G.N. (1982) Metabolic activation of aflatoxin B_1: patterns of DNA adduct formation, removal and excretion in relation to carcinogenesis. Drug Metabolism Reviews. **13**, 581.

Essigmann, J.M., Croy, R.G., Nadzan, A.M., Busby, W.F., Reinhold, V.N., Buchi, G. & Wogan, G.N. (1977) Structural identification of the major DNA adduct formed by aflatoxin B_1 *in vitro*. Procedings of the National Academy of Sciences (USA). **74**, 1870-1874.

Fantes, P.A. & Roberts, C.F. (1973) β-Galactosidase activity and lactose utilization in *Aspergillus nidulans*. Journal of General Microbiology. **77**, 471-486.

Faraj, M.K., Smith, J.E. & Harran, G. (1991) Interaction of water activity and temperature on aflatoxin production by *Aspergillus flavus* and *A. parasiticus* in irradiated maize seeds. Food Additives and Contaminants. **8**, 731-736.

Feinberg, B. & Mclaughlin, C.S. (1989) Biochemical mechanism of action of trichothecene mycotoxins. In Trichothecene Mycotoxicosis: Pathophysiological Effects. Ed. Beasley, V.R. Vol. 1. 27-35.

Forsell, J.H. & Pestka, J.J. (1985) Relation of 8-ketotrichothecene and zearalenone analog structure to inhibitors of mitogen-induced human lymphocyte blastogenesis. Applied and Environmental Microbiology. **50**, 1304-1307.

Fried, H.M. & Warner, J.R. (1981) Cloning of yeast gene for trichodermin resistance and ribosomal protein L3. Procedings of the National Academy of Sciences. (USA). **78**, 238-242.

Fronnum, F., Sterri, S., Aas, P. & Johnsen, H. (1985) Carboxyesterases, importance for detoxification of organophosphorus anticholinesterases and trichothecenes. Fundamental and Applied Toxicology. **5**, 529-538.

Gatenbeck, S. & Sierankiewicz, J. (1973) On the biosynthesis of tenuazonic acid in *Alternaria tenuisima*. Acta Chemica Scandinavica. **27**, 1825-1827.

Gekas, V. & Lopez-Levia, M. (1985) Hydrolysis of lactose: A literature review. Process Biochemistry. **20**, 2-12.

Gelderblom, W.C.A & Snyman, S.D. (1991) Mutagenicity of potentially carcinogenic mycotoxins produced by *Fusarium moniliforme*. Mycotoxin Research. 7, 46-52.

Gelderblom, W.C.A., Kriek, N.P.J., Marasas, W.F.O. & Thiel, P.G. (1991) Toxicity and carcinogenicity of the *Fusarium moniliforme* metablolite, fumonisin B_1, in rats. Carcinogenesis. **12**, 1247-1251.

Gelderblom, W.C.A., Jaskiewickz, K., Marasas, W.F.O., Thiel, P.G., Horak, R.M., Vleggaar, R. & Kriek, N.P.J. (1988) Fumonisins-novel mycotoxins with cancer promoting activity produced by *Fusarium moniliforme*. Applied and Environmental Microbiology. **54**, 1806-1811.

Giambrone, J.J. (1978) Effect of tenuazonic acid on young chickens. Poultry Science. **57**, 1554-1558.

Gitterman, C.O. (1965) Antitumour, cytotoxic and antibacterial activities of tenuazonic aicd and congeneric teramic acids. Journal of Medical Chemistry. **8**, 483-486.

Glasby, J.S. (1979) Encyclopedia of Antibiotics. Wesley, Interscience, NewYork, N.Y.

Gonzalez, M.; Pena, C. & Casas, L.T. (1990) Partial purification of β-galactosidase from yeast by an aqeuous two-phase system method. Process Biochemstry. 157-161.

Grant. P.G., Schider, D. & Davies, J.E. (1976) Mapping of trichodermin resitance in *Saccharomyces cerevisiae*: a genetic locus for a component of the 60S ribosomal subunit. Genetics. **83**, 667-673.

Greenberg, N.A. & Mahoney, R.R. (1981) Immobilisation of lactose (β-galactosidase) for use in dairy processing: a review. Process Biochemistry. **16**, 2-8.

Groopman, J.D., Fowler, K.W., Busby, W.F. & Wogan, G.N. (1981) Interaction of aflatoxin B_2 with rat liver DNA and histones *in vivo*. Carcinogenesis. **2**, 1371-1373.

Guengerich, F.P., Brian, W.R., Sair, M.A. & Ross, J.T. (1991) Expression of mammalian liver cytochrome P450 enzymes using yeast-based vectors. Methods in Enzymology. **206**, 130-145.

Guengerich, F.P., Gillam, E.M.J., Ohmori, S., Sandu, P., Brian, W.R., Sair, M.A. & Iwasaki, M. (1993) Expression of human cytochrome P450 enzymes in yeast and bacteria and relevance to studies on catalytic specificity. Toxicon. **82**, 21-37.

Gupta, R.S. & Siminovitch, L. (1978) Genetic and Biochemical characterisation of mutants of CHO cells resitant to the protein synthesis inhibitor trichodermin. Somatic Cell Genetics. **4**, 355-374.

Gurtoo, H.L., Dahms, R.P. & Paigen, B. (1978) Metabolic activation of aflatoxins related to their mutagenicity. Biochemical and Biophysical Research Communications. **81**, 965-972.

Hall, A.J. & Wild, C.P. (1994) Epidemiology of aflatoxin-related disease. In The Toxicicology of Aflatoxins. Human Health, Veterinary and Agricultural Significance. Ed. Eaton, D.L. & Groopman, J.D. Academic Press. 233-258..

Haraguchi, H., Hashimoto, K., Shibata, K., Taniguchi, M. & Oi, S. (1987) Mechanism of antifungal action of citrinin. Agricultural and Biological Chemistry. **51**, 1373-1378.

Harrison, L.R., Colvin, B.M., Greene, J.T., Newman, L.E. & Cole, R.J. (1990) Pulmonary oedema and hydrothorax in swine produced by fumonisin B_1, a toxic metabolite of *Fusarium moniliforme*. Journal of Veterinary Diagnostic Investigation. **2**, 217-221.

Hartley, R.D., Nesbitt, B.F. & O'Kelly, J. (1963) Toxic metabolites of *A. flavus*. Nature. **198**, 1056-1058.

Harwig, J. & Scott, P.M. (1971) Brine shrimp (*Artemia salina*) larvae as a screening system for fungal toxins. Applied Microbiology. **21**, 1011-1016.

Hata, S., Nishino, T., Katsuki, H., Aoyama, Y. & Yoshida, Y. (1987) Characterisation of Δ^{22} desaturation in ergosterol biosynthesis of yeast. Agricultural and Biological Chemistry. **51**, 1359-1354.

Hata, S., Nishino, T., Katsuki, H., Aoyama, Y. & Yoshida, Y. (1983) Two species of cytochrome P-450 involved in ergosterol biosynthesis of yeast. Biochemical and Biophysical Research Communications. **116**, 162-166.

Hayes, A.W. & Wyatt, E.P. (1970) Survey of the sensitivity of microorganisms to rubratoxin B. Applied and Environmental Microbiology. **20**, 164-165.

Heller, K., Schultz, C., Loser, R. & Roschenthaler, R. (1975) The inhibition of bacterial growth by ochratoxin A. Canadian Journal of Microbiology. **21**, 972-979.

Hendricks, J.D., Sinnhuber, R.O., Nioxon, J.E., Wales, J.H., Marsi, M.S. & Hsieh, D.P.H. (1980) Carcinogenic response of rainbow trout (*Salmo gairdneri*) to aflatoxin Q_1 and synergistic effect of cyclopropenoid fatty acids. Journal of the National Cancer Instituite. **64**, 523-527.

Hendrickse, R.G. (1984) The influence of aflatoxins on child health in the tropics with particular reference to kwashikor. Transactions of the Royal Society of Tropical Medicine and Hygine. **78**, 427-435.

Hesseltine, C.W. (1976) Conditions leading to mycotoxin contamination of foods and feeds. In Mycotoxins and Other Fungal Related Problems. Ed. Rodericks, J.V. Advances in Chemistry Series American Chemical Society. 149.

Hewitt, G.M. & Grootwassink, J.W.D. (1984) Simultaneus production of inulase and lactase in batch and continuous cultures of *Kluyveromyces fragilis*. Enzyme and Microbial Technology. **6**, 263-270

Hobden, A.N. & Cundliffe, E. (1980) Ribosomal resistance to the 12,13-epoxytrichothecene antibiotics in the producing organism *Myrothecium verrucaria*. Biochemical Journal. **190**, 765-770.

Hockling, A.D. (1991) Common mycotoxigenic species of *Fusarium*. In Mycotoxin Prevention and Control. Eds. Semple, R.L., Frio, A.S., Hicks, P.A. & Lazare, J.V. 25-32.

Holt, P.S., Buckley, S., Norman, J.O. & DeLoach, J.R. (1988) Cytotoxic effects of T-2 mycotoxin on cells in culture as determined by a repid colorimetric bioassay. Toxicon. **26**, 549-558.

Holzapfel, C.W. (1980) Biosynthesis of cyclopiazonic acid and related tetramic acids. In The Biosynthesis of Mycotoxins. A study in Secondary Metabolism. Ed Steyn, P.S. 327-353.

Holzapfel, C.W., Steyn, P.S., & Purchase, I.F.H. (1966) Isolation and structure of aflatoxins M_1 and M_2. Tetrahedron Letters. **25**, 2799-2803

Hsieh, D.P.H. (1987) Mode of action of mycotoxins. In Mycotoxins In Foods. Ed Krough, P. Academic Press, London. 149-176

Hsieh, D.P.H., Cullen, J.M. & Ruebner, B.H. (1984) Comparative hepatocarcinogenicity of aflatoxins B_1 and M_1 in the rat. Food and Chemical Toxicology. **22**, 1027-1028.

Ikawa, M., Carr, C. & Tatsuno, T. (1985) Trichothecene structure and toxcity to the green alga *Chlorella pyrenoidosa*. Toxicon. **23**, 535-537.

Ishii, K., Maeda, D., Kamataki, T. & Kato, R. (1986) Mutagenic activation of aflatoxin B_1 by several forms of purified cytochrome P450. Mutation Research. **174**, 85-88.

Jayaraman, A., Herbst, E.J. & Ikawa, M. (1968) The bioassay of aflatoxins and related substances with *B. megaterium* spores and chick embryos. Journal of the American Oil Chemists Society. **45**, 700-702.

Jefcoate, C.R. (1979) Measurement of substrate and inhibitor binding to microsomal P450 by optical difference spectroscopy. Methods in Enzymology. **52**, 258-279.

Jewers, K. & John, A.E. (1990) *Alternaria* mycotoxins - possible contaminants of new sorghum varieties? Tropical Science. **30**, 397-409.

Jimenez, A. & Vazquez, D. (1978) Anisomycin and related antobiotics. In Antibiotics. Ed. Hahn, F.E. Springer-Verlag. Vol. 5. 1-19.

Jimenez, A., Sanchez, L. & Vazquez, D. (1975) Simultaneous ribosomal resitance to trichodermin and anisomycin in *Saccharomyces cerevisiae* mutants. Biochimica et Biophysica Acta. **383**, 427-438.

Kalb, V.F., Loper, J.C., Dey, C.R., Wood., C.W. & Sutter, T.R. (1986) Isolation of a cytochrome P450 structural gene from *Saccharomyces cerevisiae*. Gene. **45**, 237-245.

Kapelli, O. (1986) Cytochromes P-450 of Yeasts. Microbiological Reviews. **50**, 244-258.

Kappeli, O., Sauer, M. & Fiechter, A. (1982) Convenient procedure for the isolation of highly enriched cytochrome P450-containing microsomal fractions from *Candida tropicalis*. Analytical Biochemistry. **126**, 176-182.

Kärenlampi, S., Marin, E. & Hänninen. (1980) Occurence of cytochrome-P450 in yeasts. Journal of General Microbiology. **120**, 529-533.

Kellerman, T.W., Marasas, W.F.O., Thiel, P.G., Gelderblom, W.C.A., Cawood, M. & Coetzer, J.A.W. (1990) Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B_1. Onderstepoort Journal of Veterinary Research. **57**, 269-275

Kelly, S.L., Lamb, D.C., Baldwin, B.C. & Kelly, D.E. (1993) Benzo(a)pyrene hydroxylase activity in yeast is mediated by P450 other than sterol 14α-demthylase. Biochemical and Biophysical Research Communication. **197**, 428-432.

Kelly, S.L. & Kelly, D.E. (1988) Analysis and exploitation of cytochrome P450 in yeats. Biochemical Society Transactions. **16**, 1086-1088.

Kelly, S.L., Kelly, D.E., King, D.J. & Wiseman, A. (1985) Interaction between yeast cyt. P450 and chemical carcinogens. Carcinogenesis. **6**, 1321-1325.

Kelly, D.E. & Parry, J.M. (1983) Metabolic activation of cytochrome P450/P448 in the yeast *Saccharomyces cerevisiae*. Mutation Research. **108**, 147-159.

Khachatourians, G.G., Schappert, K.T., & Koshinsky, H.A. (1989) A bioassay based on yeast cytotoxicity for the detection of trichothecene mycotoxins of agricultural importance. In Principles of Health and Safety in Agriculture. Eds. Dosman, J.A. & Cockcroft, D.W. CRC Press. 223.

Kiessling, K., Pettersson, H., Sandholm, K. & Olsen, M. (1984) Metabolism of aflatoxin, zearalenone, and three trichothecenes by intact rumen fluid, rumen proazoa and rumen bacteria. Applied and Environmental Microbiology. **47**, 1070-1073

King, D.J. & Wiseman, A. (1987) Yeast cytochrome P448 enzymes and the activation of mutagens including carcinogens. In Enzyme Induction, Mutagen Activation and Carcinogen Testing in Yeasts. Ed. Wiseman, A. Ellis Horwood, Chichester. 115-167.

King, D.J., Wiseman, A., Kelly, D.E. & Kelly, S.L. (1985) Differences in the cytochrome P450 enzymes of sterol C14 demethylase mutants of *Saccharomyces cerevisiae,* Current Genetics. **10**, 261-267.

King, R.R., McQueen, R.E., Levesque, D. & Greenhalgh, R. (1984a) Transformation of deoxynivalenol (vomitoxin) by rumen microorganisms. Journal of Agricultural and Food Chemistry. **32**, 1181-1183.

King, D.J., Azari, M.R., Wiseman, A. (1984b) Studies on the properties of highly pruified cytochrome P-448 and its dependent activity benzo(a)pyrene hydroxylase from *S. cerevisiae.* Xenobiotica. **14**, 187-206.

King, D.J., Wiseman, A., & Wilkie, D.(1983a) Studies on the genetic regulation of cytochrome P450 production in *Saccharomyces cerevisiae.* Molecular and General Genetics. **192**, 466-470.

King, D.J., Blatiak, A. & Wiseman, A. (1983b) Cytochrome P450 production and benzo(*a*)pyrene hydroxylase activity and inducibility in *Saccharomyces cerevisiae* NCYC 240 and its morphological variants NCYC 753 and NCYC 754. Biochemical Society Transactions. **11**, 710.

King, D.J., Azari, M.R. & Wisesman, A. (1982) The induction of cytochrome P448 dependent benzo(*a*)pyrene hydroxylase in *Saccharomyces cerevisiae.* Biochemical and Biophysical Research Communications. **105**, 1115-1121.

Kitabatake, N., Dot, E. & Trivedi, A.B. (1993) Toxicity evaluation of the mycotoxins, citrinin and ochratoxin A. using several animal cell lines. Biochemistry and Biophysiology. **105C**, 429-433.

Klich, M.A. & Pitt, J.I. (1988) Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. Trans. Be. Mycol. Soc. **91**, 99-108.

Kobayashi, J., Horikoshi, T., Ryu, J.C., Tashiro, F., Ishii. & Ueno, Y. (1987) The cytochrome P-450 dependent hydroxylation of T-2 toxin in various animal species. Food and Chemical Toxicology. **25**, 539-544.

Kopp, B. (1979) Biological assay for the quantitative determination of roqufortine. Z. fur Lebebsmittel-untersuchung und-forschung. **169**, 90-91.

Koshinsky, H.A. & Khachatourians, G.G. (1992) Bioassay for deoxynivalenol based on the interaction of T-2 toxin with trichothecene mycotoxins. Bulletin of Environmental Contamination and Toxicology. **49**, 246-251.

Krishnamachari, K.A.V.R., Bhat, R.V., Nagarajan, V. & Tilak, T.B.G. (1975) Hepatitis due to aflatoxicosis: An outbreak in Western India. Lancet (May). 1061-1063.

Krough, P. (1977) Ochratoxins. In Mycotoxins in Human and Animal Health. Ed Rodnicks, C.W., Hesseltine, C.W. & Mehlman, M.A. Pathotox. 489-498.

Krough, P. (1979) Ochratoxins: occurence, biological effects and and causal role in dieases. In Proceedings of the Sixth International Symposium on Animal, Plant and Microbial Toxins. Eds Eaker, D & Wadstrom, T. Pergamon Press. 673-680.

Krough, P. (1974) Mycotoxin nephropathy. In Mycotoxins. Ed Purchase, I.F.H. Elsevier.

Kuczuk, M.H., Benson, P.M., Heath, H. & Hayes, A.W. (1978) Evaluation of the mutagenic potential of mycotoxins using *Salmonella typhimurium* and *Saccharomyces cerevisiae.* Mutation Research. **53**, 11-20.

Kurata, H. (1990) Mycotoxins and mycotoxicoses: overview. In. Microbial Toxins in Fodds and Feeds. Ed. Pohland, A.E. Plenum Press, New York. 249-259

Lederberg, J. (1950) The β-galactosidase from *Escherichia coli*, strain K-12. Journal of Bacteriology. **60**, 381-392.

Lee Y.J. & Hagler, W.M. (1991) Aflatoxin and cyclopiazonic acid production by *Aspergillus flavus* isolated from contaminated maize. Journal of Food Science. **56**, 871-872.

Lee, K.S. & Röschenthaler, R. (1986) DNA damaging activity of patulin in *E. coli*. Applied and Environmental Microbiology. **52**, 1046-1054.

Lee, T.C. & Snyder, P. (1973) Phospholipid metabolism in rat liver endoplasmic reticulum. Structural analysis, turnover studies and enzymatic acitivity. Biochimica et Biophysica Acta. **291**, 71-82.

Lenz, P. Sussmuth, R. & Seibel, E. (1989) A test battery of bacterial toxicity assays and comparison with LD_{50} values. Toxicity Assessment: An International Journal. **4**, 43-52.

Lillehoj, E.B. & Ciegler, A. (1968) Aflatoxin B_1 binding and toxic effects of *Bacillus megaterium*. Journal of General Microbiology. **54**, 185-194.

Lindenmayer, A. & Smith, L. (1964) Cytochromes and other pigments of bakers yeast grown aerobically and anaerobically. Biochimica and Biophysica Acta. **93**, 445-561.

Logrieco, A., Bottalico, A., Visconti, A. & Vurro, M. (1988) Natural occurence of *Alternaria*-mycotoxins in some plant products. Microbiologie-Aliments-Nutrition. **6**, 13-17.

Lomax, L.G., Cole, R.J. & Dorner, J.W. (1984) The toxicity of cyclopiazonic acid in weaned pigs. Veterinary Pathology. **21**, 418-424.

Loveland, P.M., Wilcox, J.S., Pawlowski, N.E., Bailey, G.S. (1987) Metabolism and DNA binding of aflatoxicol and aflatoxin B_1 *in vivo* and in isolated hepatocytes from rainbow trout (*Salmo gairdneri*). Carcinogenesis. **8**, 1965-1070.

Luo, X. (1988) Food poisoning associated with *Fusarium* toxins. Proceedings of the 7th International Symposium on Mycotoxins and Phycotoxins.

Luo, Y., Yoshizawa, T. & Katayama, T. (1990) Comparative study on the natural occurence of *Fusarium* mycotoxins (trichothecenes and zearalenone) in corn and wheat from high- and low-risk areas for human esophagal cancer in China. Environmental Microbiology. **56**, 3723-

MacPherson, J.M. & Khachatourians, G.G. (1991) Partial purifiacation and charaterisation of β-galactosidase produced by *Beauveria bassiana*. Biotechnology and Applied Biochemistry. **13**, 217-230.

Madhyastha, M.S., Maraquardt, R.R. & Abramson, D. (1994a) Structure-activity relationships and interactions among trichothecene mycotoxins as assessed by a yeast bioassay. Toxicon. **32**, 1147-1152.

Madhyastha, M.S., Marquardt, R.R., Frohlich, A.A. & Borsa, J. (1994b) Optimisation of yeast bioassay for trichothecene mycotoxins. Journal of Food Protection. **57**, 490-495.

Mahoney, R.R. & Whitaker, J.R. (1978) Purification and physiochemical properties of β-galactosidase from *Kluyveromyces fragilis*. Journal of Food Science. **43**, 584-591.

Mahoney, R.R. & Whitaker, J.R. (1977) Stability and enzymatic properties of β-galactosidase from *Kluyvermyces fragilis*. Journal of Food Biochemistry. **1**, 327-350.

Mahoney, R.R., Nickerson, T.A. & Whitaker, J.R. (1974) Strain selection, growth conditions and extraction proceedures for optimum production of lactose form *Kluyveromyces fragilis*. Journal of Dairy Science. **58**, 1620-1629.

Marasas, W.F.O., Kellerman, T.W., Gelderblom, W.C.A., Coetzer, J.A.W., Thiel, P.G. & Van der Lugt, J.J. (1988) Leuoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme*. Onderstepoort Journal of Veterinary Research. **55**, 197-203.

Marasas, W.F.O., Nelson, P.E. & Tousson, T.A. (1984) Toxigenic *Fusarium* species. Identity and Mycotoxicology. University Park Pennsylvania, Pensylvania State University Press.

Marasas, W.F.O., Kriek, N.P.J., Wiggins, V.M., Steym, P.S., Towers, D.K. & Hastie, T.J. (1979) Incidence, geographic distribution, and toxigenicity of *Fusarium* species in South African corn. Phytopathology. **69**, 1081-1085.

Marchesi, S.L. Steers, E. & Shifrin, S. (1969) Purification and characterisation of the multiple forms of β-galactosidase of *Escherichia coli*. Biochimica et Biophysica Acta. **181**, 20-34.

Maron, D.M. & Ames, B.N. (1983) Revised method for the *Salmonella* mutagenicity test. Mutation Research. 113, 173-215.

Massey, T.E., Stewart, R.K., Daniels, J.M. & Liu, L. (1995) Biochemical and molecular aspects of mammalian susceptibility to aflatoxin B_1 carcinogenicity. Procedings of the Society of Experimental Biology and Medicine. **208**, 213-227.

McLaughlin, C.S., Vaughn, M.H., Campbell, I.M., Wei, C.M., Stafford, M.E. & Hanson, B.S. (1977) Inhibition of protein synthesis by trichothecenes. In Mycotoxins in Human and Animal Health. Ed. Rodricks, J.V., Hesseltine, C.W. & Mehlam, M.A. Pathotox Publishers, 263-273

Meisner, H., Cimbala, M.A. & Hanson, R.W. (1983) Decrease in renal phosphenolpyruvate caboxykinase RNA and poly (A^+) RNA level by ochratoxin A. Arch. Biochem. Biophys. **223**, 264-

Miller, J.H. (1972) Experiments in molecular genetics. Cold Spring Harbour Laboratory, Cold Spring Harbour, N.Y. 352-359.

Miller, F.A., Rightsel, W.A., Sloan, B.J., Ehrlich, J., French, J.C., Bartz, G.R. & Dickson, G.J. (1963) Antiviral activity of tenuazonic acid. Nature. **200**, 1338-1339.

Miller, F.A., Rightsel, W.A., Sloan, B.J., Ehrlich, J., French, J.C., Bartz, Q.R. & Dixon, G,J. (1963) Antiviral acitivity of tenuazonic acid. Nature. **200**, 1338-1339.

Mirocha, C.J., Pathre, S.V. & Christensen, C.M. (1979) Mycotoxins. In Secondary Products of Metabolism. Econimic Biology, Vol 3. Ed. Rose, A.H. Academic Press. 468-522.

Mirocha, C.J. & Pathre, S. (1973) Identification of the toxic principle in a sample of poefusarin. Applied Microbiology. **26**, 719-724.

Mirocha, C.J., Christensen, C.M. & Nelson, G.H. (1971) F-2 (zearalenone) oestrogenic mycotoxin from *Fusarium*. In Microbial Toxins: Algal and Fungal Toxins. Ed. Kadis, S., Ciegler, A. & Ajl, S.J. Academic Press. **7**,

Mizuno, S. (1975) Mechanism of inhibition of protein synthesis initiation by diacetoxyscirpenol and fusarenon X in the reticulocyte system. Biochimica et Biophysica Acta. **383**, 207-214.

Moroi, K., Suzuki, S., Kuga, T., Yamazaki, M. & Kanisawa, M. (1985) Reduction of ochratoxin A toxicity in mice treated with phenylalanine and phenobarbitol. Toxicology Letters. **25**, 1-8.

Moss, M.O. (1991) The environmental factors controlling mycotoxin formation. In Mycotoxins and Animal Foods. Ed. Smith, J.E. CRC Press. 38-56.

Muller, V.T. (1987) Application of skin test to guinea pig to detect T-2 toxin and diacetoxysirpenol in roughage substances. Manatshefte fur veterinarmedizin. **42**, 217-219.

Narhi, L.O. & Fulco, A.J. (1987) Identification and characterisation of two functional domains in cytochrome $P450_{BM-3}$, a catalytically self-sufficient monooxygenase induced by barbituates in *Bacillus megaterium*. Journal of Biological Chemistry. **262**, 6683-6690.

Nawaz, S., Coker, R.D. & Haswell, S.J. (1995) HPTLC - A valuable chromatographic tool for the analysis of aflatoxins. Journal of Planar Chromatography. **8**, 4-9.

Neal, G.E., Hielsch, U., Judah, D.J. & Hulbert, P.B. (1987) Conjugation of model substrates or microsomally-activated aflatoxin B with reduced glutathione, catalused by cytosolic glutathione-S-transferase in livers of rats, mice, and guinea pigs. Biochemical Pharmocology. **36**, 4269-4276.

Neal, G.E. & Green, J.A. (1983) The requirement for glutathione S-transfrease in the conjugation of activated aflatoxin B_1 during aflatoxin hepatocarcinogenesis in the rat. Chemico-Biological Interactions. **45**, 259-275.

Neal, G.E., Judah, D.J., Stripe, F. & Patterson, D.S.P. (1981) The formation of 2,3-dihydroxy-2,3-dihydro-aflatoxin B_1 by the metabolism of aflatoxin B_1 by liver microsomes isolated from certain avian and mammalian species and possible role of this metabolite in the acute toxicity of aflatoxin B_1. Toxicology and Applied Pharmacology. **58**, 431-437.

Neal, G.E. & Colley, P.J. (1979) The formation of 2,3-dihydro, 2,3-dihydroxy aflatoxin B_1 by the metabolism of aflatoxin B_1 *in vitro* by rat liver microsomes. FEBS Letters. **101**, 382-386.

Nelson, D.B., Kimbrough, R., Landrigan, P.S., Hayes, A.W., Yang, G.C., Benandies, J. (1980) Aflatoxin and Reye's syndrome: a case control study. Pediatrics. **66**, 865-869.

Nesbitt, B.F., O'Kelly, J., Sargeant, K. & Sheridan, A. (1962) Toxic metabolites of *Aspergillus flavus*. Nature **195**, 1062-1063.

Nesheim, S. & Trucksess, M.W. (1986) Thin-layer chromatography/high performance thin-layer chromatography as a tool for mycotoxin determination in modern methods. In The analysis and Structural Elucidation of Mycotoxins. Ed Cole, R.J. Academic Press. 239-264.

Neuhring, L.P., Rowland, G.N., Harrison, L.R., Cole, R.J. & Dorner, J.W. (1985) Cyclopiazonic acid mycotoxicoses in the dog, American Jouranal of Veterinary Research. **46**, 1670-1676.

Newberne, P.M. (1987) Interaction of nutrients and other factors with mycotoxins. In Mycotoxins In Foods. ED Krough, P. Academic Press, London. 188-190.

Niggli, B., Friederich, D., Hann, D. & Würgler, F.E. (1986) Endogenous promutagen activation in the yeast *Saccharomyces cerevisiae*: factors influencing aflatoxin B_1 mutagenicity. Mutation Research. **175**, 223-229.

Nishie, K., Cole, R.J. & Dorner, J.W. (1989a) Inhibitory effects of certain trichothecenes, cyclopiazonic acid and citreoviridin on *Tetrahymena pyriformis*. *In Vitro* Toxicology. **2**, 239-247.

Nishie, K., Cutler, H.G. & Cole, R.J. (1989b) Toxicity of trichothecenes, moniliformin, zearalenone/ol, griseofulvin, patulin, PR toxin and rubratoxin B on protozoan *Tetrahymena pyriformis*. Research Communications in Chemical Pathology and Pharmacology. **65**, 197-210.

Nishie, K., Cole, R.J. & Dorner, J.W. (1985) Toxicology and pharmacology of cyclopiazonic acid. Food and Chemical Toxicology. **23**, 831-839.

Norred, W.P., Porter, J.K., Dorner, J.W. & Cole, R.J. (1988) Occurence of the mycotoxin cyclopiazonic acid in meat after oral administration to chickens. Journal of Agricultural and Food Chemistry. **36**, 113-116.

Northolt, M.D., van Egmond, H.P. & Paulsch, W.E. (1977) Differences between *Aspergillus flavus* strains in growth and aflatoxin production in relation to water activity and temperature. Journal of Food Protection. **40**, 778-781.

Northolt, M.D., van Egmond, H.P. & Paulsch, W.E. (1978) Patulin production by some fungal species in relation to water activity and temperature. Journal of Food Protection. **41**, 885-890.

Northolt, M.D., van Egmond, H.P. & Paulsch, W.E. (1979a) Penicillic acid production by some fungal species in relation to water activity and temperature. Journal of Food Protection. **42**, 476-484.

Northolt, M.D., van Egmond, H.P. & Paulsch, W.E. (1979b) Ochratoxin A production by some fungal species in relation to water activity and temperature. Journal of Food Protection. **42**, 885-890.

Novotný, C & Sikyto, B. (1987) Inducible synthesis of β-galactosidase in *Kluyveromyces fragilis*. Folia Microbiologia. **32**, 6-12.

Ohta, M., Ishii, K. & Ueno, Y. (1977) Metabolism of trichothecene mycotoxins. I. Microsomal deacylation of T-2 toxin in animal tissues. Journal of Biochemistry. **82**, 1591-1598.

Ohta, M., Matsumoto, H., Ishii, K. & Ueno, Y. (1978) Metabolism of trichothecene mycotoxins. II. Substrate specificity of microsomal deacylation of trichothecene. Journal of Biochemistry. **84**, 697-706

Olivigni, F.J. & Bullerman, L.B. (1978) A microbiological assay for penicillic acid. Journal of Food Protection. **41**, 432-434.

Omura, T. & Sato, R. (1964) The carbon monoide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. Journal of Biological Chemistry. **239**, 2370-2378.

Panigrahi, S. (1993) Bioassay of mycotoxins using terrestrial and aquatic, aniaml and plant species. Food and Chemical Toxicology. **31**, 767-790.

Pearson, B.; Wolf, P.L. & Vazquez, J. (1963) A comparative study of a series of new indolyl compounds to localise β-galactosidase in tissues. Laboratory Investigation. **12**, 1249-1259.

Pier, A.C., Belden, E.L., Ellis, J.A., Nelson, E.W. & Maki, L.R. (1981) Effects of cyclopiazonic acid and aflatoxin singly and in combination on selected clinical, pathological and immunological responses of guinea pigs. Mycopathologia, **105**, 135-142.

Pier, A.C., Richard, J.L. & Cysewski, S.J. (1980) The implcations of mycotoxins in animal disease. Journal of the American Veterinary Medical Association. **176**, 719-724.

Pitt, J.I. (1991) Toxigenic *Aspergillus* and *Penicillium* species. In Mycotoxin Prevention and Control. Eds. Semple, R.L., Frio, A.S., Hicks, P.A. & Lazare, J.V. 33-43.

Pitt, J.I. & Leistner, L. (1991) Toxigenic *Penicillum* species. In Mycotoxins and Animal Foods. Ed. Smith, J.E. CRC Press. 82-94.

Pohland, A.E., Cushmac, M.E. & Andrellos, P.J. (1968) Aflatoxin B_1 hemiacetyl. Journal of the Association of Official Analytical Chemists. **51**, 907-910.

Porcer, J.M., Lafarge-Frayssinet, C., Frayssinet, C., Nurie, A., Melcion, D. & Richard-Molard, D. (1987) Determination of cytotoxicity of trichothecenes in corn by cell culture assay. Journal of the Association of Offical Analytical Chemists. **70**, 844-849.

Prelusky, D.B., Scott, P.M., Trenholm, H.L. & Lawrence, A. (1990) Minimal transmission of zearalenone to milk of dairy cows. Journal of Environmental Science and Health. **25**, 87-

Prior, M.G. (1979) Evaluation of brine shrimp (*Artemia salina*) larvae as a bioassay for mycotoxins in animal feedstuffs. Canadian Journal of Comparative Medicine. **43**, 352-355.

Purchase, I.F.H. (1971) The acute toxicity of the mycotoxin cyclopiazonic acid to rats. Toxicology and Applied Pharmocology. **18**, 114-123.

Purchase, I.F.H. (1967) Acute toxicity of aflatoxins M_1 and M_2 in one-day old ducklings. Food and Cosmetic Toxicology. **5**, 339-342.

Quillardet, P. & Hofnung, M. (1985) The SOS chromotest, a colorimetric bacterial assay for genotoxin procedures. Mutation Research. **147**, 65-78.

Rabie, C.J., van Rensborg, S.J., van der Watt, J.J. & Lübben, A. (1975) Onyalai- the possible involvement of a mycotoxin produced by *phoma sorghina* in the aetiology. South African Medical Journal. **49**, 1647-1650.

Ramsdell, H.S. & Eaton, D.L. (1990a) Mouse liver glutathione-S-transferase isoenzyme activity towards aflatoxin B_1-8,9-epoxide and benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide. Toxicology and Applied Pharmaocology. **105**, 215-225.

Ramsdell, H.S. & Eaton, D.L. (1990b) Species susceptibility fo aflatoxin B_1 carcinogenesis: Comparative knietics of microsomal biotansformation. Cancer Research. **50**, 615-620.

Raney, K.D., Shimada, T., Kim, D.H., Groopman, J.D., Harris, T.M. & Guengerich, F.P. (1992) Oxidation of aflatoxin and sterigmatocystin by human liver microsomes: Significance of aflatoxin Q_1 as a detoxication product of aflatoxin B_1. Chemical Research and Toxicology. **5**, 202-210.

Rao, L.B. & Hussain, A. (1985) Presence of cyclopiazonic acid in Kodo Millet (*Paspalum scrobicultatum*) causing "Kodo poisoning" in man and its production by associated fungi. Mycopathologia, **89**, 177-180.

Ravindranath, B. (1989) Planar chromatography. In Principles and Practice of Chromatography. Ellis Horwood Ltd. 364-400.

Reiss, J. (1986) Detection of genotoxic properties of mycotoxins with the SOS chromotest. Naturwissenschaften. **73**, 677-678.

Reiss, J. (1975a) *Bacillus subtilis*: a sensitive bioassay for patulin. Bulletin of Environmental Contamination and Toxicology. **13**, 689-691.

Reiss, J. (1975b) Mycotoxin bioassay using *B. stearothermophilus*. Journal of the Association of Offical Analytical Chemists. **58**, 624-625.

Reiss, J. (1975c) Insecticidal and larvicidal activities of the mycotoxins aflatoxin B_1, rubratoxin B, patulin and diacetoxyscirpenol towards *Drosophila melanogaster*. Chemico-Biological Interactions. **10**, 339-342

Renaud, J.P., Peyronneau, M.A., Urban, P., Truan, G., Cullin, C., Pompon, D., Beaune, P. & Mansuy, D. (1993) Recombinant yeast in drug metabolism. Toxicology. **82**, 39-52.

Reubel, G.H., Fareis, M. & Amselgruber, W.M. (1987) Cytotoxic evaluation of mycotoxins by an MTT-bioassay. Mycotoxin Research. **3**, 85-86.

Risenfield, I., Kirsch, I. & Weissman, S.H. (1985) Detection and quantitation of aflatoxin B_1 in orange juice. Food Additive and Contaminants. **2**, 253-257.

Robbana-Barnat, S., LaFarge-Frayssinet, C. & Frayssinet, C. (1989) Use of cell cultures for predicting the biological effects of mycotoxins. Cell Biology and Toxicology. **5**, 217-226.

Roebuck, B.D., Siegel, W.G. & Wogan, G.N. (1978) *In vitro* metabolism of aflatoxin B_2 by animal and human liver. Cancer Research. **38**, 999-1002.

Rohlfing, S.R. & Crawford, I.P. (1966) Purification and characterisation of β-galactosidase from *Aeromonas formicans*. Journal of Bacteriology. **91**, 1085-1097.

Röschenthaler, R, Creppy, E.E., Driesmanm, D. & Dirheimer, G. (1984) Ochratoxin A: on mechanism of action. In Toxigenic Fungi: Their Toxins and Health Hazards. Ed. Kurata, H. & Ueno, Y. Elsevier, Amsterdam. 225-

Ross, R.K., Yuan, J.M., Yu, M.C., Wogan, G.N., Quai, G.S., Tu, J.T., Groopaman, J.D., Gao, Y.T. & Henderson, B.E. (1992) Urinary aflatoxin biomarkers and risk of hepatocellualr carcinoma. Lancet. **339**, 943-946.

Ross, P.F., Rice, L.G., Plattner, R.D., Osweiler, G.D., Wilson, T.M., Owens, D.L., Nelson, H.A. & Richard, J.L. (1991) Concentrations of fumonisins in feeds associated with animal health problems. Mycopathologia. **114**, 129-135.

Ross, P.F., Nelson, P.E., Richard, J.L., Osweiler, G.D., Rice, L.G., Plattner, R.D. & Wilson, T.M. (1990) Production of fumonisins by *Fusarium moniliforme* and *F. proliferatum* isolates associated with equine leukoencephalomalacia and a pulmonary edema in swine. Applied and Environmental Microbiology. **56**, 3325-3326.

Rotman B. (1961) Measurement of activity of single molecules of β-D-galactosidase. Procedings of the National Acadamy of Sciences (USA) **47**, 1981-1985.

Saito, M. & Tatsuno, T. (1971) Toxins of *Fusarium nivale*. In Microbial Toxins. Ed Kadis, S., Ciegler, S.J. & Ajl, S.J. Academic Press. **7**,

Salhab, A.S. & Edwards, G.S. (1977) Comparative *in vitro* metabolism of aflatoxicol by liver preparations from animals and humans. Cancer Research. **37**, 1016-1021.

Salhunke D.K., Wu, D.T., Do, J.Y. & Maas, M.R. (1980) Mycotoxins in foods and feeds. In Safety of Foods. Ed. Graham, H.D. AVI. 198-264.

Sanders, M.F. (1984) A metabolic inhibition test for mycotoxins. 5th Meeting on Mycotoxins in Animal and Human Health. Ed. Moss, M.O. & Frank, M. 106-116.

Sanglard, D., Kappeli, O. & Fiechter, A. (1986) The distinction of different types of cytochrome P450 from the yeast *C. tropicalis* and *S. uvarum*. Archives of Biochemistry and Biophysics. **251**, 276-286.

Sargeant, K., Sheridan, A., O'Kelly, J & Carnaghan, R.B.A. (1961) Toxicty associated with certain samples of groundnuts. Nature. **192**, 1096-1097.

Sato, R. & Omura, T (1978) Cytochrome P450. Kodansha-Academic Press, Tokyo.

Schappert, K.T., Koshinsky, H.A. & Khachatourians, G.G. (1986) Growth inhibition of yeast by T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, diacetoxyscirpenol, verrucarol, verrucarin A and roridin A mycotoxins. Journal of the American College of Toxicology. **5**, 181-187.

Schappert K.T. & Khachatourians, G.G. (1984a) Influence of membrane on T-2 toxin toxicity in *Saccharomyces* spp. Applied and Environmental Microbiology. **47**, 681-684.

Schappert K.T. & Khachatourians, G.G. (1984b) A yeast bioassay for T-2 toxin. Journal of Microbiological Methods. **3**, 43-46.

Schappert K.T. & Khachatourians, G.G. (1983) Effects of fusariotoxin T-2 on *S. cerevisiae* and *S. carlsbergenesis*. Applied and Environmental Microbiology. **45**, 862-867.

Schenkman, J.B. (1991) Cytochrome P450-dependant monooxygenase: an overview. In Molecular Aspects of Monooxygenases and Bioactivation of Toxic Compound. Eds. Arinç, E., Schenkman, J.B. & Hodgeson, E. Series A Life Sciences Vol 2. 1-10.

Schenkman, J.B., Remmer, H. & Estabrook, R.W. (1967) Spectral studies of drug interaction with hepatic microsomal cytochrome. Molecular Pharmacology. **3**, 113-123.

Schindler, D. (1974) Two classes of inhibiors of peptidyl transferase activity in eukaryotes. Nature. **249**, 38-41

Schindler, D., Grant, P. & Davis, J. (1974) Trichodermin resitance-mutation affecting eukaryotic ribosomes. Nature. **248**, 535-536.

Schlatter, C. (1990) Past and future in mycotoxin toxicology research. Jouranl of Experimental Pathology and Toxicology. **10**, 138-144.

Schlosberg, A.S., Klinger, Y. & Malkinson, M.H. (1986) Muscovy ducklings, a partiularly sensitive avian bioassay for T-2 toxin and diacetoxyscirpenol. Avian Diseases. **30**, 820-824.

Schmutz, H.R. (1980) In situ evaluation of high performance thin-layer chromatography. In Proceeding of the International Symposium of Instrumental HPTLC. Ed Bersch, W., Hara, S., Kaiser, R.E. & Zlatkis, A. 315-345.

Schoenhard, G.L., Hendricks, J.D., Nixon, J.E., Lee, D.J., Wales, J.H., Sinnhuber, R.O. & Pawlowski, N.E. (1981) Aflatoxicol induced heparocellular carcinoma in in the rainbow trout (*Salmo gairdneri*) and the synergistic effects of cyclopropeneoid fatty acids. Cancer Research. **41**, 1011-1014.

Scott, P.M. (1991) Methods of analysis for mycotoxins- an overview. In Analysis of Oilseeds, Fats and Fatty Foods. Ed Rosell & Pritchard. Elseveir, Amsterdam.

Scott, P.M. (1989) The natural occurence of trichothecenes. In Trichothecene Mycotoxicosis: Pathophysiologic Effects. Ed. Beasley, V.R. Vol I. CRC Press. 1-36.

Scott, P.M., Harwig, J. & Blanchfield, B.J. (1980) Screening *Fusarium* strains isolated from overwintered Canadian grains for trichothecenes. Mycopathologia. **72**, 175-180.

Shiguera, H.T. & Gordon, C.N. (1963) The biological acitivity of tenuazonic aicd. Biochemistry. **2**, 1132-1137.

Shukla, T.P. (1975) β-Galactosidase technology: a solution to the lactose problem. CRC Critical Reviews in Food Technology. **1**, 325-356.

Simla, A.P., Crichton, M.B., Black, S.M., Pemble, S., Bligh, H.F.J., Beggs, J.D. & Wolf, C.R. (1993) Heterologous expression of drug-metabolising enzymes in cellular and whole animal bodies. Toxicology. **82**, 3-20.

Sinnhuber, R.O., Lee, D.J., Wales, J.H. & Landers, M.K. (1974) Hepatic carcinogenesis of aflatoxin B_1 in the rainbow trout (*salmo gairdneri*) and its enhancement by cyclopropeneoid fatty acids. Journal of the National Cancer Instituite. **53**, 1285-1288.

Sinskey, A.J. & Gomez, R.F. (1980 Use of prokaryotic and eukaryotic culture systems for examining biological activity of food constituents. In Food and Health: Science and Technology (Birch, G.G. & Parker, K.J. Eds.) Applied Science Publishers. 251-286.

Slater, T.F., Sawyer, B. & Strauli, U. (1963) Strudies on succinate-tetrazolium reductase systems. III. Points of coupling of four different tetrazolium salts. Biochimica et Biophysica Acta. 77, 383-393.

Smith, J.E. & Moss, M.O. (1985) Mycotoxins. Formation, analysis and significance. John Wiley & Sons.

Smith, K.E., Cannon, M. & Cundliffe, E. (1975) Inhibition at the initiation level of eukaryotic protein synthesis by T-2 toxin. FEBS Letters. **50**, 8-12.

Sorenson, W.G., Tucker, J.D. & Simpson, J.P. (1984) Mutageinicity of the teramic mycotoxin cyclopiazonic acid. Applied and Environmetnal Microbiology. **47**, 1355-1357.

Spiro, R.C. (1970) Glycoproteins. Annual Review of Biochemistry. **39**, 599-638.

Stafford, M.E. & McLaughlin, C.S. (1973) Trichodermin, a possible inhibtor of the termination process of protein synthesis. Jouranl of Cell Physiology. **82**, 121-

Steyn, P.S. (1984) Ochratoxins and related dihydroisocoumarins. In Mycotoxins: Production, Isolation, Separation and Purification. Ed. Betina, V. Elsevier, Amsterdam. 183-216.

Steyn, P.S. (1980) The Biosynthesis of mycotoxins, A sudy in secondary metabolism. Academic Press.

Steyn, P.S. & Rabie, C.J. (1976) Characterisation of magnesium and calcium tenuazonate from *Phoma sorghina*. Phytochemistry, **15**, 1877-1979.

Stolloff, L. (1972) Analytical methods for mycotoxins. Clinical Toxicology. **5**, 465-494.

Stoloff, L., Verett, M.J., Dantzman, J. & Reynolda, E.F. (1972) Toxicological study of aflatoxin P_1 using fertile chicken egg. Toxicology and Applied Pharmacology. **23**, 528-531

Stone, P.E., Rudbidge, T.W. & MacDonald, K.D. (1986) *Rhodotorula rubra* bioassay for T-2 toxin: increased sensitivity and wider application. Jouranl of Microbiological Methods. **5**, 59-63.

Storm, D.R.; Rosenthal, K.S. & Swanson, P.E. (1977) Polymyxin and related peptide antibiotics. Annul Review of Biochemistry. **46**, 723-763.

Stott, W.T. & Bullerman, L.B. (1975a) Microbiological assay of patulin using *Bacillus megaterium*. Journal of the Association of Offical Analytical Chemists.**58**, 497-499.

Stott, W.T.& Bullerman, L.B. (1975b) Patulin, a mycotoxin of potential concern in foods. Journal of Milk Food Technology. **38**, 695-705.

Stryer, L. (1981) Biochemistry. Second Edition. Freemann and Compnay, New York.

Sukroongreung, S., Schappert, K.T. & Khachoatourians, G.G. (1984) Survey of the sensitivity of twelve yeast genera toward T-2 toxin. Applied and Environmental Microbiology. **48**, 416-419.

Swanson, S.P. & Corley, R.A. (1989) The disribution, metabolism, and excretion of trichothecene mycotoxins. In Trichothecene Mycotoxicoses. Pathophysiological Effects. Ed. Beasley, V.R. CRC Press. Volume 1, 37-61

Swanson, S.P., Helaszek, C., Buck, W.B., Rodd, H.D. & Haschek, W.M. (1988) The role of intestinal microflora in the metabolism of trichothecenes. Food and Chemical Toxicology. **26**, 823-829.

Swanson, S.P., Nicolletti, J., Rodd, H.D., Buck, W.B., Cote, L.M. & Yoshizawa. (1986) Gas chromatographic analysis of milk for deoxynivalenol and its metabolite, DOM-1. Jouranl of the Association of Official Analytical Chemists. **69**, 41-43

Swenson, D.H., Lin, J.K., Miller, E.C. & Miller, J.A. (1977) Aflatoxin B_1 2,3-oxide as a probable intermediate in the covalent binding of aflatoxins B_1 and B_2 to rat liver DNA and ribosomal RNA *in vivo*. Cancer Research. **37**, 172-181.

Szabo, G. & Davis, R. (1964) Studies on the β-galactosidase activity of *Saccharomyces fragilis*. Journal of General Microbiology. **37**, 99-112.

Tamm, C. (1980) The biosynthesis of the cytochalasins. In The Biosynthesis of Mycotoxins. A study in Secondary Metabolism. Ed Steyn, P.S. 269-297.

Tamm, C. & Tori, M. (1984) Trichothecenes. In Mycotoxins. Production, Isolation, Separation and Purification. Ed. Betina, V. Elsevier. 131-182.

Tamm, C. & Breitenstein, W. (1980) The biosynthesis of trichothecene mycotoxins. In The Biosynthesis of Mycotoxins. A study in Secondary Metabolism. Ed Steyn, P.S. 69-101.

Terse, P.S., Madhyastha, M.S., Zurovac, O., Stringfellow, D., Marquardt, R.R. & Kemppainen, B.W. (1993) Comparison of *in vitro* and *in vivo* biological activities of mycotoxins. Toxicon. **31**, 913-919

Thiel, P.G., Marasas, W.F.O., Sydenham, E.W., Shephard, G.S. & Gelderblom, W.C.A. (1992) The implications of naturally occuring levels of fumonisins in corn for human and animal health. Mycopathologia, **117**, 3-9.

Thompson, W.L. & Wannemacher, R.W. (1986) Structure-function relationships of 12,13-epoxytrichothecene mycotoxins in cell culture: comparison to whole animal lethality. Toxicon. **24**, 985-994.

Tingle, M. & Halvorson, H.O. (1972) Mutants in *Saccharomyces lactis* controlling both β-glucosidase and β-galactosidase activities. Genetic Research. **19**, 27-32.

Tiwari, R.P., Dham, C.K., Bhalla, T.C., Saini, S.S. & Vadehra, D.V. (1985) Mechanism of action of aflatoxin B_1 in *Bacillus megaterium*. Applied and Environmental Microbiology. **49**, 904-907.

Ueno, Y. (1991) Biochemical mode of action of mycotoxins. In Mycotoxins and Animal Foods. Ed Smith, J.E. & Henderson, R.S. CRC Press. 437-453.

Ueno, Y. (1984) Toxicological features of T-2 toxin and related trichothecenes. Fundamental and Applied Toxicology. **4**, S124-S132.

Ueno, Y. (1983) General Toxicology. In Developments in Food Science. IV. Elsevier. 135-146.

Ueno, Y. (1977) Trichothecenes. In Mycotoxins in Human and Animal Health. Ed. Rodericks, J.V., Hesseltine, C.W. & Mehlman, M.A. Pathotox Publishers. 189-228.

Ueno, Y & Ueno, I. (1978) Toxicology and biochemistry of mycotoxins. In Toxicology, Biochemistry and Pathology of Mycotoxins. Ed Uraguchi, K. & Yamazaki, M. John Wiley and Sons. 107-188.

Ueno, Y. & Kubota, K. (1976) DNA-attacting ability of carcinogenic mycotoxins in recombination-deficient mutant cells of *Bacillus subtillis*. Cancer Research. **36**, 445-451

Ueno, Y., Nakayama, K., Ishii, K., Tashiro, F., Minoda, T., Omori, T. & Komagata, K. (1983a) Metabolism of T-2 toxin in *Curtobacterium* strains. Applied and Environmental Microbiology. **46**, 120-126.

Ueno, Y., Tashiro, F. & Kabayashi, T. (1983b) Species differences in zearalenone reductase activity. Food and Chemical Toxicology. **21**, 167-173.

Ueno, Y., Kubota, K., Ito, I. & Nakamura, Y. (1978) Mutagenicitiy of carcinogenic mycotoxins in *Salmonella typhimurium*. Cancer Research, **38**, 536-542.

Ueno, Y., Nakajima, M., Sakai, K., Ishii, K., Sato, M. & Shimada, N. (1973) Comparative toxicology of trichothecene mycotoxins. Inhibition of protein synthesis in animal cells. Journal of Biochemistry (Tokyo) **74**, 285-296.

Ueno, Y., Hosoya, M., Morita, Y., Ueno, I. & Tasuno, T. (1968) Inhibition of protein synthesis in rabbit reticulocytes by nivalenol, a toxic principle isolated from *Fusarium nivale*-growing rice. Jouranl of Biochemistry. **64**, 479-

Umeda, M., Yamamoto, T. & Saito, M. (1972) DNA-strand breakage of HeLa cells induced by several mycotoxins. Japanese Jouranl of Experimental Medicine. **42**, 527-535.

Uwaifo, A.O.& Bassir, O. (1978) Toxic effects of aflatoxin B_1 on *Bacillus brevis*. Biochemistry and Experimental Biology. **14**, 275-382

Uwajima, T.; Yagi, H. & Terada, O. (1972) Purification, crystallisation and some properties of β-galactosidase from *Saccharomyces fragilis*. Agricultural and Biological Chemistry. **36**, 570-577.

Vaara, M. & Vaara, T. (1983) Sensitisation of Gram-negative bacteria to antibiotics and complement by a non-toxic oligopeptide. Nature. **303**, 526-528.

VanRensberg, S.J., Cook-Mozarrari, P., Vn Schalkwyk, D.J., Can Der Watt, J.J., Vencent, T.J. & Purchase, I.F. (1985) Hepatocellular carcinoma and dietary aflatoxin in Mozambique and Transkei. British Journal of Cancer. **51**, 713-726.

Vesely, D., Vesela, D. & Jelinek, R. (1984) Use of chick embryo in screening for toxin-producing fungi. Mycopathologia. **88**, 135-140.

Viitasala, L. & Gyllenberg. (1968) Toxicity of aflatoxins to *B. megaterium*. Lebensm.-Wiss. Technol. 12, 113-114.

Vleggaar, R. & Steyn, P.S. (1980) The biosynthesis of some miscellaneous mycotoxins. In The Biosynthesis of Mycotoxins. A study in Secondary Metabolism. Ed Steyn, P.S. 395-422.

Wallenfals K. & Weil R. (1972) β-Galactosidase. in The Enzymes. Ed. Boyer, P.D. Academic Press. N.Y. **7**, 617-663.

Wallenfels, K. & Malhorta, O.P. (1960) β-galactosidase. In The Enzymes (Eds. Boyer, P.D.; Lardy, H. & Myrback, K.) Academic Press. **4**, 409-430

Watson, D.H. & Lindsay, D.G. (1982) A critical review of biological methods for the detection of fungal toxins in foods and foodstuffs. Journal of the Science of Food and Agriculture. **33**, 59-67.

Wei, R. & Chu, F.S. (1985) Modification of *in vitro* metabolism of T-2 toxin by esterase inhibitors. Applied and Environmental Microbiology. **50**, 115-

Wei, C.M. & McLaughlin, C.S. (1974) Structure-function relationships in the 12,13-epoxytrichothecenes. Novel inhibitors of protein synthesis. Biochemical and Biophysical Research Communications. **57**, 838-844.

Wei, C.M., Campbell, I.M., McLaughlin, C.S. & Vaughn, M.H. (1974) Binding of trichodermin to mammalian ribosomes and its inhibition by other 12,13-epoxytrichothecenes. Molecular and Cellular Biochemistry. **31**, 215-219.

Wendorff, W.L. & Amundson, C.C. (1971) Characterisation of β-galactosidase from *Saccharomyces fragilis*. Journal of Milk Food Technology. **34**, 300-306.

Wendorff, W.L., Admundson, C.H. & Olsen, N.F. (1970) Nutrient requirements and growth conditions for production of lactase enzyme by *Saccharomyces fragilis*. Journal of Milk Food Technology. **33**, 451-454.

WHO. (1990) In Selected Mycotoxins: Ochrotoxins, Trichothecens, Ergot. Environmental Health Criteria, **105**. 71-163.

WHO. (1979) Environmental Health Criteria 11. Mycotoxins. WHO Publications Geneva. 28-50.

Wilson, D. & Abramson, D. (1992) Mycotoxins. In Storage of Cereal Grains and Their Products. Ed. Sauer, D.B. American Association of Cereal Chemists. 340-391.

Wiseman, A. & Woods, L.F.J. (1979) Benzo(a)pyrene metabolites formed by the action of yeast cytochrome P450/P448. Journal of Chemical and Technical Biotechnology. **29**, 320-324.

Wong, Z.A. & Hsieh, D.P.H. (1980) The comparative metabolism and toxicokinetics of aflatoxin B_1 in the monkey, rat and mouse. Toxicology and Applied Pharmacology. **55**, 115-125.

Wong, Z.A. & Hsieh, D.P.H. (1976) Mutagenicity of aflatoxins related to their metabolism and carcinogenic potenrial. Procedings of the National Academy of Sciences (USA). **73**, 2241-2244.

Yagen, B & Bialer, M. (1993) Metabolism and pharmacokinetics of T-2 toxin and related trichothecenes. Drug Metabolism Reviews. **25**, 281-323.

Yamazaki, M. (1980) The biosynthesis of neurotropic mycotoxins. In The Biosynthesis of Mycotoxins. A study in Secondary Metabolism. Ed Steyn, P.S. 193-220.

Yates, I.E. (1986) Bioassay systems and their use in the diagnosis of mycotoxicoses. In Diagnosis of Mycotoxicoses Eds. Richard, J.L. & Thurston, J.R. Martinus Nijhoff. 333-381.

Yates I.E. (1985) Cytotoxicity and mutagenicity of aflatoxins B_1, B_2, G_1, and G_2 in *Photobacteriium phosphoreum*. Journal of Microbiological Methods. **3**, 181-186.

Yates, I.E. & Poter, J.K. (1982) Bacterial bioluminesence as a bioassay for mycotoxins. Applied and Environmental Microbiology. **44**, 1072-1075.

Yeh, F.S., Yu, M.C., Mo, C.C., Luo, S., Tong, M.J. and Henderson, B. (1989) Hepatitis B virus, aflatoxins and hepatocellular carcinoma in Southern Guangxi, China. Cancer Research. **49**, 2506-2509.

Yoshida, Y. & Aoyama, Y. (1984) Yeast cytochrome P-450 catalysing lanosterol 14α-demethylation. I. Purification and spectral properties. Journal of Biological Chemistry. **259**, 1655-1660

Yoshizawa, T., Sakamoto, T. & Kuwamura, K. (1985) Structures of deepoxytrichothecene metabolites from 3' hydroxy HT-2 toxin and T-2 tetraol in rats. Applied and Environmental Microbiology. **50**, 676-679.

Yoshizawa, T., Sakamoto, T. & Okamoto, K. (1984) *In vitro* formation of 3'hydroxy T-2 and 3'hydroxy HT-2 toxins from T-2 toxins by liver homogenates from mice and monkeys. Applied and Environmental Microbiology. **47**, 130-134.

Yoshizawa, T., Swanson, S.P., Mirocha, C.J. (1980) *In vitro* metabolism of T-2 toxin in rats. Applied and Environmental Microbiology. **40**, 901-906.

APPENDICES

APPENDIX 1
REPRODUCIBILITY OF THE YEAST BIOASSAY

T-2 toxin	Control		0.1ng/ml		1ng/ml		2.5ng/ml	
Time (hrs)	Growth (A_{560})							
	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV
0	0.201 ± 0.013	6.55	0.214 ± 0.006	2.58	0.210 ± 0.009	4.04	0.224 ± 0.005	2.02
1	0.249 ± 0.014	5.49	0.243 ± 0.019	7.95	0.249 ± 0.008	3.34	0.261 ± 0.004	1.55
2	0.333 ± 0.016	4.93	0.345 ±0.013	3.74	0.341 ± 0.005	1.32	0.357 ± 0.014	3.98
3	0.501 ± 0.018	3.69	0.528 ± 0.025	4.74	0.519 ± 0.003	0.49	0.530 ± 0.018	3.43
4	0.658 ± 0.027	4.17	0.684 ± 0.021	3.00	0.681 ± 0.002	0.22	0.687 ± 0.022	3.22
5	0.852 ± 0.028	3.28	0.867 ± 0.013	1.49	0.879 ± 0.007	0.80	0.875 ± 0.032	3.62
6	1.018 ± 0.022	2.20	1.051 ± 0.050	4.79	1.016 ± 0.019	1.91	1.025 ± 0.034	3.33
7	1.095 ± 0.022	1.99	1.113 ± 0.056	4.12	1.078 ± 0.041	3.78	1.096 ± 0.008	0.76
8	1.155 ± 0.022	1.89	1.171 ± 0.023	1.93	1.162 ± 0.001	0.09	1.175 ± 0.004	0.34
9	1.204 ± 0.011	0.88	1.201 ± 0.022	1.79	1.203 ± 0.001	0.10	1.191 ± 0.007	0.57
10	1.228 ± 0.012	1.02	1.223 ± 0.011	0.91	1.232 ± 0.017	1.41	1.217 ± 0.012	0.98
10	β-galactosidase activity (cleavage of Xgal A_{666}-A_{560})							
	0.797 ± 0.056	6.98	0.797 ± 0.023	2.87	0.797 ± 0.032	4.06	0.736 ± 0.018	2.38
10	Cell viability (cleavage of MTT A_{560}-A_{666})							
	0.715 ± 0.120	16.76	0.691 ± 0.145	21.02	0.644 ± 0.133	20.58	0.513 ± 0.265	51.67

T-2 toxin	5ng/ml		7.5ng/ml		10ng/ml		25ng/ml	
Time (hrs)	Growth (A_{560})							
	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV
0	0.209 ± 0.006	2.66	0.217 ± 0.006	2.66	0.207 ± 0.008	3.68	0.207 ± 0.005	2.17
1	0.245 ± 0.012	4.98	0.258 ± 0.012	4.98	0.241 ± 0.011	4.35	0.253 ± 0.011	4.33
2	0.344 ± 0.014	4.21	0.336 ± 0.014	4.21	0.320 ± 0.012	3.62	0.315 ± 0.006	1.80
3	0.489 ± 0.031	6.38	0.477 ± 0.031	6.38	0.448 ± 0.021	4.61	0.374 ± 0.008	2.04
4	0.624 ± 0.01	1.58	0.590 ± 0.010	1.58	0.556 ± 0.038	6.84	0.416 ± 0.018	4.33
5	0.776 ± 0.012	1.6	0.741 ± 0.012	1.60	0.676 ± 0.033	4.94	0.471 ± 0.024	5.19
6	0.946 ± 0.015	1.6	0.904 ± 0.015	1.60	0.800 ± 0.018	2.28	0.535 ± 0.006	1.13
7	1.028 ± 0.022	2.17	1.004 ± 0.022	2.17	0.922 ± 0.013	1.37	0.649 ± 0.037	5.77
8	1.109 ± 0.009	0.81	1.105 ± 0.009	0.81	1.055 ± 0.040	3.79	0.771 ± 0.033	4.31
9	1.145 ± 0.014	1.23	1.141 ± 0.014	1.23	1.105 ± 0.007	0.59	0.855 ± 0.009	1.09
10	1.184 ± 0.008	0.68	1.170 ± 0.008	0.68	1.095 ± 0.005	0.47	0.945 ± 0.038	4.00
10	β-galactosidase activity (cleavage of Xgal A_{666}-A_{560})							
	0.708 ± 0.014	1.989	0.070 ± 0.023	32.30	0.006 ±		-0.02 ± 0.00	0.00
10	Cell viability (cleavage of MTT A_{560}-A_{666})							
	0.521 ± 0.152	29.18	0.427 ± 0.073	17.01	0.337 ± 0.014	4.05	0.303 ± 0.017	5.48

T-2 toxin	50ng/ml		75ng/ml		100ng/ml		250ng/ml	
Time (hrs)	Growth (A_{560})							
	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV
0	0.217 ± 0.008	3.60	0.215 ± 0.009	4.07	0.204 ± 0.009	4.22	0.218 ± 0.007	3.25
1	0.255 ± 0.008	3.17	0.243 ± 0.011	4.34	0.212 ± 0.011	5.27	0.219 ± 0.004	1.84
2	0.305 ± 0.010	3.18	0.295 ± 0.011	3.73	0.273 ± 0.004	1.60	0.284 ± 0.012	4.23
3	0.358 ± 0.022	6.15	0.319 ± 0.015	4.78	0.293 ± 0.006	1.94	0.299 ± 0.015	4.86
4	0.365 ± 0.024	6.58	0.312 ± 0.021	6.79	0.311 ± 0.003	1.11	0.284 ± 0.01	3.54
5	0.387 ± 0.023	5.84	0.306 ± 0.011	3.43	0.287 ± 0.008	2.61	0.287 ± 0.018	6.2
6	0.446 ± 0.029	6.42	0.310 ± 0.014	4.56	0.284 ± 0.004	1.53	0.284 ± 0.021	7.21
7	0.467 ±0.037	7.91	0.300 ± 0.013	4.29	0.272 ± 0.009	3.13	0.267 ± 0.017	6.21
8	0.498 ± 0.014	2.77	0.290 ± 0.013	4.59	0.258 ± 0.009	3.31	0.250 ± 0.016	6.21
9	0.536 ± 0.034	6.29	0.308 ± 0.009	2.92	0.261 ± 0.011	4.02	0.252 ± 0.017	6.76
10	0.620 ± 0.029	4.70	0.303 ± 0.015	5.06	0.264 ± 0.011	3.97	0.252 ± 0.018	7.27
10	β-galactosidase activity (cleavage of Xgal A_{666}-A_{560})							
	-0.020 ± 0.00	0.00	-0.02 ± 0.00	0.00	-0.02 ±-0.00	0.00	-0.02 ± 0.00	0.00
10	Cell viability (cleavage of MTT A_{560}-A_{666})							
	0.205 ± 0.039	19.10	0.221 ± 0.017	7.69	0.147 ± 0.012	7.81	0.132 ± 0.006	4.30

Table 1a Reproducibility of the yeast bioassay: effect of T-2 toxin on growth, β-galactosidase activity and cell viability -Within-assay variation, experiment 1.

T-2 toxin	Control		0.1ng/ml		1ng/ml		2.50ng/ml	
Time (hrs)	Growth (A_{560})							
	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV
0	0.202 ± 0.011	5.47	0.212 ± 0.008	3.86	0.196 ± 0.011	5.68	0.203 ± 0.015	7.19
1	0.247 ± 0.009	3.68	0.246 ± 0.005	1.83	0.254 ± 0.012	4.85	0.237 ± 0.004	1.7
2	0.342 ± 0.015	4.28	0.349 ± 0.024	6.97	0.351 ± 0.010	2.85	0.322 ± 0.021	6.37
3	0.358 ± 0.019	3.53	0.548 ± 0.027	4.86	0.524 ± 0.024	4.64	0.473 ± 0.015	3.25
4	0.698 ± 0.017	2.46	0.699 ± 0.013	1.89	0.705 ± 0.009	1.21	0.641 ± 0.009	1.43
5	0.884 ± 0.021	3.39	0.896 ± 0.027	3.02	0.854 ± 0.022	2.52	0.818 ± 0.015	1.88
6	1.007 ± 0.004	0.4	1.011 ± 0.007	0.69	1.005 ± 0.004	0.4	0.951 ± 0.020	2.14
7	1.100 ± 0.013	1.14	1.096 ± 0.013	1.17	1.07 ± 0.032	3.00	1.047 ± 0.034	3.27
8	1.166 ± 0.013	1.12	1.175 ± 0.020	1.67	1.172 ± 0.014	1.16	1.133 ± 0.012	1.02
9	1.217 ± 0.013	1.03	1.213 ± 0.016	1.28	1.228 ± 0.008	0.64	1.185 ± 0.007	0.61
10	1.233 ± 0.013	1.04	1.228 ± 0.019	1.53	1.242 ± 0.007	0.56	1.227 ± 0.009	0.69
10	β-galactosidase activity (cleavage of Xgal A_{666}-A_{560})							
	0.773 ± 0.064	8.26	0.744 ± 0.062	8.29	0.824 ± 0.037	4.43	0.746 ± 0.073	9.83
10	Cell viability (cleavage of MTT A_{560}-A_{666})							
	0.739 ± 0.114	15.36	0.671 ± 0.049	7.31	0.741 ± 0.073	9.87	0.659 ± 0.126	19.12

T-2 toxin	5ng/ml		7.5ng/ml		10ng/ml		25ng/ml	
Time (hrs)	Growth (A_{560})							
	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV
0	0.190 ± 0.018	9.53	0.217 ± 0.005	2.11	0.206 ± 0.009	4.25	0.191 ± 0.013	6.92
1	0.229 ± 0.006	2.656	0.245 ± 0.010	4.10	0.231 ± 0.010	4.11	0.237 ± 0.016	6.83
2	0.316 ± 0.005	1.58	0.311 ± 0.011	3.54	0.297 ± 0.005	1.78	0.275 ± 0.012	4.39
3	0.442 ± 0.010	2.30	0.415 ± 0.012	2.86	0.364 ± 0.029	8.06	0.329 ± 0.016	4.85
4	0.606 ± 0.010	1.72	0.543 ± 0.030	5.50	0.457 ± 0.027	5.88	0.383 ± 0.018	4.59
5	0.781 ± 0.018	2.35	0.708 ± 0.021	3.02	0.540 ± 0.020	3.66	0.425 ± 0.005	1.25
6	0.892 ± 0.013	1.147	0.829 ± 0.012	1.46	0.650 ± 0.028	4.32	0.458 ± 0.009	1.98
7	0.976 ± 0.011	1.10	0.946 ± 0.023	2.42	0.738 ± 0.017	2.31	0.500 ± 0.018	3.65
8	1.038 ± 0.054	5.23	1.003 ± 0.003	0.25	0.813 ± 0.017	2.03	0.549 ± 0.026	4.73
9	1.124 ± 0.011	0.98	1.073 ± 0.056	5.18	0.906 ± 0.014	1.56	0.615 ± 0.029	4.75
10	1.196 ± 0.007	0.59	1.151 ± 0.024	2.05	0.998 ± 0.010	0.96	0.693 ± 0.028	3.98
10	β-galactosidase activity (cleavage of Xgal A_{666}-A_{560})							
	0.777 ± 0.118	15.24	0.622 ± 0.132	21.18	0.080 ± 0.061	77.19	-0.02 ± 0.00	0.00
10	Cell viability (cleavage of MTT A_{560}-A_{666})							
	0.730 ± 0.090	12.38	0.681 ± 0.139	20.42	0.642 ± 0.030	4.71	0.312 ± 0.019	5.94

T-2 toxin	50ng/ml		75ng/ml		100ng/ml		250ng/ml	
Time (hrs)	Growth (A_{560})							
	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV
0	0.209 ± 0.015	7.37	0.195 ± 0.009	4.47	0.194 ± 0.007	3.51	0.209 ± 0.012	5.55
1	0.219 ± 0.010	4.40	0.210 ± 0.012	5.71	0.221 ± 0.010	4.32	0.227 ± 0.013	5.67
2	0.252 ± 0.010	3.98	0.228 ± 0.005	2.17	0.248 ± 0.007	2.92	0.244 ± 0.009	3.88
3	0.300 ± 0.006	1.89	0.252 ± 0.008	3.00	0.264 ± 0.021	7.88	0.274 ± 0.011	3.83
4	0.323 ± 0.013	4.02	0.299 ± 0.003	0.84	0.276 ± 0.013	4.79	0.297 ± 0.005	1.78
5	0.345 ± 0.021	6.19	0.288 ± 0.005	1.71	0.292 ± 0.007	2.37	0.279 ± 0.012	4.19
6	0.360 ± 0.019	5.36	0.295 ± 0.009	3.15	0.287 ± 0.012	4.00	0.261 ± 0.009	3.34
7	0.379 ± 0.019	5.02	0.293 ± 0.007	2.32	0.267 ± 0.014	5.20	0.254 ± 0.012	4.75
8	0.383 ± 0.018	4.59	0.278 ± 0.007	2.53	0.257 ± 0.018	7.08	0.240 ± 0.006	2.55
9	0.405 ± 0.007	1.62	0.288 ± 0.003	0.87	0.267 ± 0.007	2.76	0.249 ± 0.007	2.90
10	0.423 ± 0.009	2.02	0.282 ± 0.009	3.2	0.261 ± 0.009	3.51	0.253 ± 0.011	4.40
10	β-galactosidase activity (cleavage of Xgal A_{666}-A_{560})							
	-0.020 ± 0.00	0.00	-0.020 ± 0.00	0.00	-0.020 ± 0.00	0.00	-0.020 ± 0.00	0.00
10	Cell viability (cleavage of MTT A_{560}-A_{666})							
	0.242 ± 0.038	15.88	0.178 ± 0.012	6.74	0.177 ± 0.008	4.52	0.141 ± 0.022	15.52

Table 1b Reproducibility of the yeast bioassay: effect of T-2 toxin on growth, β-galactosidase activity and cell viability -Within-assay variation, experiment 2.

T-2 toxin	Control		0.1ng/ml		1ng/ml		2.5ng/ml	
Time (hrs)	Growth (A_{560})							
	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV
0	0.189 ± 0.010	5.12	0.190 ± 0.006	2.89	0.181 ± 0.007	4.00	0.184 ± 0.009	4.69
1	0.221 ± 0.013	5.72	0.233 ± 0.022	9.44	0.225 ± 0.009	3.88	0.215 ± 0.012	5.64
2	0.299 ± 0.015	4.98	0.292 ± 0.014	4.76	0.292 ± 0.009	3.04	0.281 ± 0.013	4.71
3	0.401 ± 0.021	5.19	0.412 ± 0.008	1.96	0.386 ± 0.013	3.34	0.361 ± 0.033	9.14
4	0.515 ± 0.018	3.47	0.505 ± 0.024	4.84	0.516 ± 0.011	2.04	0.495 ± 0.018	3.69
5	0.713 ± 0.016	2.21	0.730 ± 0.023	3.10	0.690 ± 0.016	2.36	0.706 ± 0.008	1.14
6	0.898 ± 0.019	2.08	0.913 ± 0.034	3.67	0.864 ± 0.019	2.18	0.859 ± 0.014	1.57
7	1.005 ± 0.007	0.72	1.006 ± 0.004	0.43	0.996 ± 0.016	1.56	0.990 ± 0.022	2.19
8	1.080 ± 0.016	1.52	1.086 ± 0.008	0.70	1.058 ± 0.010	0.91	1.051 ± 0.009	0.86
9	1.155 ± 0.015	1.32	1.162 ± 0.015	1.32	1.125 ± 0.008	0.69	1.121 ± 0.014	1.27
10	1.205 ± 0.014	1.13	1.214 ± 0.007	0.59	1.190 ± 0.002	0.17	1.192 ± 0.013	1.10
10	β-galactosidase activity (cleavage of Xgal A_{666}-A_{560})							
	0.769 ± 0.100	12.99	0.860 ± 0.040	4.66	0.757 ± 0.038	4.96	0.806 ± 0.153	19.02
10	Cell viability (cleavage of MTT A_{560}-A_{666})							
	0.743 ± 0.110	14.87	0.799 ± 0.134	16.74	0.815 ± 0.107	13.13	0.696 ± 0.087	12.51

T-2 toxin	5ng/ml		7.5ng/ml		10ng/ml		25ng/ml	
Time (hrs)	Growth (A_{560})							
	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV
0	0.189 ± 0.007	3.82	0.184 ± 0.008	4.49	0.176 ± 0.011	6.40	0.188 ± 0.008	4.02
1	0.220 ± 0.007	3.28	0.225 ± 0.006	2.99	0.226 ± 0.011	4.99	0.222 ± 0.010	4.30
2	0.296 ± 0.007	2.25	0.296 ± 0.018	8.23	0.270 ± 0.003	1.19	0.265 ± 0.006	2.15
3	0.396 ± 0.006	1.43	0.385 ± 0.017	6.28	0.326 ± 0.010	3.07	0.301 ± 0.010	3.32
4	0.508 ± 0.009	1.68	0.499 ± 0.002	0.69	0.399 ± 0.007	1.78	0.348 ± 0.014	3.95
5	0.688 ± 0.013	1.89	0.651 ± 0.004	1.50	0.473 ± 0.024	5.03	0.385 ± 0.012	3.21
6	0.872 ± 0.024	2.77	0.828 ± 0.020	7.24	0.558 ± 0.024	4.32	0.449 ± 0.015	3.24
7	0.988 ± 0.012	1.17	0.939 ± 0.012	4.22	0.717 ± 0.012	1.64	0.506 ± 0.013	2.58
8	1.057 ± 0.006	0.55	1.037 ± 0.017	6.16	0.851 ± 0.011	1.27	0.580 ± 0.027	4.74
9	1.111 ± 0.011	0.95	1.09 ± 0.009	3.14	0.982 ± 0.017	1.73	0.658 ± 0.031	4.66
10	1.161 ± 0.005	0.41	1.132 ± 0.012	4.2	1.025 ± 0.017	1.64	0.751 ± 0.037	4.96
10	β-galactosidase activity (cleavage of Xgal A_{666}-A_{560})							
	0.613 ± 0.083	13.62	0.003 ± 0.002	57.28	-0.020 ± 0.00	0.00	-0.020 ± 0.00	0.00
10	Cell viability (cleavage of MTT A_{560}-A_{666})							
	0.573 ± 0.053	9.34	0.546 ± 0.076	13.94	0.478 ± 0.023	4.74	0.403 ± 0.068	16.87

T-2 toxin	50ng/ml		75ng/ml		100ng/ml		250ng/ml	
Time (hrs)	Growth (A_{560})							
	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV
0	0.171 ± 0.010	5.76	0.185 ± 0.008	4.49	0.179 ± 0.004	1.96	0.185 ± 0.013	6.98
1	0.226 ± 0.008	3.58	0.202 ± 0.006	2.99	0.220 ± 0.014	6.30	0.215 ± 0.017	7.99
2	0.251 ± 0.009	3.57	0.223 ± 0.018	8.23	0.237 ± 0.004	1.52	0.233 ± 0.011	4.72
3	0.288 ± 0.011	3.82	0.263 ± 0.017	6.28	0.266 ± 0.005	1.89	0.258 ± 0.008	2.91
4	0.304 ± 0.015	5.06	0.301 ± 0.002	0.69	0.270 ± 0.001	0.37	0.264 ± 0.009	3.43
5	0.326 ± 0.022	6.79	0.291 ± 0.004	1.50	0.272 ± 0.008	2.97	0.272 ± 0.011	4.18
6	0.342 ± 0.009	2.64	0.276 ± 0.020	7.24	0.265 ± 0.008	3.05	0.257 ± 0.006	2.28
7	0.377 ± 0.022	5.82	0.275 ± 0.012	4.22	0.260 ± 0.006	2.25	0.261 ± 0.009	3.34
8	0.396 ± 0.008	1.96	0.270 ± 0.017	6.16	0.262 ± 0.007	2.70	0.264 ± 0.008	3.18
9	0.407 ± 0.008	1.85	0.276 ± 0.009	3.14	0.262 ± 0.007	2.67	0.261 ± 0.009	3.51
10	0.416 ± 0.008	1.93	0.282 ± 0.012	4.20	0.267 ± 0.013	4.91	0.259 ± 0.010	3.90
10	β-galactosidase activity (cleavage of Xgal A_{666}-A_{560})							
	-0.020 ± 0.00	0.00	-0.020 ± 0.00	0.00	-0.020 ± 0.00	0.00	-0.020 ± 0.00	0.00
10	Cell viability (cleavage of MTT A_{560}-A_{666})							
	0.252 ± 0.060	23.78	0.141 ± 0.025	17.72	0.167 ± 0.028	16.82	0.147 ± 0.017	11.62

Table 1c Reproducibility of the yeast bioassay: effect of T-2 toxin on growth, β-galactosidase activity and cell viability -Within-assay variation, experiment 3.

T-2 toxin	Control		0.1ng/ml		1ng/ml		2.5ng/ml	
Time (hrs)	Growth (A_{560})							
	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV
0	0.197 ± 0.013	6.35	0.205 ± 0.013	6.11	0.196 ± 0.015	7.70	0.203 ± 0.019	9.55
1	0.239 ± 0.017	7.28	0.241 ± 0.016	6.61	0.243 ± 0.016	6.57	0.238 ± 0.021	8.89
2	0.325 ± 0.024	7.37	0.329 ± 0.032	9.59	0.328 ± 0.028	8.62	0.320 ± 0.036	11.16
3	0.480 ± 0.062	12.81	0.496 ± 0.066	13.32	0.476 ± 0.069	114.55	0.455 ± 0.082	18.02
4	0.624 ± 0.082	13.13	0.630 ± 0.095	15.09	0.634 ± 0.089	14.12	0.608 ± 0.088	14.53
5	0.816 ± 0.078	9.58	0.831 ± 0.079	9.536	0.808 ± 0.090	11.17	0.800 ± 0.077	9.58
6	0.975 ± 0.057	5.87	0.992 ± 0.068	6.89	0.962 ± 0.074	7.74	0.945 ± 0.075	7.94
7	1.067 ± 0.047	4.37	1.072 ± 0.055	5.16	1.048 ± 0.047	4.53	1.045 ± 0.051	4.84
8	1.134 ± 0.042	3.73	1.144 ± 0.046	4.06	1.131 ± 0.056	4.91	1.120 ± 0.055	4.90
9	1.192 ± 0.030	2.49	1.192 ± 0.028	2.33	1.185 ± 0.047	3.97	1.166 ± 0.035	2.98
10	1.222 ± 0.018	1.46	1.222 ± 0.013	1.07	1.221 ± 0.026	2.11	1.212 ± 0.019	1.53
10	β-galactosidase activity (cleavage of Xgal A_{666}-A_{560})							
	0.780 ± 0.075	9.64	0.800 ± 0.063	7.91	0.793 ± 0.042	5.33	0.763 ± 0.091	11.98
10	Cell viability (cleavage of MTT A_{560}-A_{666})							
	0.732 ± 0.113	15.45	0.720 ± 0.118	16.40	0.733 ± 0.119	16.21	0.623 ± 0.174	28.02

T-2 toxin	5ng/ml		7.5ng/ml		10ng/ml		25ng/ml	
Time (hrs)	Growth (A_{560})							
	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV
0	0.196 ± 0.014	7.15	0.206 ± 0.017	8.41	0.196 ± 0.017	8.80	0.195 ± 0.012	6.17
1	0.231 ± 0.013	5.81	0.243 ± 0.016	6.46	0.233 ± 0.011	4.85	0.237 ± 0.017	7.33
2	0.318 ± 0.022	7.06	0.314 ± 0.020	6.40	0.296 ± 0.023	7.65	0.285 ± 0.024	8.50
3	0.443 ± 0.044	9.85	0.426 ± 0.043	10.08	0.380 ± 0.057	15.03	0.335 ± 0.034	10.05
4	0.579 ± 0.055	9.46	0.544 ± 0.043	7.88	0.470 ± 0.073	15.44	0.382 ± 0.033	8.60
5	0.748 ± 0.047	6.26	0.700 ± 0.041	5.89	0.563 ± 0.092	16.38	0.427 ± 0.040	9.32
6	0.904 ± 0.037	4.06	0.854 ± 0.040	4.64	0.670 ± 0.108	16.07	0.481 ± 0.042	8.75
7	0.997 ± 0.027	2.76	0.963 ± 0.033	3.44	0.792 ± 0.098	12.43	0.552 ± 0.076	13.83
8	1.068 ± 0.042	3.93	1.048 ± 0.045	4.31	0.906 ± 0.115	12.68	0.633 ± 0.107	16.95
9	1.127 ± 0.018	1.62	1.102 ± 0.042	3.80	0.998 ± 0.088	8.78	0.709 ± 0.113	15.94
10	1.180 ± 0.016	1.38	1.151 ± 0.021	1.87	1.039 ± 0.045	4.29	0.796 ± 0.118	14.83
10	β-galactosidase activity (cleavage of Xgal A_{666}-A_{560})							
	0.699 ± 0.102	14.58	0.231 ± 0.302	130.41	0.022 ± 0.054	249.72	-0.020 ± 0.000	0.00
10	Cell viability (cleavage of MTT A_{560}-A_{666})							
	0.608 ± 0.132	21.73	0.551 ± 0.141	25.52	0.486 ± 0.134	27.50	0.339 ± 0.060	17.63

T-2 toxin	50ng/ml		75ng/ml		100ng/ml		250ng/ml	
Time (hrs)	Growth (A_{560})							
	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV	$\bar{x}$ ± s.d.	%CV
0	0.199 ± 0.023	11.72	0.198 ± 0.015	7.51	0.192 ± 0.012	6.39	0.204 ± 0.018	8.68
1	0.233 ± 0.018	7.86	0.218 ± 0.021	9.52	0.218 ± 0.011	5.07	0.220 ± 0.012	5.56
2	0.269 ± 0.028	10.45	0.249 ± 0.037	14.72	0.253 ± 0.017	6.60	0.254 ± 0.025	9.97
3	0.316 ± 0.035	10.98	0.278 ± 0.033	12.03	0.275 ± 0.018	6.54	0.277 ± 0.020	7.30
4	0.331 ± 0.031	9.49	0.304 ± 0.012	4.09	0.286 ± 0.020	7.13	0.282 ± 0.016	5.68
5	0.353 ± 0.033	9.37	0.295 ± 0.010	3.54	0.284 ± 0.011	3.88	0.279 ± 0.014	4.86
6	0.383 ± 0.051	13.45	0.294 ± 0.020	6.71	0.279 ± 0.013	4.53	0.267 ± 0.017	6.45
7	0.408 ± 0.050	12.29	0.289 ± 0.015	5.05	0.266 ± 0.010	3.74	0.261 ± 0.012	
8	0.426 ± 0.056	13.19	0.279 ± 0.014	5.13	0.259 ± 0.011	4.22	0.251 ± 0.014	5.57
9	0.449 ± 0.068	15.02	0.291 ± 0.015	5.30	0.263 ± 0.008	2.94	0.254 ± 0.012	4.60
10	0.486 ± 0.101	20.87	0.289 ± 0.015	5.14	0.264 ± 0.010	3.76	0.255 ± 0.012	4.85
10	β-galactosidase activity (cleavage of Xgal A_{666}-A_{560})							
	-0.020 ± 0.000	0.00	-0.020 ± 0.000	0.00	-0.020 ± 0.000	0.00	-0.020 ± 0.000	0.00
10	Cell viability (cleavage of MTT A_{560}-A_{666})							
	0.233 ± 0.046	19.68	0.180 ± 0.038	21.32	0.164 ± 0.020	12.46	0.140 ± 0.016	11.10

Table 1d Reproducibility of the yeast bioassay: effect of T-2 toxin on growth, β-galactosidase activity and cell viability - Cummulative data, between-assay variation.

APPENDIX 2
STRUCTURE-ACTIVITY RELATIONSHIPS AMONGST THE TRICHOTHECENE MYCOTOXINS

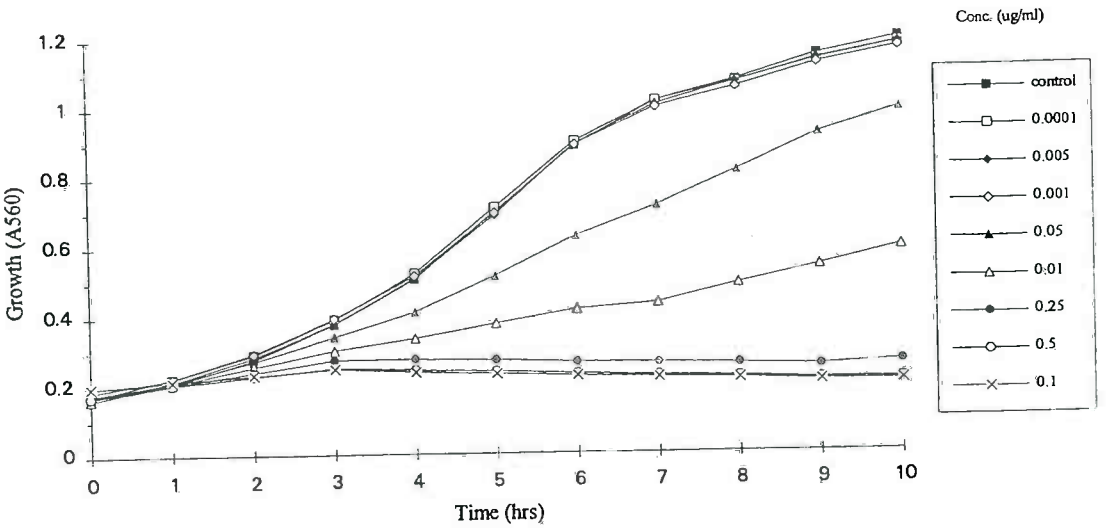

Figure 2a Effect of verrucarin A on growth of *K. marxianus*.

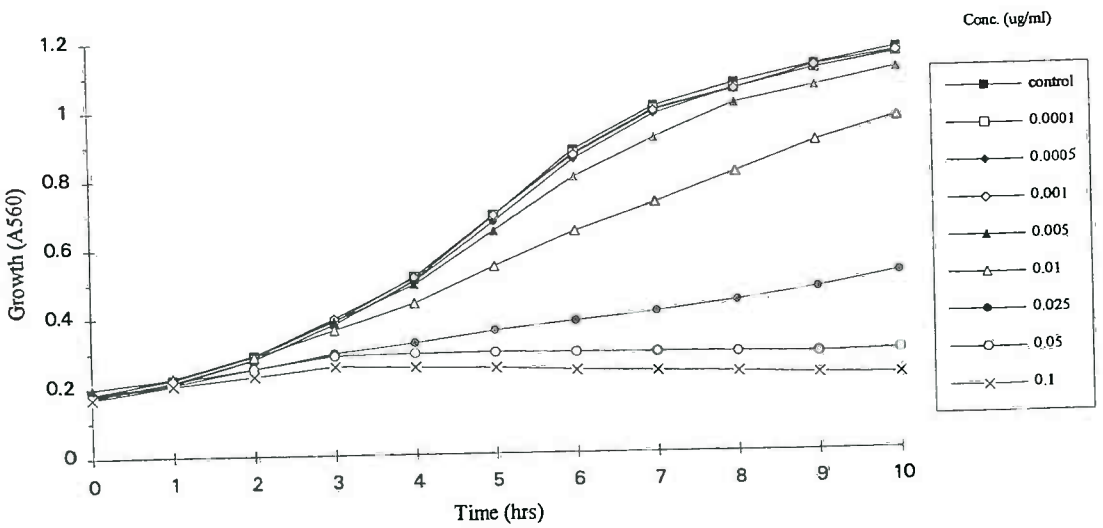

Figure 2b Effect of roridin A on growth of *K. marxianus*.

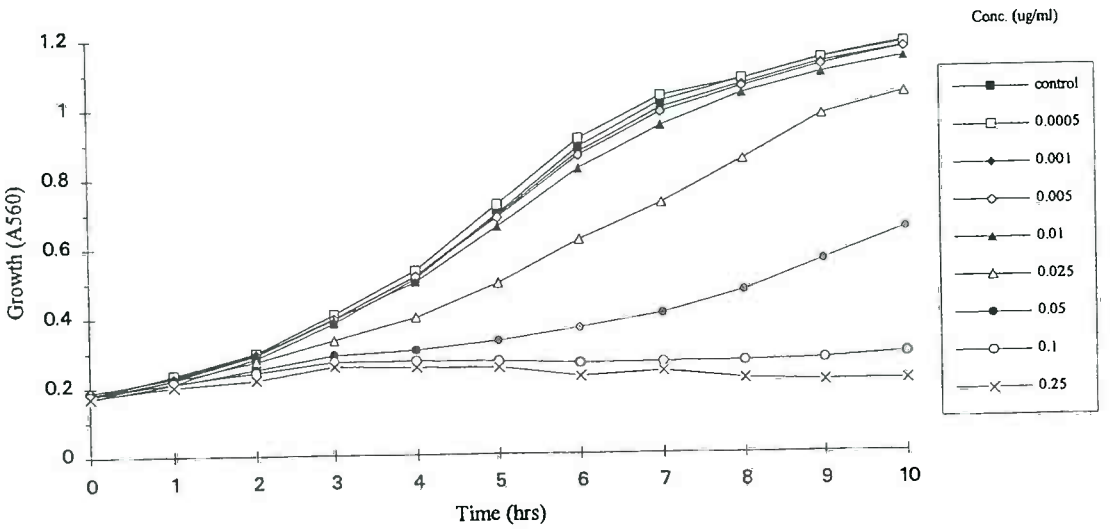

Figure 2c Effect of T-2 toxin on growth of *K. marxianus*.

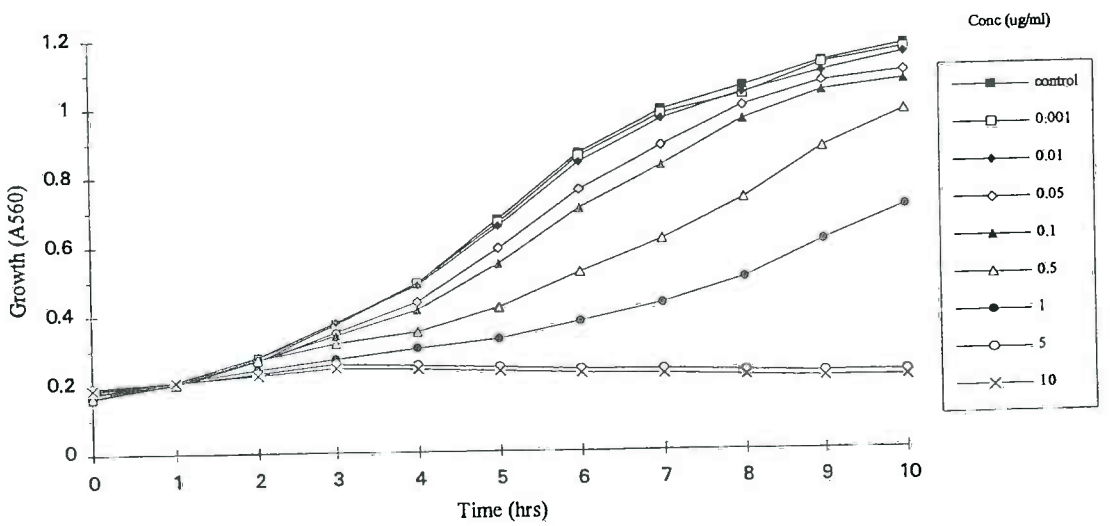

Figure 2d Effect of HT-2 toxin on growth of *K. marxianus*.

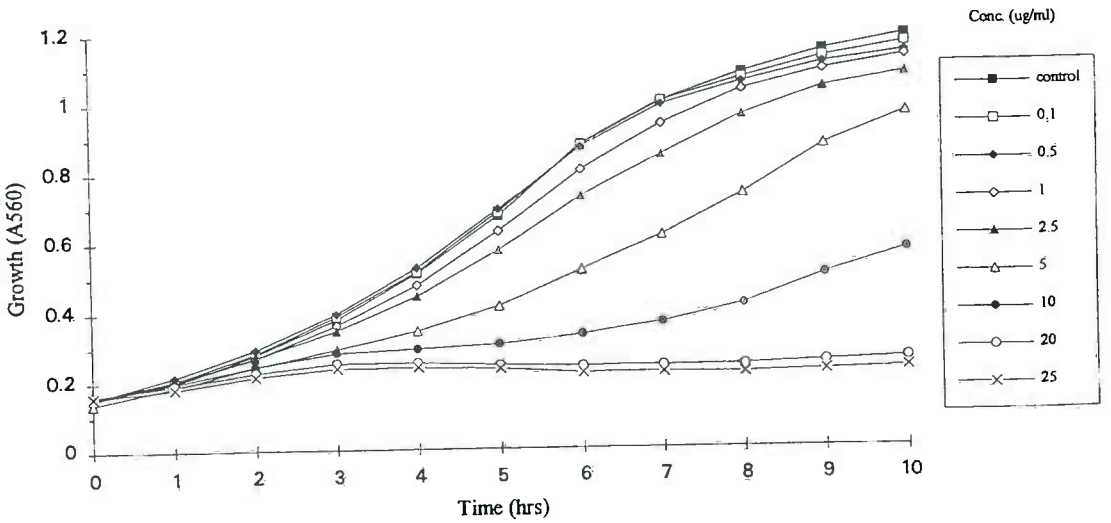

Figure 2e Effect of acetyl T-2 toxin on growth of *K. marxianus*.

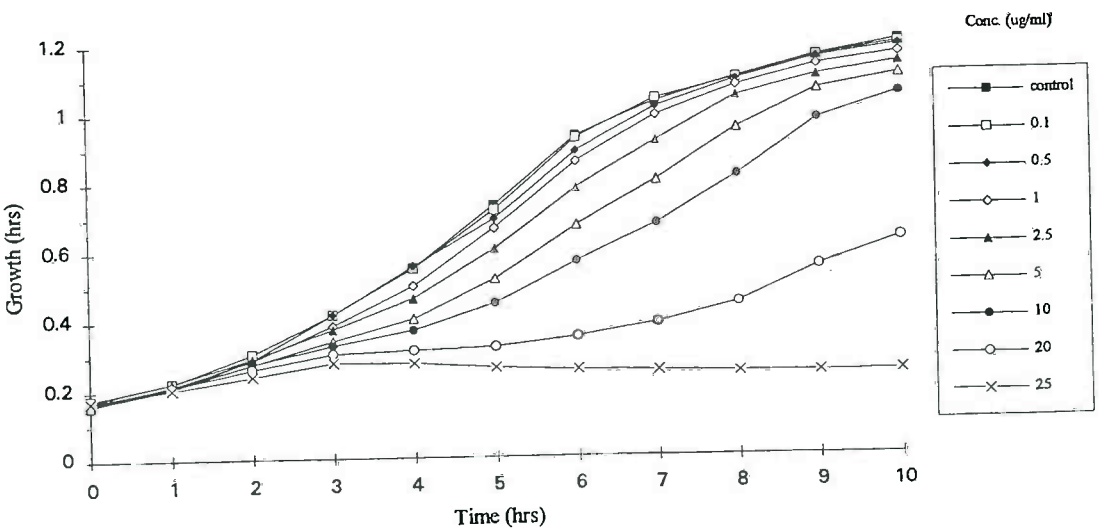

Figure 2f Effect of neosolaniol on growth of *K. marxianus*.

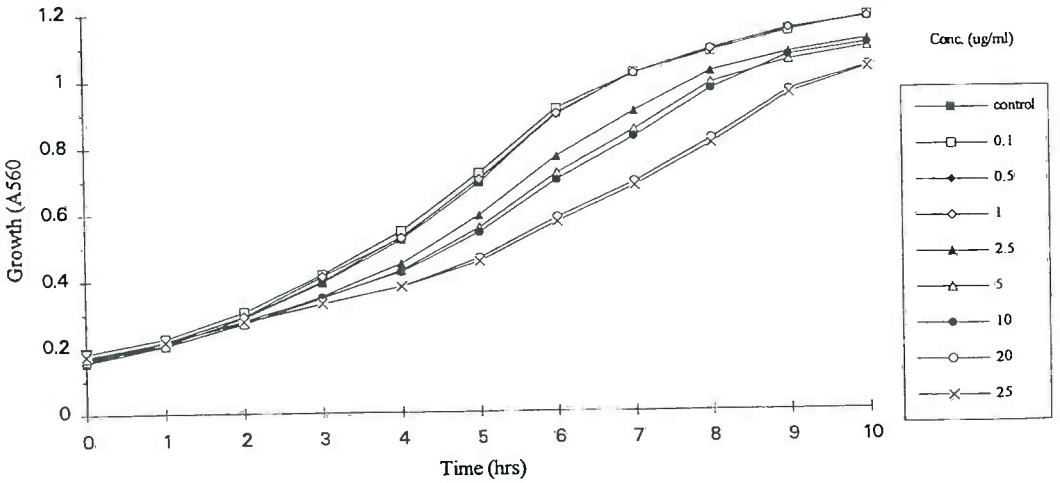

Figure 2g Effect of T-2 triol on growth of *K. marxianus*.

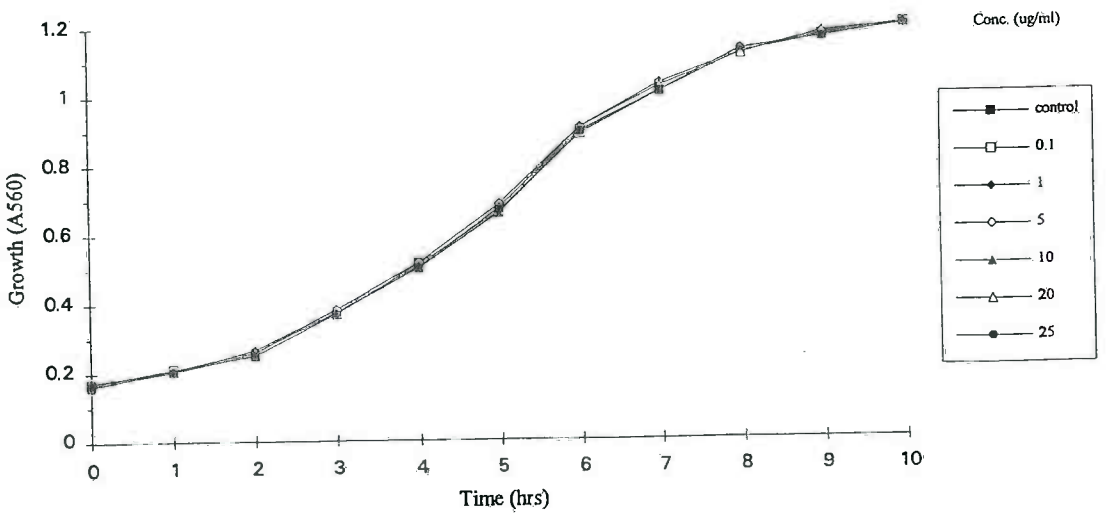

Figure 2h Effect of T-2 tetraol on growth of *K. marxianus*.

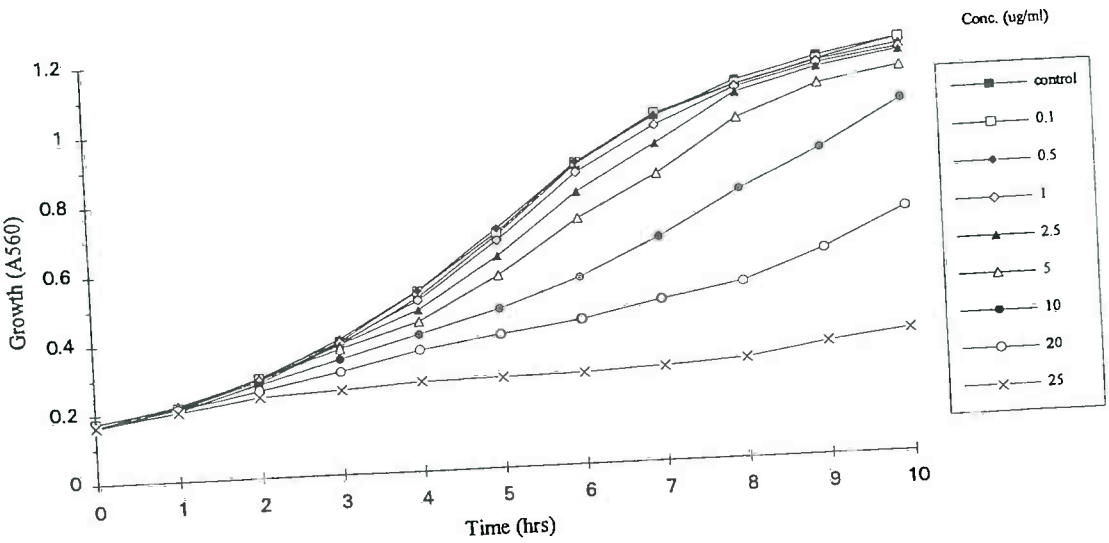

Figure 2i Effect of fusaranon-X on growth of *K. marxianus*.

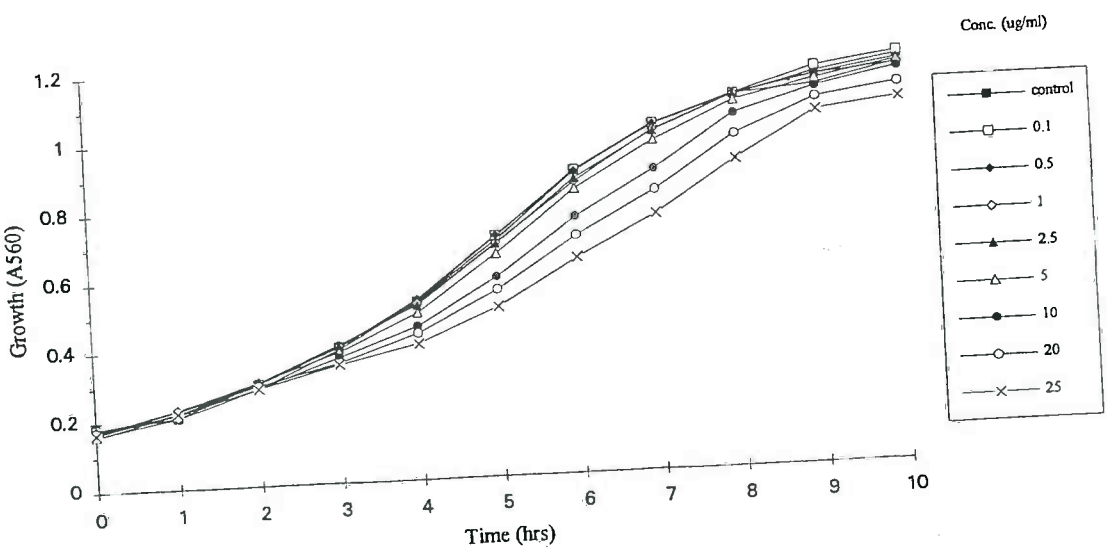

Figure 2j Effect of deoxynivalenol on growth of *K. marxianus*.

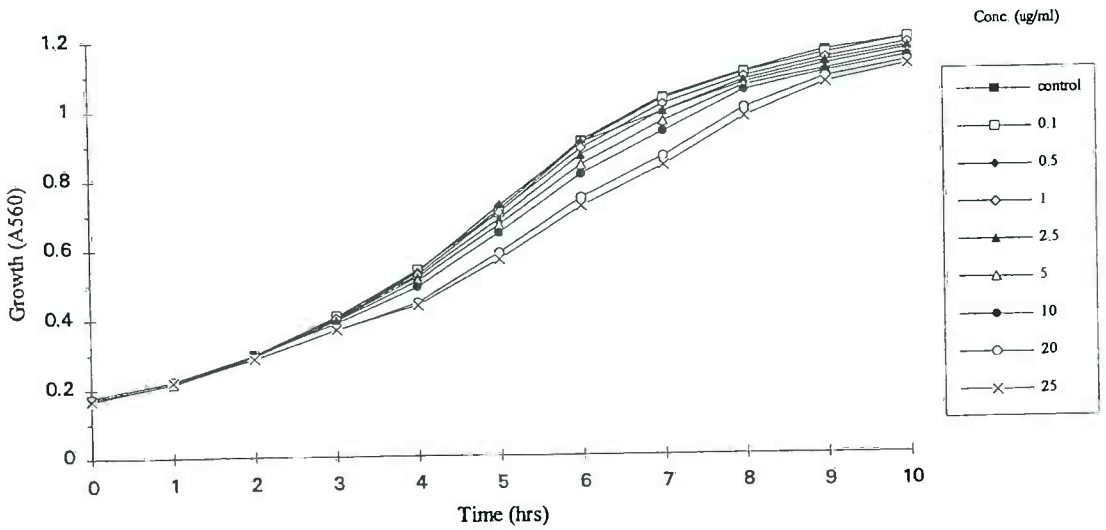

Figure 2k Effect of nivalenol on growth of *K. marxianus*.

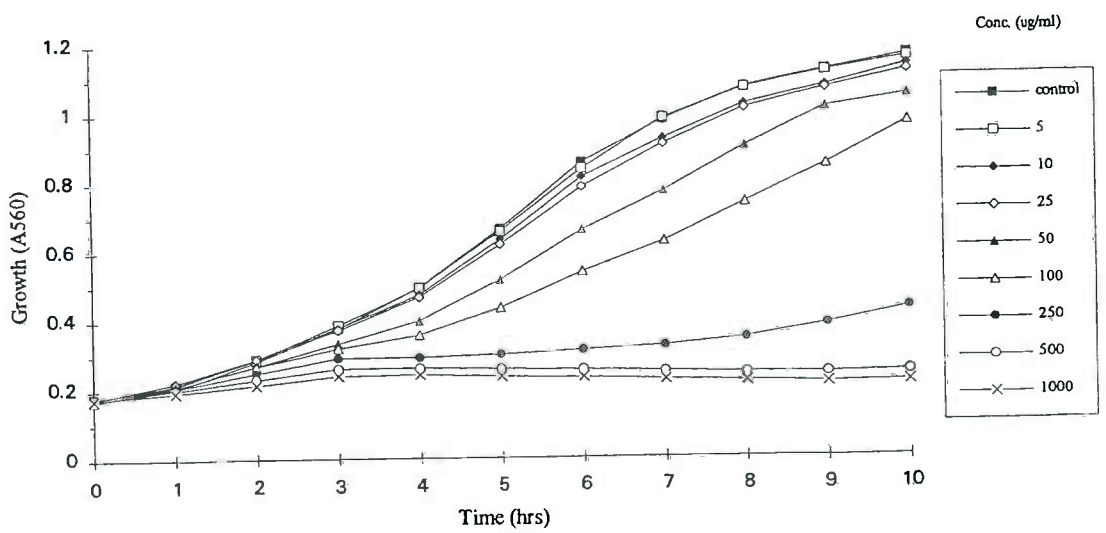

Figure 2l Effect of diacetoxyscirpenol on growth of *K. marxianus*.

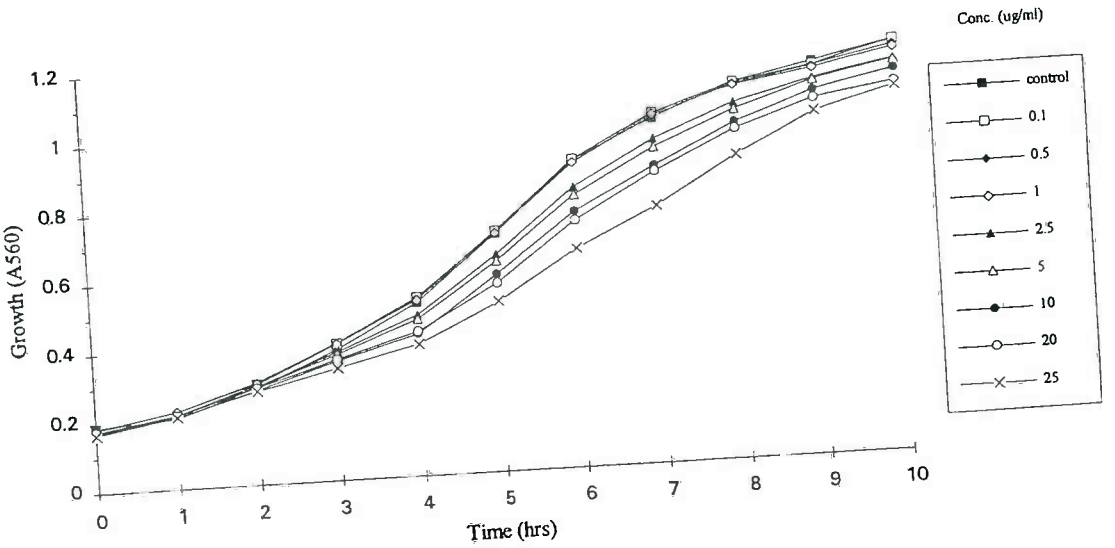

Figure 2m Effect of scirpentriol on growth of *K. marxianus*.

Conc. of toxin	Percentage inhibition of growth												
	SCR	DAS	AcT-2	T-2	HT-2	TRI	TET	NEO	FUS	NIV	DON	VER	ROR
0.1ng/ml												2	1
0.5ng/ml				0								1	1
1ng/ml				1	0							2	1
5ng/ml		0		3								20	7
10ng/ml		2		4	2							58	20
25ng/ml		4		14								91	65
50ng/ml		11		52	6							96	88
100ng/ml	-2	19	2	89	9	1	0	2	1	-2	-1	99	95
250ng/ml		73		96									
500ng/ml	1	93	5		18	-1	1	2	2	4	4		
1µg/ml	2	96	5		48	-1	0	3	3	2	4		
2.5µg/ml	4		11			5	0	7	5	3	2		
5µg/ml	4		20		97	8	1	9	8	6	3		
10µg/ml	10		60		98	8	2	15	17	6	3		
20µg/ml	7		91			15	3	56	47	7	9		
25µg/ml	11		93			16	3	93	81	8	13		

Table 2a Percentage inhibition of growth by the trichothecenes. Data used for the construction of dose-response curves in the structure-activity study. Figures are the average of at least 2 replicate wells, and were calculated using equation 3 (page 81).

Conc. of toxin	Percentage inhibition of β-galactosidase activity												
	SCR	DAS	AcT-2	T-2	HT-2	TRI	TET	NEO	FUS	NIV	DON	VER	ROR
0.1ng/ml												-2	7
0.5ng/ml				-1								3	3
1ng/ml				3	1							4	1
5ng/ml		-2		-1								103	56
10ng/ml		4		20	3							103	103
25ng/ml		3		100								103	103
50ng/ml		96		103	33							103	103
100ng/ml	-7	103	1	103	96	-2	1	-2	7	-1	3		
250ng/ml		103		103									
500ng/ml	-7	103	2		103	-3	2	3	-4	-1	-9		
1µg/ml	0	103	10		103	2	4	2	4	-2	4		
2.5µg/ml	-5		94			5	4	56	4	-9	-9		
5µg/ml	31		103		103	48	-4	88	83	2	3		
10µg/ml	96		103		103	91	1	98	103	8	6		
20µg/ml	95		103			98	0	103	103	36	94		
25µg/ml	98		103			98	0	103	103	78	100		

Table 2b Percentage inhibition of β-galactosidase activity by the trichothecenes. Data used for the construction of dose-response curves in the structure-activity study. Figures are the average of at least 2 replicate wells, and were calculated using equation 4 (page 82).

Toxin	Relative Toxicity					
	Inhibition of Growth			Inhibition of β-galactosidase		
	NEL	EC_{50}	MIC	NEL	EC_{50}	MIC
VER	1	1	1	1	1	1
ROR	1	2.25	2.5	1	2	2
T-2	1	6.25	5	5	6	10
HT-2	10	125	50	10	35	100
AcT-2	100	1000	>250	100	750	1000
NEO	100	2500	>250	500	1000	4000
TRI	1000	>3125	>250	1000	2500	>5000
TET	>25000	>3125	>250	>25000	>12500	>5000
DAS	100	22.5	10	6.5	15	20
SCR	100	>3125	>250	2500	3000	>5000
FUS	100	2750	>250	700	1750	4000
NIV	100	>3125	>250	4000	7000	>5000
DON	100	>3125	>250	4000	10500	5000

Table 2c Relative toxicity of the trichothecene mycotoxins. Relative toxicity being the relative toxin concentration required to elicit an effect in relation to verrucarin A (VER), the most potent of the trichothecenes tested.

APPENDIX 3

UPTAKE AND BINDING OF AFLATOXIN B_1 AND T-2 TOXIN BY *K. MARXIANUS* AND *B. MEGATERIUM*

Methanol	0%		0%		5%		8.5%		15%	
PMBS	0		15µg/ml		15µg/ml		15µg/ml		15µg/ml	
Extract	AFB_1 in each sample (ng)									
	$\bar{x} \pm sd$	% CV	$\bar{x} \pm sd$	% CV	$\bar{x} \pm sd$	% CV	$\bar{x} \pm sd$	% CV	$\bar{x} \pm sd$	% CV
Supernatant	4180.04 ± 40.45	0.97	3812.88 ± 129.99	3.41	3775.71 ± 11.57	2.93	4069.77 ± 60.61	1.49	4092.86 ± 150.26	3.67
Wash 1	440.40 ± 7.23	1.64	465.08 ± 46.69	10.04	460.25 ± 8.65	1.88	392.41 ± 16.90	4.31	378.63 ± 39.03	10.31
Wash 2	138.93 ± 5.42	3.90	129.99 ± 9.42	7.25	128.87 ± 10.29	7.98	118.69 ± 14.67	12.36	92.40 ± 14.14	15.31
Wash 3	62.15 ± 4.65	7.49	53.68 ± 7.33	13.65	47.95 ± 5.74	11.97	30.74 ± 6.39	20.79	38.42 ± 4.65	12.09
Wash 4	22.86 ± 2.54	11.11	14.63 ± 0.77	5.27	13.79 ± 0.30	2.21	12.28 ± 1.88	15.32	7.64 ± 0.70	9.17
Wash 5	9.52 ± 1.39	14.56	11.53 ± 1.64	14.23	8.50 ± 2.35	27.62	9.38 ± 1.75	18.63	6.10 ± 0.09	14.51
Cells	28.39 ± 3.07	10.81	24.79 ± 4.10	16.54	26.17 ± 5.43	20.75	17.84 ± 2.33	13.04	13.30 ± 0.74	5.58
	Percentage AFB_1 in each sample									
Sn	83.68 ± 0.81	0.97	83.69 ± 3.17	3.80	82.58 ± 2.74	3.32	91.67 ± 0.27 2.80	3.05	89.52 ± 3.64	4.06
Wash 1	8.82 ± 0.14	1.64	10.17 ± 0.98	9.65	10.07 ± 0.23	2.27	8.58 ± 0.34	3.92	8.28 ± 0.82	9.92
Wash 2	2.78 ± 0.11	3.90	2.84 ± 0.22	7.64	2.82 ± 0.21	7.59	2.60 ± 0.31	11.97	2.02 ± 0.30	14.92
Wash 3	1.24 ± 0.09	7.49	1.17 ± 0.16	14.03	1.05 ± 0.12	11.59	0.67 ± 0.14	20.41	0.84 ± 0.10	11.70
Wash 4	0.46 ± 0.05	11.11	0.32 ± 0.02	5.66	0.30 ± 0.01	2.60	0.27 ± 0.04	14.93	0.17 ± 0.01	8.78
Wash 5	0.19 ± 0.03	14.56	0.25 ± 0.04	14.62	0.19 ± 0.05	27.24	0.20 ± 0.04	18.25	0.13 ± 0.00	1.12
Cells	0.57 ± 0.06	10.81	0.54 ± 0.09	16.93	0.57 ± 0.12	20.37	0.40 ± 0.03	8.53	0.29 ± 0.02	5.19
% Recovery	98 ± 4	3.70	99 ± 3	2.81	98 ± 2	2.48	102 ± 2	1.86	101 ± 2	2.36

Table 3a Effect of methanol and PMBS on the uptake and binding of AFB_1 by *K. marxianus*.

Methanol	0%		5%		8.5%		15%	
Sample	AFB_1 in each sample (ng)							
	$\bar{x} \pm sd$	% CV	$\bar{x} \pm sd$	% CV	$\bar{x} \pm sd$	% CV	$\bar{x} \pm sd$	% CV
Supernatant	3230.04 ± 101.43	0.03	3379.84 ± 146.59	0.04	3348.97 ± 46.65	0.01	3301.94 ± 144.36	0.04
Wash 1	492.29 ± 10.05	0.02	472.08 ± 37.32	0.08	438.74 ± 1.15	0.00	392.56 ± 1.38	0.00
Wash 2	110.01 ± 15.55	0.14	92.68 ± 8.72	0.09	87.85 ± 11.72	0.13	88.23 ± 6.35	0.07
Wash 3	30.33 ± 4.32	0.14	22.21 ± 0.92	0.04	18.81 ± 2.04	0.11	22.98 ± 4.41	0.19
Wash 4	10.03 ± 2.32	0.23	11.82 ± 1.36	0.11	13.98 ± 2.80	0.20	15.74 ± 0.06	0.00
Wash 5	1.85 ± 0.53	0.29	1.70 ± 0.88	0.52	2.16 ± 0.11	0.05	2.12 ± 0.30	0.14
Cells	7.29 ± 1.06	0.15	11.06 ± 0.81	0.07	13.90 ± 1.36	0.10	11.92 ± 7.13	0.60
	Percentage AFB_1 in each sample							
Sn	73.01 ± 1.09	0.01	75.35 ± 1.780	0.02	74.69 ± 0.43	0.01	73.62 ± 1.77	0.02
Wash 1	11.13 ± 0.367	0.03	10.52 ± 0.062	0.06	9.79 ± 0.22	0.02	8.76 ± 0.14	0.02
Wash 2	2.49 ± 0.37	0.15	2.07 ± 0.015	0.07	1.96 ± 0.22	0.11	1.97 ± 0.01	0.05
Wash 3	0.69 ± 0.11	0.16	0.50 ± 0.01	0.02	0.42 ± 0.04	0.09	0.51 ± 0.01	0.17
Wash 4	0.23 ± 0.05	0.21	0.26 ± 0.03	0.01	0.31 ± 0.07	0.22	0.35 ± 0.01	0.02
Wash 5	0.04 ± 0.01	0.30	0.04 ± 0.02	0.053	0.05 ± 0.00	0.07	0.05 ± 0.01	0.12
Cells	0.16 ± 0.02	0.14	0.25 ± 0.02	0.090	0.31 ± 0.04	0.12	0.27 ± 0.16	0.61
% Recovery	87.75 ± 1.33	0.02	88.98 ± 2.55	0.03	87.52 ± 0.50	0.01	85.51 ± 1.65	0.02

Table 3b Effect of methanol on the uptake and binding of AFB_1 by *B. megaterium*.

Organism	*K. marxianus*		*B. megaterium*	
Sample	T-2 toxin in each sample (μg)			
	$\bar{x}$ ± sd	% CV	$\bar{x}$ ± sd	% CV
Supernatant	52.965 ± 1.246	2.35	43.420 ± 0.559	1.29
Wash 1	3.208 ± 0.437	13.63	3.391 ± 0.578	17.05
Wash 2	0.253 ± 0.017	6.61	0.677 ± 0.040	5.92
Wash 3	0.143 ± 0.034	23.52	0.141 ± 0.010	7.40
Wash 4	0.039 ± 0.006	14.66	0.041 ± 0.003	6.09
Wash 5	n.d.		n.d.	
Cells	0.013 ± 0.003	24.75	0.009 ± 0.001	1.11
Percentage T-2 toxin in each sample				
Sn	95.96 ± 2.359	2.46	83.90 ± 1.079	1.29
Wash 1	5.82 ± 0.797	13.70	6.55 ± 1.118	17.05
Wash 2	0.46 ± 0.029	6.43	1.31 ± 0.077	5.92
Wash 3	0.26 ± 0.061	23.70	0.27 ± 0.020	4.40
Wash 4	0.07 ± 0.010	14.64	0.08 ± 0.005	6.09
Wash 5	n.d.		n.d. ±	
Cells	0.02 ± 0.006	25.05	0.02 ± 0.002	11.11
% Recovery	102 ± 3	2.86	92 ± 2	1.89

n.d. = not detected

Table 3c Uptake and binding of T-2 toxin by K. marxianus and B. megaterium in the presence of 5% methanol.

ELSEVIER

Journal of Microbiological Methods 35 (1999) 207–218

Journal of Microbiological Methods

A novel colorimetric yeast bioassay for detecting trichothecene mycotoxins

Kathryn H. Engler[a,1], Ray Coker[b], Ivor H. Evans[a,*]

[a]University of Greenwich, Wellington Street, Woolwich, London, SE18 6PF, UK
[b]Natural Resources Institute, Chatham Maritime, Chatham, Kent, ME4 4TB, UK

Received 18 September 1998; accepted 14 December 1998

Abstract

A novel colorimetric microbial bioassay for toxicity has been developed; it shows particular sensitivity to trichothecene mycotoxins. The assay uses inhibition of expression of β-galactosidase activity within the yeast *Kluyveromyces marxianus* as a sensitive toxicity indicator, cultures remaining yellow, rather than turning deep green-blue, in the presence of X-gal, a chromogenic substrate. The assay is conducted in standard microtitre plates, permitting small volumes (160 μl) and many replicates, and can be scored either automatically by a plate-reader, or by eye. Factors likely to affect the efficacy of the bioassay, including carbon source, solvents, inoculum cell density, and the use of membrane-modulating agents (MMAs), were assessed. Polymyxin B nonapeptide was the most effective toxicity-enhancing MMA tested, enabling the trichothecene mycotoxin, verrucarin A, to be detected at a concentration of about 1 ng/ml. The assay's reproducibility was examined using polymyxin B sulfate, a cheaper MMA, and another trichothecene mycotoxin, T2 toxin: reproducibility and sensitivity were better for the β-galactosidase X-gal endpoint than for an alternative chromogenic toxicity indicator, the respiratory substrate 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT).

Keywords: Bioassay; Colorimetric; Mycotoxins; Toxicity; Yeast

1. Introduction

Over the past few decades it has been increasingly recognised by the agricultural and food industries that contamination of feeds and foodstuffs by toxic fungal metabolites — mycotoxins — is a significant problem for human and animal health and economic well-being (Coker, 1997). While precise and sensitive chemical methods are available for measuring toxin levels, these tend to employ expensive laboratory-based instrumentation. There is a clear need for inexpensive portable methods for detecting toxins, known and unknown, in the field, in the places where foods are grown, stored, processed and distributed, and in modestly equipped laboratory environments. Bioassays, especially those using microorganisms, are prime candidates for such methods, as they have the potential to be inexpensive, rapid, sensitive to a wide range of toxic substances — including as yet uncharacterised toxicants — and are free from ethical objections. The work described

*Corresponding author. Tel.: +44-181-331-8214; fax: +44-181-331-8305.
E-mail address: i.h.evans@gre.ac.uk (Ivor H. Evans)
[1]Present address: Respiratory and Systemic Infection Laboratory, Central Public Health Laboratory, 61, Colindale Avenue, London, NW9 5HT, UK.

0167-7012/99/$ – see front matter
PII: S0167-7012(98)00119-5

here was aimed at developing a microbial toxicity bioassay with these characteristics.

Microbiological bioassays for mycotoxins were surveyed by Yates in the mid-1980s (Yates, 1986) and by Watson and Lindsay (1982): they often rely on classical microbiological methodology, such as disk diffusion assays using Petri dishes, or culture flasks and relatively large amounts of growth medium. Typically, mycotoxins are detected as zones of inhibition on plates or spectrophotometrically, as reduction in absorbance of liquid cultures, compared to controls. Burmeister and Hesseltine (1970) and Schappert and Khachatourians (1983) found that strains of *Saccharomyces* were sensitive to T2 toxin, which is produced by *Fusarium sporotrichioides*, and to other fusariotoxins; T2 toxin has been implicated in the aetiology of Alimentary Toxic Aleukia (ATA) and, together with other trichothecenes, exhibits significant immunosuppressive activity (Coker, 1997). Khachatourians et al. went on to survey 12 different yeast genera, including *Saccharomyces* and *Kluyveromyces*, for T2 toxin sensitivity and found that a particular strain of *K. marxianus*, GK1005, was the most sensitive of all strains examined (Sukroongreeung et al., 1984). Schappert and Khachatourians (1984a) therefore proposed the use of *K. marxianus* GK1005 for use in a disk-diffusion type bioassay for T2 toxin. More recently published work (Madhyastha et al., 1994a,b) has confirmed the finding that *K. marxianus* is particularly sensitive to trichothecene mycotoxins, whereas bacterial species, for example *Bacillus brevis*, are very insensitive to these toxins. *K. marxianus* GK1005, with its proven response to trichothecene mycotoxins, was therefore selected for the work reported here.

As *K. marxianus* is a lactose-utilising yeast, with a well-characterised (intracellular) β-galactosidase activity, and as there are readily available chromogenic substrates for this enzyme, the β-galactosidase system was thought likely to provide a particularly promising approach to the development of a colorimetric bioassay for toxicity. An appealing feature of the use of β-galactosidase is that in many microorganisms the enzyme is inducible, so the toxicity signal — suppression of β-galactosidase activity — should be generated by a wide variety of toxicants interfering with any one of the many different cellular functions required for successful induction and expression of the β-galactosidase gene. Consideration was also given to the possible use of colorimetric assays for dehydrogenase enzymes involved in mitochondrial functions, as many toxicants are known to inhibit mitochondrial activities (Bruce et al., 1987). Other objectives of the bioassay development work reported here included miniaturisation to the scale of the microtitre dish, to reduce costs, increase testing capacity, and allow a degree of automation, and incorporation of agents to enhance sensitivity of detection, by facilitating toxin penetration into the cells. A yeast-based bioassay for trichothecenes, using microtitre dishes, has recently been reported (Binder et al., 1997), but no permeabilising agents were used, and the endpoint was inhibition of growth.

2. Materials and methods

2.1. Organism and cultivation medium

Kluyveromyces marxianus GK1005 was obtained from the Ministry of Fisheries and Food (Nobel House, 17 Smith Square, London, SW1P 3JR). The yeast was routinely maintained and grown on 1% (w/v) yeast extract, 1% (w/v) bacteriological peptone, and 2% (w/v) glucose (YPD), solidified when required with 2% (w/v) agar. Cultures used for inoculation in bioassay development and assessment experiments were prepared by adding a single-cell colony from an agar plate to 50 ml of liquid medium, identical to that to be used in the subsequent experiment, in a 250-ml flask. The culture was incubated in a rotary incubator for 16 h at 200 rev./min; *K. marxianus* cultures were always grown at 35°C.

2.2. Chemicals

Mycotoxins were from Sigma-Aldrich (Poole, Dorset, UK) and were dissolved in spectroscopic grade methanol to make stock solutions at, typically, 0.1 mg/ml. Absolute mycotoxin concentrations were verified by UV absorbance. Cetyl trimethyl ammonium bromide (CTAB) and polymyxin B sulphate (PMBS) were also from Sigma, whereas polymyxin B nonapeptide (PBN) was from ICN Biomedicals

(Thame, Oxfordshire, UK). Stock solutions of CTAB, PMBS and PBN, were prepared in water, filter-sterilised, and kept no more than a day. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), from Calbiochem Novabiochem (Beeston, Nottinghamshire, UK), was dissolved in dimethylformamide (DMF) at 100 mg/ml and stored at −20°C in the dark. This stock was used to make a working solution of 20 mg/ml X-gal in aqueous DMF (2 parts water:3 parts DMF) immediately before each assay; excess working solutions of X-gal were discarded after each experiment. *o*-Nitrophenyl-β-D-galactopyranoside (ONPGal) was from Sigma and was dissolved in water at 4 mg/ml immediately before use, and subsequently discarded. 3-(4,5-Dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) was from Sigma, and was prepared as a 5 mg/ml stock solution in phosphate-buffered saline (Dulbecco's formula — 'PBS', from Flow Laboratories, Irvine, UK) and filter-sterilised prior to use. All other chemicals were from Sigma.

2.3. Determination of cell density

Cell density was determined by measurement of absorbance at 560 nm using a Titertek Multiscan Plus MKII microtitre plate-reader from Labsystems (Basingstoke, Hampshire, UK). This was interfaced with an Amstrad microcomputer and Titresoft 1.01 software, also from Labsystems. A_{560} was calibrated by direct haemocytometer counts, and one A_{560} unit corresponded to 1.1×10^9 cells.

2.4. Determination of β-galactosidase activity

Cells were first permeabilised by the addition of 5 μl of 0.1% (w/v) sodium dodecyl sulfate (SDS) and 3 μl chloroform to each well of the microtitre plate. Either 1 μl of 100 mg/ml X-gal in DMF (in vivo experiments and experiments examining the effects of different carbon sources), 5 μl of 20 mg/ml X-gal in DMF (effects of altering glucose concentration and inoculum cell density), or 8 μl of 20 mg/ml X-gal in aqueous DMF (methanol and ethanol toxicity experiments and the standardised bioassay) were then added, the contents of the wells mixed, and the plates incubated at 35°C in a plate-shaker (Wesbart, UK) for a maximum of 30 min. The plates were read on the Titertek plate-reader using a test filter at 666 nm and a reference filter at 560 nm. β-Galactosidase activity was expressed as product formation ($A_{666} - A_{560}$), as a function of cell density (A_{560}) if required.

2.5. Determination of mitochondrial activity

Sixteen μl of MTT in PBS were added to each well of the microtitre plate and the plate was incubated statically at 35°C for 4 h, after which the medium was removed and 200 μl dimethylsulfoxide were added to each well. Thorough mixing and dissolution of the MTT-cleavage product was effected by repeated pipetting. The plates were read on the Titertek plate reader using a test filter at 560 nm and a reference filter at 666 nm.

2.6. Standard bioassay procedure

Growth medium (136 μl; 1% (w/v) yeast extract, 1% (w/v) bacteriological peptone, and 50 mM glucose — 'YPD-50'), containing PMBS to give a final assay concentration of 15 μg/ml, was added to the wells of a microtitre plate (sterile, flat-bottomed, MR24A from Dynex (formerly Dynatech) Labs, Billingshurst, West Sussex, UK). Eight μl of mycotoxin stock solution or methanol (controls) were then added, followed by 16 μl of yeast inoculum, to yield an initial assay cell density of $\sim 2 \times 10^8$ cells per ml. Blank wells contained 152 μl of medium and 8 μl of methanol. The plate was mixed, A_{560} was measured, then the plate was sealed with a Mylar plate sealer (ICN Biomedicals) and incubated at 35°C for the duration of the assay. Cell density was regularly monitored during the assay. When the control (mycotoxin-free) cultures reached stationary phase (after about 10 h, with an A_{560} of ca. 1.2), the cultures were assayed for enzyme activity (β-galactosidase or mitochondrial (MTT-cleavage) activity).

2.7. Spectrum of X-gal cleavage product

A single colony of *K. marxianus* GK1005 was inoculated into 50 ml 1% yeast extract, 1% bacteriological peptone, 50 mM lactose, in a 250-ml flask, and incubated for 16 h at 35°C and 200 rev./min in an orbital shaker. Samples (10 ml) of the

culture were transferred into each of two universal bottles, 0.2 ml of chloroform and 0.1 ml 0.1% w/v SDS were added to each bottle, to permeabilise the cells, 20 μl X-gal (100 mg/ml in DMF) were added to one bottle, and 20 μl DMF were added to the other. Both bottles were then incubated at 35°C and 200 rev./min in an orbital shaker, until indigo precipitate was clearly visible in the bottle containing X-gal. Using the solvent (DMF) control sample as a reference, the absorption spectrum of the X-gal-containing sample was then determined in a Lambda 3 spectrophotometer (Perkin-Elmer).

3. Results and discussion

3.1. Selection of chromogenic substrate for β-galactosidase

Preliminary experiments assessed the use of *o*-nitrophenyl-β-D-galactopyranoside (ONPGal) as a chromogenic β-galactosidase substrate, using an assay based on that of Miller (1972). These experiments showed that *K. marxianus* GK1005 does indeed express β-galactosidase activity when growing on carbon sources such as lactose and galactose, but the assay was rather cumbersome: because of poor cell permeability to ONPGal, a permeabilisation step using chloroform and SDS was required, and the colour of the reaction product, *o*-nitrophenol, needed to be intensified by addition of alkaline buffer at the end of the reaction (Engler, 1996). Another disadvantage was that *o*-nitrophenol is yellow, and thus similar in colour to YPD medium. Consequently, ONPGal was discarded as a β-galactosidase substrate for the bioassay.

Pearson et al. (1963) introduced the use of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) as a chromogenic β-galactosidase substrate, initially for histochemical work. X-gal is a colourless compound which β-galactosidase cleaves to a halogenated indoxyl which is then rapidly oxidised by the oxygen normally present in solution to a bis-indigo product that is intensely blue-green, insoluble, light-fast and stable. X-gal is now very widely used, especially in molecular genetic work, as a substrate for the β-galactosidase of *E. coli*. It was therefore decided to assess the use of X-gal in the *K. marxianus* GK1005 bioassay.

3.2. Use of X-gal in the bioassay

The recommended solvent for X-gal is dimethylformamide (DMF), so experiments were undertaken to examine the growth sensitivity of *K. marxianus* GK1005 to DMF. Fig. 1 is a representative result from these experiments, and shows that all levels of DMF tested were toxic, but that 1% v/v, though slowing growth, did not affect the final cell yield. Therefore, 1% v/v was selected as the highest DMF concentration that could be tolerated during the active growth phase of a bioassay. This level was important to establish because the original bioassay concept was to have all reagents, growth medium, cells, and potentially toxic samples, present at the beginning of the assay, both in order to simplify the assay operation as far as possible and to facilitate its development into a pre-packaged kit form.

When X-gal was used as an in vivo substrate — cleavage by the intracellular β-galactosidase enzyme requiring prior penetration of the cell by the substrate — it was found that appearance of detectable levels of the blue bis-indigo product took over 24 h. However, when ONPGal was used as the substrate, high levels of β-galactosidase were registered after 7 h growth, in identical culture conditions. Slow

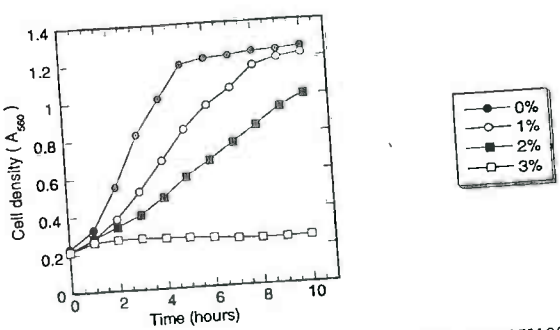

Fig. 1. Effect of DMF on growth of *K. marxianus* GK1005. Samples (140 μl) of overnight culture of GK1005 diluted 10-fold with fresh YPD-50 medium were inoculated into the wells of a microtitre dish. Identical volumes (10 μl) of water (●), or aqueous DMF (giving final concentrations of 1% v/v (○), 2% v/v (■), 3% v/v (□)) were added to individual wells and the plate was incubated for 10 h at 35°C, A_{560} of the wells being measured hourly.

uptake of X-gal by the cells is the likely explanation of the disparity. When the permeabilisation step employed in the ONPGal assay (treatment with chloroform and SDS) was used, the blue cleavage product of X-gal was clearly visible 30 min after permeabilisation, and β-galactosidase activity was detected in cultures after the same growth times as when using ONPGal. In order to minimise the response time of the assay it was decided to adopt a two-stage approach: initial incubation of cells in growth medium and potential toxicants for a time period known to produce high levels of β-galactosidase in controls, then visualisation of β-galactosidase via a permeabilisation step (which would itself damage or kill cells). Thus the β-galactosidase assay protocol adopted for the bioassay was: addition of 5 μl 0.1% w/v SDS and 3 μl chloroform to the microtitre plate well, followed by 8 μl of 20 mg/ml X-gal in aqueous DMF (found to give high product levels in derepressed control cells; Engler, 1996); the plate was then sealed with a Mylar plate-sealer, incubated in the shaker-incubator at 35°C for 30 min, and scanned for absorbance at 560 nm and 666 nm (see below).

It was also necessary to determine appropriate wavelengths for measuring the absorbance of the bis-indigo product of X-gal when using a microtitre plate-reader. The absorbance spectrum in the visible light region of bis-indigo was therefore determined, and showed an essentially single broad peak with a maximum at ca. 660 nm (Fig. 2). The Multiscan Plus Mk II microtitre plate reader used in the bioassay work permits measurement of sample absorbances at two wavelengths; 666 nm was used to measure bis-indigo absorption, whereas 560 nm was a suitable wavelength for estimating cell light-scattering and, consequently, cell density/growth. ($A_{666} - A_{560}$) was therefore used as a measure of β-galactosidase activity.

3.3. Effect of carbon source on β-galactosidase activity in GK1005

In microorganisms, β-galactosidase is typically an inducible enzyme, with lactose or allolactose being the inducer molecule, but glucose can also be involved in regulation of the enzyme, through glucose (catabolite repression) effects. The pattern of

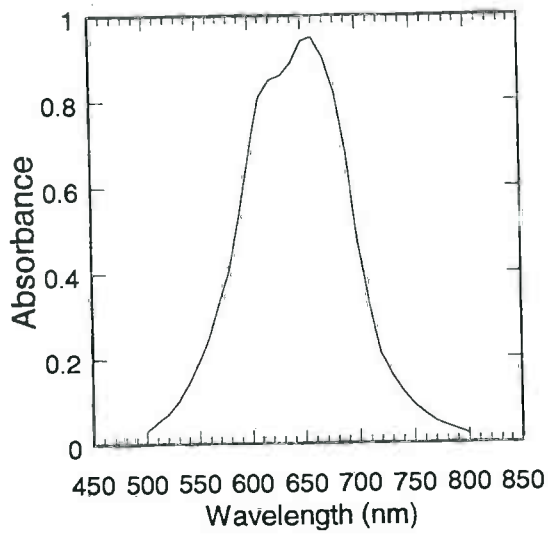

Fig. 2. Absorption spectrum of cleavage product produced by β-galactosidase hydrolysis of X-gal.

β-galactosidase expression was examined in *K. marxianus* GK1005 growing in batch cultures, with three different carbon sources — lactose, galactose and glucose. Representative results are given in Fig. 3a and b, and show that the outcomes were quite similar for all three types of culture, the growth curves being typically sigmoid, and β-galactosidase activity exhibiting low initial activity in lag phase, complete suppression of activity during log phase, then sharp induction during entry into stationary phase, levels continuing to rise even when growth had virtually ceased (9–10 h). These results indicated that glucose could be an acceptable carbon source for the bioassay, as the culture does, in effect, switch from no detectable activity (mid-log) to high levels of activity (stationary), and so could give a good colorimetric indication of growth inhibition/toxicity.

An experiment was performed to examine the relationship between initial glucose concentration in the growth medium and derepression of β-galactosidase activity. The results in Fig. 4 show that between 25 mM and 200 mM the pattern of repression, in the first hour, was virtually identical, and that, not unexpectedly, β-galactosidase activity was derepressed in the four cultures in order of increasing glucose concentration. Since both 25 mM and 50

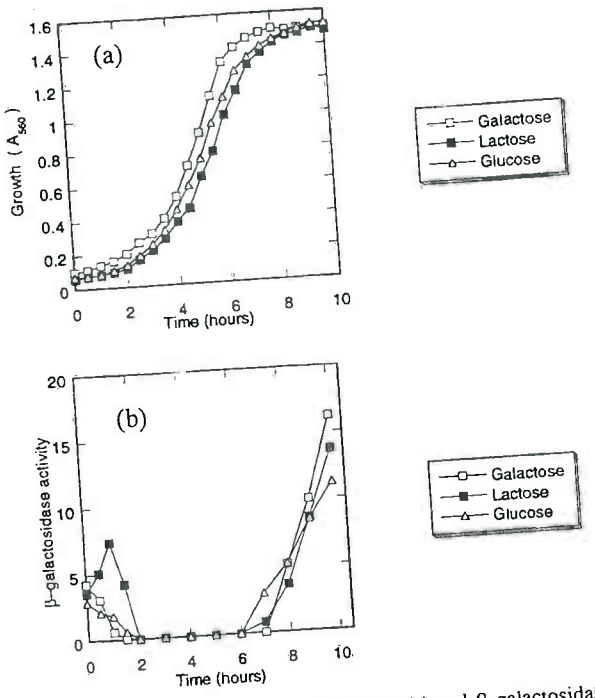

Fig. 3. Effect of carbon source on growth (a) and β-galactosidase activity $((A_{666} - A_{560})/A_{560}$ per min, × 100) (b) of *K. marxianus* GK1005. Carbon sources: galactose (□), lactose (■), and glucose (△). All wells were in triplicate, and datum points are the mean of three values.

mM glucose gave very similar final derepression levels of β-galactosidase at 10 h, 50 mM was chosen as the standard glucose level in the bioassay, as it is closer to the 2% w/v level normally used in yeast growth media.

3.4. Effect of initial cell density/inoculum size

It was anticipated that while lower starting cell densities would have the merit of higher toxin-cell ratios, the time taken to reach substantial β-galactosidase levels in controls would be longer, so extending the time needed for the bioassay. β-Galactosidase derepression was monitored in four cultures with different initial cell densities and, on the basis of the results (see Fig. 5), 2×10^8 cells/ml was selected as an appropriate starting cell density for the bioassay.

3.5. Choice of mycotoxin solvent

Another factor relevant to the bioassay is the nature and acceptable level of solvent for delivery of mycotoxin standards or potentially mycotoxin-contaminated food samples. Methanol and ethanol are both cheap and effective solvents for compounds such as mycotoxins, so the growth inhibitory effects of these two solvents on *K. marxianus* GK1005 were therefore tested in the experiment reported in Fig. 6. Solvent effects on β-galactosidase activity were also monitored and are reported in the same figure. Evidently, methanol is less inhibitory, in respect of both parameters, than ethanol, and can be used up to 5% v/v in the bioassay without affecting either

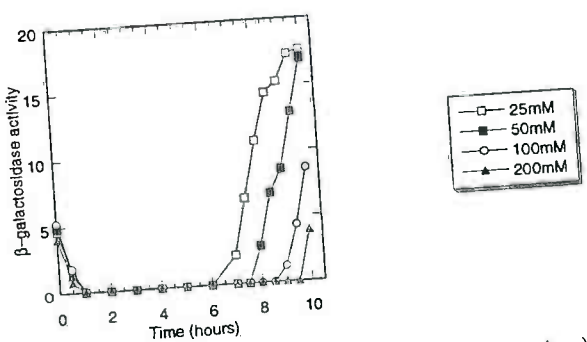

Fig. 4. Derepression of β-galactosidase activity $((A_{666} - A_{560})/A_{560}$ per min, × 100) in cultures of *K. marxianus* GK1005 with different initial glucose concentrations: 25 mM (□), 50 mM (■), 100 mM (○), and 200 mM (▲). All wells were in triplicate, and datum points are the mean of three values.

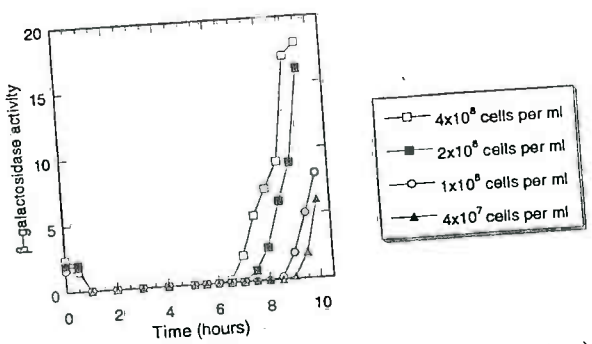

Fig. 5. Derepression of β-galactosidase activity $((A_{666} - A_{560})/A_{560}$ per min, × 100) in cultures of *K. marxianus* GK1005 with different initial cell concentrations: 4×10^8/ml (□), 2×10^8/ml (■), 1×10^8/ml (○), 4×10^7/ml (▲). All wells were in triplicate, and datum points are the mean of three values.

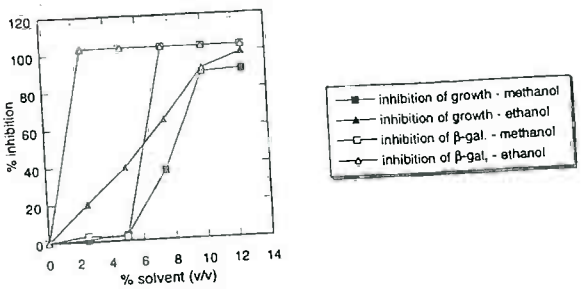

Fig. 6. Inhibition of growth and β-galactosidase activity of cultures of *K. marxianus* GK1005 by methanol and ethanol: inhibition of growth (■) and β-galactosidase activity (□) by methanol, and inhibition of growth (▲) and β-galactosidase activity (△) by ethanol. Percentage inhibition of growth was calculated as $100 - ((\Delta A(\text{alcohol})/\Delta A(\text{control})) \times 100)$ where $\Delta A(\text{alcohol})$ = average change in absorbance of wells containing a given alcohol concentration (final A_{560} − start A_{560}), and $\Delta A(\text{control})$ = average change in absorbance of control wells (final A_{560} − start A_{560}). Percentage inhibition of β-galactosidase activity was calculated as $100 - (A_{666} - A_{560}(\text{alcohol})/A_{666} - A_{560}(\text{control})) \times 100)$ where $A_{666} - A_{560}(\text{alcohol})$ = average absorbance of the cleaved X-gal product at a given alcohol concentration, and $A_{666} - A_{560}(\text{control})$ = average absorbance of the cleaved X-gal product for the control wells. All wells were in triplicate.

growth or β-galactosidase activity significantly. Methanol is also a suitable solvent choice for the bioassay, as it is often used in the isolation of mycotoxins from potentially contaminated food samples, prior to further analysis. An interesting feature of the results presented in Fig. 6 is that β-galactosidase activity is appreciably more sensitive to inhibition than growth: it was indeed hoped that suppression of β-galactosidase activity would prove a more sensitive indicator of toxicity than growth.

3.6. Assessment of membrane-modulating agents (MMAs)

Chemicals (membrane-modulating agents or MMAs) which interact with cell (especially plasma) membranes, enhancing permeability, have previously been used to enhance bioassay sensitivity to toxins. For example, Schappert and Khachatourians (1984b) examined the effects of ethanol, Triton X-100, and cetyl trimethylammonium bromide (CTAB) on the sensitivity of growth of *Saccharomyces* spp. to the trichothecene T2 toxin; they found that CTAB, in particular, significantly increased sensitivity. Schappert and Khachatourians then went on to incorporate CTAB in a disk diffusion bioassay for T2 toxin, based on *K. marxianus* GK1005 (Schappert and Khachatourians, 1984a). Boguslawski (1985) showed that the cyclic peptide moiety of polymyxin B sulfate (PMBS), polymyxin B nonapeptide (PBN), significantly enhanced the sensitivity of *Saccharomyces cerevisiae* to antibiotics and inhibitors such as erythromycin, rifampicin, and ethidium bromide. Subsequently, Connolly and Corry (1990) looked at promotion of sensitivity of a conductimetric *K. marxianus* bioassay by three MMAs, CTAB, PMBS and PBN. They found increased sensitivity with all three MMAs, the order of effectiveness being PBN ≈ PMBS > CTAB. In later bioassay work using *K. marxianus*, MMAs were not employed (Madhyastha et al., 1994a,b). In the work reported here, experiments were undertaken to assess the efficiency of CTAB, PMBS and PBN on enhancing two, somewhat different, outputs of the bioassay: growth, as measured by A_{560}, and β-galactosidase activity, as

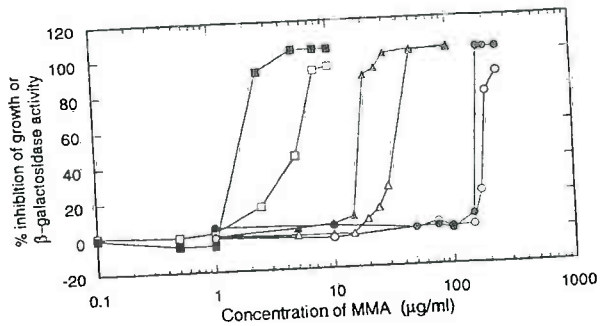

Fig. 7. Inhibition of growth of and β-galactosidase activity of *K. marxianus* GK1005 by the membrane-modulating agents CTAB, PMBS and PBN: growth inhibition by CTAB (□), PMBS (△), and PBN (○), and β-galactosidase inhibition by CTAB (■), PMBS (▲), and PBN (●). Percentage inhibition of growth was calculated as $100 - (\Delta A(\text{MMA})/\Delta A(\text{control}) \times 100)$ where $\Delta A(\text{MMA})$ = average change in absorbance of wells containing a given MMA concentration (final A_{560} − start A_{560}), and $\Delta A(\text{control})$ = average change in absorbance of control wells (final A_{560} − start A_{560}). Percentage inhibition of β-galactosidase activity was calculated as $100 - (A_{666} - A_{560}(\text{alcohol})/A_{666} - A_{560}(\text{control}) \times 100)$ where $A_{666} - A_{560}(\text{MMA})$ = average absorbance of the cleaved X-gal product at a given MMA concentration, and $A_{666} - A_{560}(\text{control})$ = average absorbance of the cleaved X-gal product for the control wells. All wells were in triplicate.

measured by $(A_{666} - A_{560})$. Firstly, in order to establish useable concentrations for the MMAs, the inhibitory effects per se of the three agents were determined. The experiment was carried out in microtitre dishes, each well being replicated three times. The results are shown as % inhibition curves in Fig. 7. All three agents were capable of completely inhibiting both growth and β-galactosidase activity at appropriate concentrations, the order of toxicity being PBN < PMBS < CTAB. The data indicated that maximal concentrations that could be used in the bioassay without significantly inhibiting growth or β-galactosidase activity were 1 μg/ml for CTAB, 15 μg/ml for PMBS and 150 μg/ml for PBN. The value for CTAB is comparable to the previous findings — 0.5 μg/ml in the work by Schappert and Khachatourians (1984a) and around 1 μg/ml for the conductimetric studies (Connolly and Corry, 1990). Again, for the conductimetric bioassay, Connolly and Corry (1990) found roughly comparable upper tolerance limits for PMBS and PBN — 25 μg/ml and 100 μg/ml, respectively.

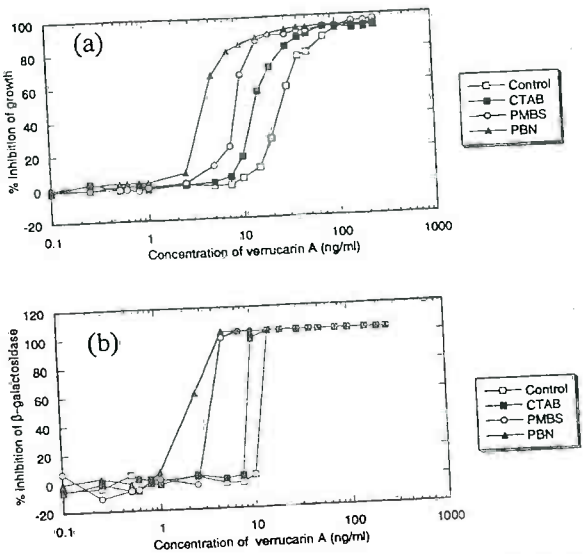

Fig. 8. Dose–response curves for the inhibition of growth (a) and β-galactosidase activity (b) of cultures of *K. marxianus* GK1005 by the mycotoxin verrucarin A in the presence of three different MMAs: control (□), 1 μg/ml CTAB (■), 15 μg/ml PMBS (○), and 150 μg/ml PBN (▲). Percentage inhibition of growth was calculated as $100 - (\Delta A(\text{toxin})/\Delta A(\text{control}) \times 100)$ where ΔA(toxin) = average change in absorbance of wells containing a given toxin concentration (final A_{560} − start A_{560}), and ΔA(control) = average change in absorbance of control wells (final A_{560} − start A_{560}). Percentage inhibition of β-galactosidase activity was calculated as $100 - (A_{666} - A_{560}(\text{toxin})/A_{666} - A_{560}(\text{control}) \times 100)$ where $A_{666} - A_{560}(\text{toxin})$ = average absorbance of the cleaved X-gal product at a given toxin concentration, and $A_{666} - A_{560}(\text{control})$ = average absorbance of the cleaved X-gal product for the control wells. All wells were in triplicate.

The usefulness of the three MMAs in enhancing sensitivity to a mycotoxin was then tested. The trichothecene verrucarin A was selected for this test, earlier results having indicated that, in a non-colorimetric yeast bioassay, it is one of the more potent trichothecenes (Dell, 1993). Microtitre plates were set up with a range of verrucarin A concentrations (0.1 ng/ml to 250 ng/ml, final bioassay concentration) in triplicate; replicate series contained either 1 μg/ml CTAB, 15 μg/ml PMBS, or 150 μg/ml PBN, and there were also a number of control wells, also in triplicate. The plates were incubated for a total of 10 h at 35°C, cell density (A_{560}) being monitored regularly, and, at the end of the incubation, β-galactosidase activity was determined by the standard procedure. Dose–response curves were

Table 1
Effect of MMAs on verrucarin A toxicity to *K. marxianus* GK1005

MMA	Inhibition of growth by verrucarin A			Inhibition of β-galactosidase activity by verrucarin A		
	NEL (ng/ml)	EC_{50} (ng/ml)	MIC (ng/ml)	NEL (ng/ml)	EC_{50} (ng/ml)	MIC (ng/ml)
Control	10	28	100	10	11	15
CTAB (1 μg/ml)	5	13	75	7.5	8.5	15
PMBS (15 μg/ml)	2.5	9	40	2.5	3.5	7.5
PBN (150 μg/ml)	1	4	25	1	2	5

NEL (nil effect level) is the highest toxin concentration at which no inhibition is detected; EC_{50} (effective concentration, 50) is the toxin concentration giving 50% inhibition; MIC (minimal inhibitory concentration) is the lowest concentration of toxin giving 100% inhibition.

constructed for the inhibition of growth and β-galactosidase activity and are shown in Fig. 8a and b. Evidently all three MMAs potentiate verrucarin A toxicity, but they vary in their potency: CTAB is least effective and PBN is most effective. Comparison of Fig. 8a and Fig. 8b also shows that inhibition of β-galactosidase activity is a more sensitive indicator of toxicity than inhibition of growth. This is clearly demonstrated in the numerical comparison of the data sets for the two parameters given in Table 1. Three different features of inhibition were used for comparison of the dose–response curves. These are defined here as NEL — nil-effect level — the highest toxin concentration at which no inhibition is detected; EC_{50} — the concentration of toxin giving 50% inhibition; MIC (minimal inhibitory concentration) — the lowest concentration of toxin giving 100% inhibition. On all three measures the MMAs tested increased the inhibitory potency of verrucarin A. PBN, the most effective MMA, increased the bioassay sensitivity between threefold (MIC for β-galactosidase) and about 10-fold (NEL for β-galactosidase). PBN was about twice as effective as PMBS, which was about twice as effective as CTAB, which enhanced toxicity about twofold, compared with the solvent control. The lowest concentration of verrucarin A that could be detected, using PBN and a β-galactosidase endpoint, was about 1 ng/ml. This can be compared with 10 ng/ml for a recent bioassay using swine kidney cells (Hanelt et al., 1994), indicating that the PBN–yeast bioassay developed here can be more sensitive than some tissue culture bioassays — which are usually considered to be among the most sensitive of all bioassays. However, because of the cost of PBN, the cheaper PMBS was used in all further experiments evaluating the bioassay.

3.7. Reproducibility of the bioassay

The bioassay's reproducibility was assessed using T2 toxin, as this has been perhaps the most widely used trichothecene in testing and developing various bioassays. As well as growth and β-galactosidase, a third parameter was used as a toxicity indicator: this was respiratory activity, as registered by the activity of mitochondrial succinate dehydrogenase. This enzyme can be assayed through its ability to cleave the tetrazolium ring of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), to MTT formazan, an insoluble dye with a λ_{max}, after solubilisation, of 560 nm. A wide variety of toxicants have

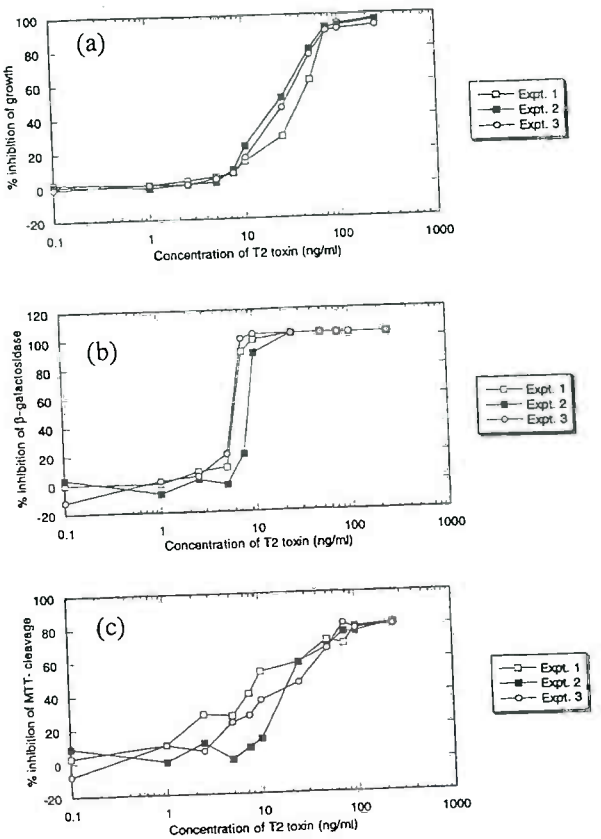

Fig. 9. Dose–response curves for the inhibition of growth (a), β-galactosidase activity (b), and MTT-cleavage activity (c), of cultures of *K. marxianus* GK1005 by the mycotoxin T2-toxin, for three independent experiments: experiment 1 (□), experiment 2 (■), and experiment 3 (○). Percentage inhibition of growth was calculated as $100 - (\Delta A(\text{toxin})/\Delta A(\text{control}) \times 100)$ where $\Delta A(\text{toxin})$ = average change in absorbance of wells containing a given toxin concentration (final A_{560} − start A_{560}), and $\Delta A(\text{control})$ = average change in absorbance of control wells (final A_{560} − start A_{560}). Percentage inhibition of β-galactosidase activity was calculated as $100 - (A_{666} - A_{560}(\text{toxin})/A_{666} - A_{560}(\text{control}) \times 100)$ where $A_{666} - A_{560}(\text{toxin})$ = average absorbance of the cleaved X-gal product at a given toxin concentration, and $A_{666} - A_{560}(\text{control})$ = average absorbance of the cleaved X-gal product for the control wells. Percentage inhibition of MTT-cleavage activity was calculated as $100 - (A_{560} - A_{666}(\text{toxin})/A_{560} - A_{666}(\text{control}) \times 100)$ where $A_{560} - A_{666}(\text{toxin})$ = average absorbance of the product of cleaved MTT at a given toxin concentration, and $A_{560} - A_{666}(\text{control})$ = average absorbance of the product of cleaved MTT for the control wells. All wells were in triplicate.

been shown to interfere with mitochondrial function, the mitochondrion often being a primary target (Bruce et al., 1987), so it was of interest to compare the responses of these two rather different colorimetric toxicity indicators. The bioassay, using the three parameters as toxicity indicators, was repeated on three different occasions, the effects of T2 concentrations between 0.1 μg/ml and 250 μg/ml being monitored. In each experiment each toxin concentration was assessed in triplicate, control wells also being triplicated. This allowed both within-assay and between-assay variation to be estimated. The growth of *K. marxianus* GK1005 was monitored over 10 h, after which β-galactosidase and MTT cleavage activities were determined. The mean ($\bar{x}$), standard deviation (S.D.) and coefficient of variation (%CV) for replicate wells for the three toxicity parameters were determined to assess within-assay variation; $\bar{x}$, S.D. and %CV for all replicate wells from the three separate experiments, were also determined, to assess between-assay variation (all numerical data given in Engler, 1996). Percentage inhibition of growth, β-galactosidase activity, and MTT cleavage activity were calculated for the three separate experiments

Table 2
NEL, EC_{50} and MIC concentrations of T2 toxin determined from the dose–response curves (Fig. 9a–c) produced from assays performed on three separate occasions, using *K. marxianus* GK1005

Endpoint	Experiment 1			Experiment 2			Experiment 3		
	NEL (ng/ml)	EC_{50} (ng/ml)	MIC (ng/ml)	NEL (ng/ml)	EC_{50} (ng/ml)	MIC (ng/ml)	NEL (ng/ml)	EC_{50} (ng/ml)	MIC (ng/ml)
Growth	1.0	40	100	2.0	24	100	1.0	28	100
β-Galactosidase	1.0	6	25	5	8.5	25	0.75	5.8	10
Cell viability	<0.1	9	>250	1.0	20	>250	1.8	28	>250

NEL (nil effect level) is the highest toxin concentration at which no inhibition is detected; EC_{50} (effective concentration, 50) is the toxin concentration giving 50% inhibition; MIC (minimal inhibitory concentration) is the lowest concentration of toxin giving 100% inhibition.

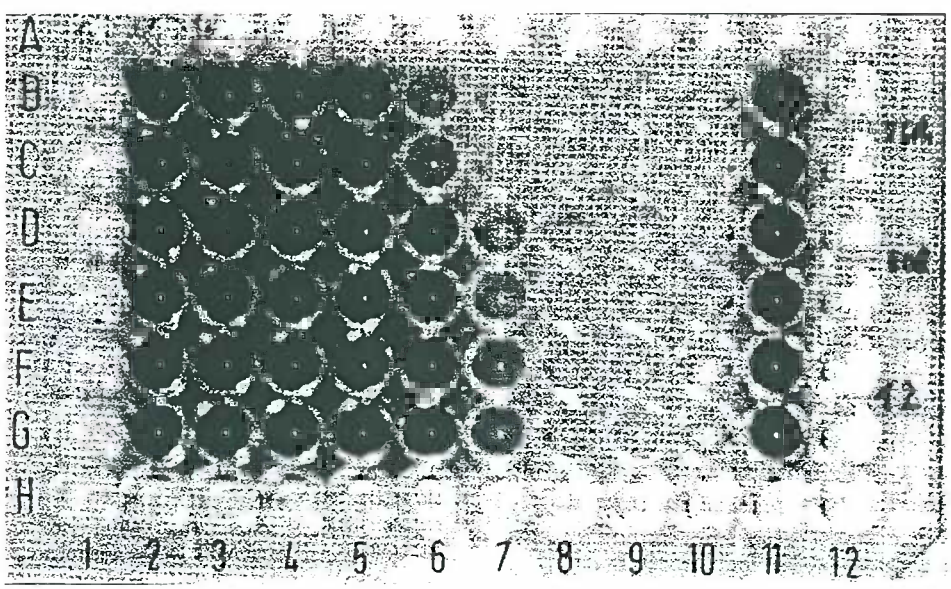

Fig. 10. Photograph of a bioassay result using the methodology developed and described here: verrucarin A — rows B and C; roridin A — rows D and E; T2 toxin — rows F and G. Concentrations of verrucarin A and roridin A were 0, 0.1, 0.5, 1.0, 5.0, 10, 25, 50, 100 and 0 ng/ml in columns 2–11, respectively. Concentrations of T2 toxin were 0, 0.5, 1.0, 5.0, 10, 25, 50, 100, 250 and 0 ng/ml, in the same columns.

and were used to construct the dose–response curves given in Fig. 9a–c. For growth, within-assay variation (%CV) was $< 5\%$ in most cases and $< 10\%$ in all cases; between-assay variation was greater, with %CV $> 10\%$ often seen. In the case of β-galactosidase activity, within-assay variation was low (%CV mainly $< 10\%$), but, again, between-assay variation was much greater. By contrast, MTT-cleavage showed high levels of both within- and between-assay variation. Comparison of NEL, EC_{50} and MIC for the three parameters (Table 2), as well as inspection of Fig. 9a–c, shows that inhibition of β-galactosidase is the clearest and most sensitive toxicity indicator, with a minimum detection limit of around 5 ng/ml. This is approaching the sensitivity shown by tissue-culture assays such as that of Porcher et al. (1987) using murine lymphocytes, where the sensitivity is about 1 ng/ml. Other yeast-based bioassays have reported T2 sensitivity of 40 ng/ml (Schappert and Khachatourians, 1984a) and 20 ng/disk (Madhyastha et al., 1994b) in disk-diffusion bioassays, 5 ng/ml using conductimetry (Connolly and Corry, 1990), and 90 ng/ml using microtitre dishes and a growth inhibition endpoint (Binder et al., 1997).

To conclude, a novel yeast bioassay has been developed which signals toxicity colorimetrically. An example of the bioassay in use is shown in Fig. 10. The bioassay is very sensitive to at least some potential mycotoxin contaminants of food and feed, and may therefore be particularly suitable for adaptation into kit form, subject to the performance of the bioassay in the presence of food and feed matrices. The assay shows acceptable reproducibility and sensitivity comparable to the levels given by tissue-culture trichothecene bioassays. It is likely that sensitivity can be improved further by varying factors such as the use of MMAs and inoculum cell density.

Acknowledgements

This publication is an output from a research project partly funded by the Department for International Development of the United Kingdom. However, the Department for International Development can accept no responsibility for any information provided or views expressed. The authors would also like to acknowledge the award of an SERC-CASE studentship to K.E, and the help of the staff of the mycotoxin section, NRI, in particular Victor Medlock and John Gibbs, and also Geoff Cooper, University of Greenwich.

References

Binder, J., Horvath, E.M., Heidegger, J., et al., 1997. A bioassay for comparison of the toxicity of trichothecenes and their microbial metabolites. Cereal Res. Commun. 25, 489–491.

Boguslawski, G., 1985. Effects of polymyxin B nonapeptide on growth and permeability of the yeast *Saccharomyces cerevisiae*. Mol. Gen. Genet 199, 401–405.

Bruce, I.J., Evans, I.H., Wilkie, D., 1987. Testing for selective toxicity and mutagenicity against the mitochondrion in *Saccharomyces cerevisiae*. Soc. Appl. Bacteriol. Technical Handbook Series 23, 109–135.

Burmeister, H.R., Hesseltine, C.W., 1970. Biological assays for two mycotoxins produced by *Fusarium tricinctum*. Appl. Microbiol. 20, 437–440.

Coker, R.D., 1997. Mycotoxins and their control: constraints and opportunities. NRI Bulletin 73. Natural Resources Institute, Central Avenue, Chatham Maritime, Chatham, Kent, ME4 4TB.

Connolly, P., Corry, J.E.L., 1990. Effect of polymyxin B nonapeptide and polymyxin B sulphate on trichothecene mycotoxin sensitivity of yeast using a conductimetric instrument. Int. J. Food Microbiol. 10, 73–90.

Dell, M.P.K., 1993. The Development of Methods for the Quality Control of Foodstuffs Contaminated with Mycotoxins. Ph.D. Thesis, University of Greenwich, London.

Engler, K., 1996. Development and Assessment of a Yeast Bioassay for the Detection of Mycotoxins. Ph.D. Thesis, University of Greenwich, London.

Hanelt, M., Gareis, M., Kollarczik, B., 1994. Cytotoxicity of mycotoxins evaluated by the MTT-cell culture assay. Mycopathologia 128, 167–174.

Madhyastha, M.S., Marquardt, R.R., Masci, A., Borsa, J., Frohlich, A.A., 1994a. Comparison of toxicity of different mycotoxins to several species of bacteria and yeasts: use of *Bacillus brevis* in a disc diffusion assay. J. Food Prot. 57, 48–53.

Madhyastha, M.S., Marquardt, R.R., Frohlich, A.A., Borsa, J., 1994b. Optimisation of yeast bioassay for trichothecene mycotoxins. J. Food Prot. 57, 490–495.

Miller, J.H., 1972. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Pearson, B., Wolf, P.L., Vasquez, J., 1963. A comparative study of a series of new indolyl compounds to localise β-galactosidase in tissues. Lab. Invest. 12, 1249–1259.

Porcher, J.M., Lafarge-Frayssinet, C., Frayssinet, C., Nurie, A., Melcion, D., Richard-Molard, D., 1987. Determination of cytotoxicity of trichothecenes in corn by cell culture assay. J. Assoc. Offic. Anal. Chem. 70, 844–849.

Schappert, K.T., Khachatourians, G.G., 1983. Effect of fusariotoxin T-2 on *S. cerevisiae* and *S. carlsbergensis*. Appl. Environ. Microbiol. 45, 862–867.

Schappert, K.T., Khachatourians, G.G., 1984a. A yeast bioassay for T2-toxin. J. Microbiol. Methods 3, 43–46.

Schappert, K.T., Khachatourians, G.G., 1984b. Influence of membrane on T2-toxin toxicity in *Saccharomyces* spp. Appl. Environ. Microbiol. 47, 681–684.

Sukroongreeung, S., Schappert, K.T., Khachatourians, G.G., 1984. Survey of the sensitivity of twelve yeast genera towards T2-toxin. Appl. Environ. Microbiol. 48, 416–419.

Watson, D.H., Lindsay, D.G., 1982. A critical review of biological methods for the detection of fungal toxins in foods and feedstuffs. J. Sci. Food Agric. 33, 59–67.

Yates, I.E., 1986. Bioassay systems and their use in diagnosis of mycotoxicoses. In: Richards, J.L., Thurston, J.R. (Eds.), Diagnosis of Mycotoxicoses. Martinus Nijhoff, Dordrecht, Holland, pp. 333–381.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1999, p. 1854–1857
0099-2240/99/$04.00+0

Vol. 65, No. 5

A Colorimetric Technique for Detecting Trichothecenes and Assessing Relative Potencies

KATHRYN H. ENGLER,[1]† RAYMOND D. COKER,[2] AND IVOR H. EVANS[1]*

University of Greenwich, Woolwich, London, SE18 6PF,[1] and Natural Resources Institute, Chatham, Kent, ME4 4TB,[2] United Kingdom

Received 4 August 1998/Accepted 3 March 1999

We tested a novel colorimetric toxicity test, based on inhibition of β-galactosidase activity in the yeast *Kluyveromyces marxianus*, for sensitivity to a range of mycotoxins. A variety of trichothecene mycotoxins could be detected. The order of toxicity established with this bioassay was verrucarin A > roridin A > T-2 toxin > diacetoxyscirpenol > HT-2 toxin > acetyl T-2 toxin > neosolaniol > fusarenon X > T-2 triol > scirpentriol > nivalenol > deoxynivalenol > T-2 tetraol. The sensitivity of detection was high, with the most potent trichothecene tested, verrucarin A, having a 50% effective concentration (concentration of toxin causing 50% inhibition) of 2 ng/ml. Other mycotoxins (cyclopiazonic acid, fumonisin B_1, ochratoxin A, patulin, sterigmatocystin, tenuazonic acid, and zearalenone) could not be detected at up to 10 μg/ml, nor could aflatoxins B_1 and M_1 be detected at concentrations up to 25 μg/ml. This test should be useful for trichothecene detection and for studies of relevant interactions—both between trichothecenes themselves and between trichothecenes and other food constituents.

Bioassays have become increasingly useful for mycotoxin detection (20, 21) as a precursor to chemical analysis. Bioassays provide a rapid means for screening samples and allow the analyst to make an informed decision when selecting a more detailed chemical analysis procedure (2). *Kluyveromyces marxianus* (GK1005) is particularly sensitive to the trichothecene mycotoxins (17). This yeast has been used in disk diffusion bioassays (15) and a conductimetric bioassay (4) for the detection of trichothecene mycotoxins.

We recently developed a colorimetric bioassay that uses the inhibition of expression of β-galactosidase as a toxicity indicator (5, 6). With a colorimetric substrate used for the β-galactosidase, toxicity is registered by the *K. marxianus* cultures remaining yellow, rather than turning blue-green, allowing both visual and spectrophotometric detection. Our objectives were (i) to evaluate this technique for various mycotoxins, (ii) to establish dose-response relationships for a group of trichothecene mycotoxins with a range of different substituents, and (iii) to substantiate the usefulness of this bioassay in mycotoxin detection and investigation.

MATERIALS AND METHODS

Organism and media. *K. marxianus* GK1005 was obtained from the Ministry of Food and Fisheries, London, United Kingdom. The yeast was routinely maintained and grown on 1% (wt/vol) yeast extract, 1% (wt/vol) bacteriological peptone, and 2% (wt/vol) glucose (YPG), solidified when required with 2% (wt/vol) agar. Cultures for inoculation of the bioassay were prepared by adding a single colony from an agar plate to 50 ml of YPG-50 liquid medium in a 250-ml flask and incubating this mixture in a rotary incubator for 16 h at 35°C and 200 rpm. (YPG-50 medium contained 1% [wt/vol] yeast extract, 1% [wt/vol] bacteriological peptone, and 50 mM glucose.) For the bioassay procedure, YPG-50 was supplemented from a stock solution of polymyxin B sulfate (PMBS) (ICN Biomedicals, Ltd., Thame, Oxfordshire, United Kingdom) to give a final bioassay PMBS concentration of 15 μg/ml. Stock solutions of PMBS were prepared in water, filter-sterilized, and kept no more than 1 day.

Mycotoxin standards. Mycotoxins (Sigma-Aldrich Chemical Company, Ltd., Poole, Dorset, United Kingdom) were diluted in spectroscopy-grade methanol at, typically, 0.1 mg/ml. Absolute concentrations were verified by UV absorbance. In the initial experiments, we used aflatoxin B_1 (AFB_1), aflatoxin M_1, (AFM_1), citrinin (CIT), cyclopiazonic acid (CPA), deoxynivalenol (DON), diacetoxyscirpenol (DAS), fumonisin B_1 (FB_1), ochratoxin A (OTA), patulin (PAT), roridin A (ROR), sterigmatocystin (STG), T-2 toxin (T-2), tenuazonic acid (TEN), verrucarin A (VER), and zearalenone (ZEA). Each mycotoxin standard was tested at final assay concentrations of 10 μg/ml to 0.1 ng/ml (serial 10-fold dilutions); a 25-μg/ml test concentration also was included for AFB_1 and AFM_1. For the trichothecene structure-activity study, we used acetyl T-2 (AcT-2), DON, DAS, fusarenon X (FUS), HT-2 toxin (HT-2), neosolaniol (NEO), nivalenol (NIV), ROR, scirpentriol (SCR), T-2 tetraol (TET), T-2 triol (TRI), T-2, VER, at final assay concentrations of 25 μg/ml to 0.1 ng/ml.

Assay procedure. One hundred thirty-six microliters of PMBS-supplemented YPG-50 medium was added to the wells of a microtiter plate. Eight microliters of mycotoxin stock solution or methanol (control wells) was added, followed by 16 μl of yeast inoculum, to yield an initial cell density of 2×10^8 cells/ml. Blank wells contained 152 μl of medium and 8 μl of methanol. Plates were mixed, and cell density was determined; the plates were sealed with Mylar plate sealers (ICN Biomedicals, Ltd.) and incubated in a plate shaker (Wesbart Ltd., Billinghurst, West Sussex, United Kingdom) at 35°C for the duration of the assay. Cell density was monitored throughout the assay. When the control wells reached stationary phase (~10 h, with an A_{560} of ca. 1.2), the cultures were assayed for β-galactosidase activity.

Determination of cell density. Cell density was determined by measuring A_{560} with a Titertek Multiscan Plus Mk II microtiter plate reader (Labsystems, Ltd., Basingstoke, Hampshire, United Kingdom) connected to an Amstrad microcomputer and Titresoft 1.01 software (Labsystems). A_{560} was calibrated by direct hemocytometer counts, and 1 A_{560} unit corresponded to 1.1×10^9 cells/ml.

Determination of β-galactosidase activity. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (Calbiochem Novobiochem, Ltd., Beeston, Nottinghamshire, United Kingdom) was dissolved in dimethylformamide (DMF) at 100 mg/ml and stored in the dark at −20°C. This stock solution was used to prepare a working solution of 20 mg of X-Gal per ml in aqueous DMF (2 parts water to 3 parts DMF) immediately before each assay. Cells were permeabilized by the addition of 5 μl of 0.1% (wt/vol) sodium dodecyl sulfate and 3 μl of chloroform to each well. Eight microliters of the X-Gal working solution was then added to each well, and the plates were incubated at 35°C in the plate shaker for 20 min. Finally, the plates were read on the microtiter plate reader by using a test filter at 666 nm and a reference filter at 560 nm.

Construction and use of dose-response curves. Dose-response curves were constructed for the inhibition of growth and for β-galactosidase activity. The percentage of inhibition of a given end point was determined by comparison with that of the methanol controls. For each toxin concentration, at least two replicate wells were used, and for the methanol controls, at least 12 replicates were used.

* Corresponding author. Mailing address: University of Greenwich, Wellington St., Woolwich, London SE18 6PF, United Kingdom. Phone: 0181-331-8214. Fax: 0181 331 8305. E-mail: I.H.Evans@gre.ac.uk.

† Present address: Respiratory and Systemic Infection Laboratory, Central Public Health Laboratory, London NW9 5HT, United Kingdom.

Three parameters were calculated by using the dose-response curves: (i) the no-effect level (NEC), i.e. the highest concentration of toxin at which no inhibition was detected, (ii) the 50% effective concentration (EC_{50} [the concentration of toxin at which 50% inhibition was observed]), and (iii) the MIC (i.e., the lowest concentration of toxin at which 100% inhibition was detected).

RESULTS

Detection of mycotoxins by the colorimetric bioassay. Initially we evaluated 14 mycotoxins known as natural contaminants of foods or feeds or previously reported to be toxic to *K. marxianus* (10, 11, 14). Of the 14 mycotoxins tested, only five trichothecenes could be detected by the colorimetric yeast bioassay: DON (25 μg/ml), DAS (1 μg/ml), ROR (1 μg/ml), T-2 (1 μg/ml), and VER (0.1 μg/ml). None of the nontrichothecene mycotoxins were detected, including CPA, FB_1, OTA, PAT, STG, TEN, and ZEA, the latter at up to 10 μg/ml, and AFB_1 and AFM_1 at up to 25 μg/ml.

Structure-activity relationships among the trichothecene mycotoxins. Thirteen mycotoxins were used to determine if structure-activity relationships existed within the trichothecene group of mycotoxins. Dose-response curves (Fig. 1) were used to estimate the NEC, EC_{50}, and MIC of each trichothecene. The curves provide six different estimates of toxicity for each compound (Table 1). For the most potent toxins (VER, ROR, T-2, DAS, HT-2, and AcT-2), all six evaluations gave the same relative order of toxicity. For the less potent toxins, MIC and EC_{50}s for inhibition of growth (Table 1) sometimes could not be determined, which made the exact order of toxicity impossible to establish on this basis. However, the β-galactosidase assay was more sensitive than the growth assay (Table 1), and an unambiguous order of toxicity could be determined by using EC_{50}s for the inhibition of β-galactosidase activity. This order was VER > ROR > T-2 > DAS > HT-2 > AcT-2 > neosolanio (NEO) > FUS > TRI > scirpentriol (SCR) > nivalenol (NIV) > DON > TET.

DISCUSSION

The insensitivity of *K. marxianus* to the nontrichothecene mycotoxins has been previously noted with disk diffusion assays (10, 11, 15). The apparent insensitivity of *K. marxianus* to many mycotoxins, contrasting with the good sensitivity to at least some trichothecene mycotoxins, suggests that it might be exploited in a selective bioassay for tricothecenes (11).

Our colorimetric bioassay exhibits as great or greater sensitivity than the other yeast bioassays. For example, considering the most potent trichothecene, VER, the colorimetric bioassay gave a MIC of 5 ng/ml (Table 1) compared with a MIC of 120 ng/ml reported by Schappert et al. (16) for a disk diffusion assay. Expressed slightly differently, the colorimetric bioassay gave an EC_{50} of 0.32 ng/well, compared with the minimum reported detection level (4-mm inhibition zone diameter) of 5 ng/disk reported by Madhyastha et al. (11) for an optimized disk bioassay. The β-galactosidase–colorimetric end point, which contributes the main novelty of the bioassay used here, is more sensitive than inhibition of cell growth—the end point used in the other yeast bioassays. This sensitivity can be seen when the inhibition of β-galactosidase dose-response curves (Fig. 1B and D) is inflected more sharply and at lower toxin concentrations than the curves for growth inhibition (Fig. 1A and C); the effect is quantitatively displayed as EC_{50}s and MICs (Table 1). The value of the β-galactosidase end point is particularly clear for TRI, which was virtually undetected on the basis of growth inhibition.

The most potent toxins in our assay (Table 1) were VER and ROR, which have a macrocylic ring between the C-6 and C-4 positions and no substituents at the C-3, C-7 and C-8 positions. T-2 was the most potent of the nonmacrocyclic trichothecenes tested, followed by DAS. T-2 and DAS both possess acetoxy groups at the C-4 and C-15 positions, together with a hydroxy group at the C-3 position; potency declines greatly when these groups are absent and/or when keto or hydroxy moieties are at the C-8 position (Table 1). The HT-2 results show that replacement of the C-4 acetoxy (T-2) by a hydroxy (HT-2) causes a modest loss in potency (6-fold), whereas the same substitution

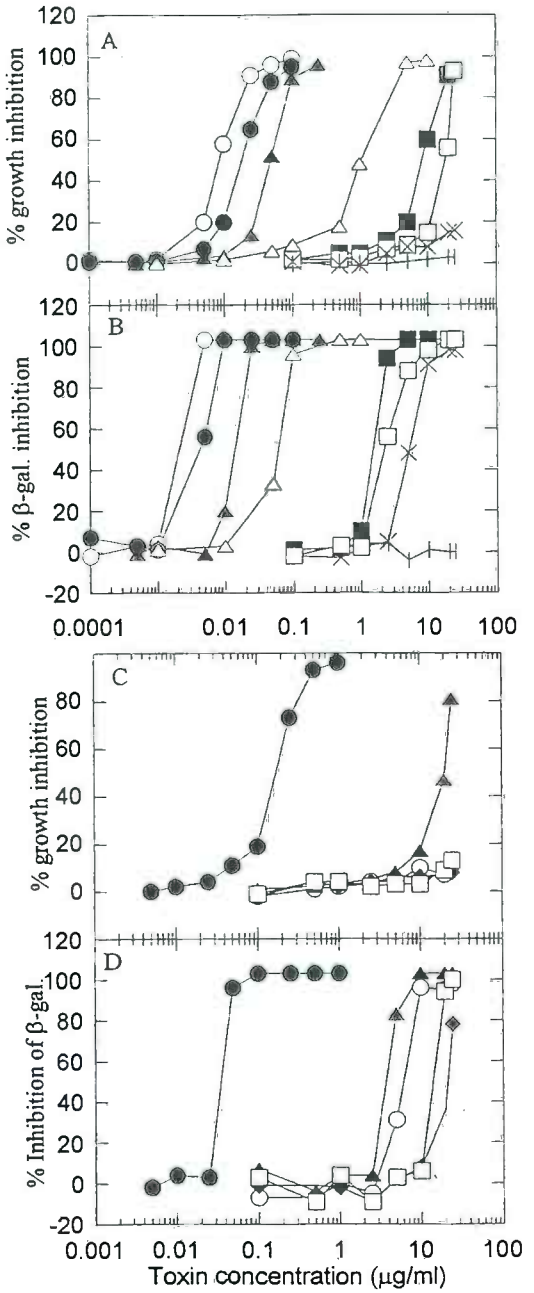

FIG. 1. Inhibition of growth and β-galactosidase activity in *K. marxianus* by trichothecene mycotoxins. Standard deviations for all data points are <10% the value of the point. (A and B) Inhibition of growth (A) and β-galactosidase activity (B) of *K. marxianus* by VER (○), ROR (●), T-2 (▲), HT-2 (△), AcT-2 (■), NEO (□), TRI (X), and TET (I). (C and D) Inhibition of growth (C) and β-galactosidase activity (D) of *K. marxianus* by DAS (●), SCR (○), FUS (▲), NIV (◆), and DON (□).

TABLE 1. NEC, EC_{50}, and MIC estimates for inhibition of growth and β-galactosidase activity of *K. marxianus* by trichothecenes[a]

Toxin type	Inhibition (μg/ml) of:						Relative toxicity
	Growth			β-Galactosidase induction			
	NEC	EC_{50}	MIC	NEC	EC_{50}	MIC	
Group 1 (no oxygen function at the C-8 position [R_1])							
DAS	0.005	0.18	1.0	0.0065	0.03	0.1	15
SCR	0.1	>25	>25	2.5	6.0	10	3,000
Group 2 (non-keto oxygen function at the C-8 position [R_1])							
T-2	0.001	0.05	0.25	0.005	0.012	0.025	6
HT-2	0.01	1	5	0.01	0.07	0.5	35
AcT-2	0.1	8.0	>25	0.1	1.5	5	750
NEO	0.5	20	>25	0.5	2.0	20	1,000
TRI	1.0	>25	>25	0.8	5.0	20	2,500
TET	>25	>25	>25	>25	>25	>25	>12,500
Group 3 (ketone at the C-8 position [R_1])							
FUS	0.1	22	>25	0.7	3.5	10.0	1,750
NIV	0.1	>25	>25	4.0	14	>25	7,000
DON	0.1	>25	>25	4.0	21	25	10,500
Group 4 (macrocyclic trichothecenes)							
VER	0.001	0.008	0.1	0.001	0.002	0.005	1
ROR	0.001	0.018	0.25	0.001	0.004	0.01	2

[a] The trichothecenes are arranged in the four chemical groups of Tamm and Tori (18) and are ranked, within each group, in order of potency. The relative toxicities of the individual toxins are also given, based on their EC_{50}s, with VER being 1.

at C3 (T-2 changed to AcT-2) causes a much more dramatic potency reduction (over 100-fold). VER, ROR, T-2 and DAS stand out as the most potent of the trichothecenes in our yeast system.

The overall results obtained here are in general agreement with those from other investigations. A study using *K. marxianus* in a disk diffusion assay showed the orders of toxicity to be VER > ROR > T-2 > HT-2 > TRI > TET (16) and T-2 > DAS > HT-2 > AcT-2 > FUS > TRI > NEO > NIV > DON > TET (12). By using the *Chlorella* growth inhibition assay, AcT-2 and NEO inhibited growth at 1 mg/ml, whereas TET, NIV, and DON had no effect (9). Two studies of trichothecene lymphotoxicity gave results strongly paralleling those from our yeast system: one showed a similar decrease in toxicity with substitution at C-4 (FUS > NIV > DON) (7). The other study highlighted the importance for potency of a hydroxy at C-3, together with acetoxy groups at C-4 and C-15 (T-2 and DAS), and the decrease in toxicity that occurs when these groups are absent and/or when there are keto or hydroxy moieties at C-8 (1). The similarity of the responses of the two systems indicates the potential application of our test to the evaluation of trichothecene lymphotoxicity. Again, measuring inhibition of protein synthesis in cultured Vero (animal) cells, the order of toxicity was shown to be VER > ROR > T-2 > DAS > HT-2 > NEO > FUS > SCR > TRI > DON > AcT-2 > NIV > TET (19). This ranking is very similar to that of our colorimetric bioassay. Our bioassay can also detect some trichothecenes with a sensitivity comparable to chemical methods. For example, the EC_{50} for T-2 toxin in our assay is approximately 10 ng/ml, compared to 20 to 25 ng per "spot" needed for detection on a thin-layer chromatography plate (3), 10 ng needed for detection by high-performance liquid chromatography (by UV absorption of the *p*-nitrobenzoate derivative) (3), and approximately 20 ng needed for detection by electron impact-selective ion monitoring-mass spectrometry detection (3).

In summary, our colorimetric bioassay is highly sensitive to a number of trichothecene mycotoxins and showed essentially similar results to, but greater sensitivity than, other yeast (11, 15) and animal tissue culture (7, 8, 13, 19) bioassays. The simplicity, speed, ease of replication, and quantification of results mean this assay is particularly well suited to studies of possible synergistic and antagonistic interactions between trichothecene mycotoxins and between trichothecenes and other toxicants and food components.

ACKNOWLEDGMENTS

This research was supported in part by a SERC-CASE studentship to K.E. and the Department for International Development of the United Kingdom.

We also thank J. Gibbs and M. Nagler for technical advice and assistance and Geoff Cooper, School of Chemical and Life Sciences, for help with computations.

REFERENCES

1. **Bondy, G. S., M. N. Beremand, S. P. McCormick, and J. J. Pestka.** 1991. Murine lymphocyte proliferation impaired by substituted neosolaniols and calonectrins—*Fusarium* metabolites associated with trichothecene biosynthesis. Toxicon **29**:1107–1113.
2. **Coker, R. D.** 1997. Mycotoxins and their control: constraints and opportunities. NRI bulletin 73. Natural Resources Institute, Chatham, Kent, United Kingdom.
3. **Coker, R. D.** The chromatography of mycotoxins. *In* I. Wilson (ed.), Encyclopedia of separation science, in press.
4. **Connolly, P., and J. E. L. Corry.** 1990. Effect of polymyxin B nonapeptide and polymyxin B sulphate on trichothecene mycotoxin sensitivity of yeast using a conductimetric instrument. Int. J. Food Microbiol. **10**:73–90.
5. **Engler, K.** 1996. Ph.D. thesis. University of Greenwich, London, United Kingdom.
6. **Engler, K., R. D. Coker, and I. H. Evans.** A novel colorimetric yeast bioassay for detecting trichothecene mycotoxins. J. Microbiol. Methods, in press.
7. **Forsell, J. H., and J. J. Pestka.** 1985. Relation of 8-ketotrichothecene and zearalenone analog structure to inhibitors of mitogen-induced human lymphocyte blastogenesis. Appl. Environ. Microbiol. **50**:1304–1307.
8. **Hanelt, M., M. Gareis, and B. Kollarczik.** 1994. Cytotoxicity of mycotoxins evaluated by the MTT-cell culture assay. Mycopathologia **128**:167–174.
9. **Ikawa, M., C. Carr, and T. Tatsuno.** 1985. Trichothecene structure and toxicity to the green alga *Chlorella pyrenoidosa*. Toxicon **23**:535–537.
10. **Madhyastha, M. S., R. R. Marquardt, A. Masci, J. Borsa, and A. A. Frohlich.**

1994. Comparison of toxicity of different mycotoxins to several species of bacteria and yeasts: use of *Bacillus brevis* in a disc diffusion assay. J. Food Prot. **57**:48–53.
11. **Madhyastha, M. S., R. R. Marquardt, A. A. Frohlich, and J. Borsa.** 1994. Optimisation of yeast bioassay for trichothecene mycotoxins. J. Food. Prot. **57**:490–495.
12. **Madhyastha, M. S., R. R. Marquardt, and D. Abramson.** 1994. Structure-activity relationships and interactions among trichothecene mycotoxins as assessed by yeast bioassay. Toxicon **32**:1147–1152.
13. **Porcher, J. M., C. Lafarge-Frayssinet, C. Frayssinet, A. Nurie, D. Melcion, and D. Richard-Molard.** 1987. Determination of cytotoxicity of trichothecenes in corn by cell culture assay. J. Assoc. Off. Anal. Chem. **70**:844–849.
14. **Schappert, K. T., and G. G. Khachatourians.** 1983. Effect of fusariotoxin T-2 on *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*. Appl. Environ. Microbiol. **45**:862–867.
15. **Schappert, K. T., and G. G. Khachatourians.** 1984. A yeast bioassay for T2-toxin. J. Microbiol. Methods **3**:43–46.
16. **Schappert, K. T., H. A. Koshinsky, and G. G. Khachatourians.** 1986. Growth inhibition of yeast by T-2, HT-2, T-2 triol, T-2 tetraol, diacetoxyscirpenol, verrucarol, verrucarin A, and roridin A mycotoxins. J. Am. Coll. Toxicol. **5**: 181–187.
17. **Sukroongreeung, S., K. T. Schappert, and G. G. Khatchatourians.** 1984. Survey of sensitivity of twelve yeast genera toward T-2 toxin. Appl. Environ. Microbiol. **48**:416–419.
18. **Tamm, C., and M. Tori.** 1984. Trichothecenes, p. 131–182. *In* V. Betina (ed.), Mycotoxins. Production, isolation, separation and purification. Elsevier Scientific Publications, Amsterdam, The Netherlands.
19. **Thompson, W. L., and R. W. Wannemacher.** 1986. Structure-function relationships of 12,13-epoxytrichothecene mycotoxins in cell culture: comparison to whole animal lethality. Toxicon **24**:985–994.
20. **Watson, D. H., and D. G. Lindsay.** 1982. A critical review of biological methods for the detection of fungal toxins in foods and feedstuffs. J. Sci. Food Agric. **33**:59–67.
21. **Yates, I. E.** 1986. Bioassay systems and their use in diagnosis of mycotoxicoses, p. 333–381. *In* J. L. Richards and J. R. Thurston (ed.), Diagnosis of mycotoxicoses. Martinus Nijhoff, Dordrecht, The Netherlands.

OUTPUTS

The project outputs, which have been described in the previous sections, may be summarised as follows:

- a simple, highly specific *K. marxianus* micro-titre dish bioassay for the detection of the trichothecene mycotoxins which shows superior sensitivity and end-point detection when compared with published procedures
- methods for isolating preparations of purified microsomal particles from *K. marxianus* and *Saccharomyces cerevisiae*
- measurements of the cytochrome P450 content of the yeast microsomal preparations (a) using carbon monoxide difference spectroscopy and (b) by spectroscopic analysis of benzo(α)pyrene binding to P450
- a description of the uptake and metabolism of aflatoxin B_1 and T-2 toxin by *K. marxianus* and *Bacillus megaterium*
- an understanding of the effect of methanol and polymyxin B sulphate on the permeability of *K. marxianus* to aflatoxin B_1 and T-2 toxin
- a comparison between the effectiveness of the simple yeast bioassay and a sophisticated turbidimetric device (the Bioscreen) for the detection of aflatoxin B_1 and T-2 toxin
- data describing the structure/activity relationship between the simple yeast bioassay and selected trichothecene mycotoxins
- preliminary evidence of the sensitivity of *Bacillus megaterium* towards aflatoxin B_1

CONTRIBUTION OF OUTPUTS

The project outputs contribute towards the development of strategies which improve the food security of poor households. Specifically, bioassays have an important role to play in the control of food safety, providing simple, cost-effective means of monitoring toxicity, and a vehicle for evaluating the toxic mechanism. For example, the project outputs have led to the production of a simple, effective bioassay for the detection of the trichothecene mycotoxins together with an understanding of the

uptake, binding and metabolic fate of aflatoxin B_1 and T-2 toxin when applied to cultures of *K. marxianus* and *Bacillus megaterium*.

A better understanding of the behaviour of toxins will facilitate the establishment of preventative, food safety management systems which can be applied in rural areas in developing countries.

The concept of the yeast bioassay is being further developed, using HEFCE funds, utilising yeasts which have been genetically engineered to express human cytochrome P450 monooxygenases. The application of the yeast bioassay will be extended to additional mycotoxins, with varying metabolic profiles, in order to study the possibility of discriminating between toxins, and of evaluating the results of interactions between them.

The following attached publications have been produced.