REFERENCES

Adams, G. C., Surve-Iyer, R. S., and Iezzoni, A. F. 2002. Ribosomal DNA sequence divergence and group I introns within the *Leucostoma* species *L. cinctum*, *L. persoonii*, and *L. parapersoonii* sp. nov., ascomycetes that cause Cytospora canker of fruit trees. *Mycologia* 94: 947-967.

Anastassiadis, S., Aivasidis, A., and Wandrey, C. 2003. Continuous gluconic acid production by isolated yeast-like mould strains of *Aureobasidium pullulans*. *Appl. Microbiol. Biotechnol.* 61: 110-7.

Anastassiadis, S., Aivasidis, A., Wandrey, C., and Rehm, H.J. 2005. Process optimization of continuous gluconic acid fermentation by isolated yeast-like strains of *Aureobasidium pullulans*. *Biotechnol. Bioeng.* 91: 494-501.

Anastassiadis, S. and Rehm, H. J. 2006. Continuous gluconic acid production by the yeast-like *Aureobasidium pullulans* in a cascading operation of two bioreactors. *Appl. Microbiol. Biotechnol.* 73: 541-8.

Anderson, K. L. 1995. Biochemical analysis of starch degradation by *Ruminobacter amylophilus* 70. *Appl. Environ. Microbiol.* 61: 1488-1491.

Andrews, J. H., Harris, R. F., Spear, R. N., Lau, G. W., and Nordheim, E. V. 1994. Morphogenesis and adhesion of *Aureobasidium pullulans*. *Can. J. Microbiol.* 40: 6-17.

Andrews, J. H., Spear, R. N., and Nordheim, E. V. 2002. Population biology of *Aureobasidium pullulans* on apple leaf surfaces. *Can .J. Microbiol.* 48: 500-513.

Audet, J., Lounes, M., and Thibault, J. 1996. Pullulan fermentation in a reciprocating plate bioreactor. *Bioprocess. Eng.* 15: 209-214.

Badr-Eldin, S. M., El-Tayeb, O. M., El-Masry, H. G., Mohamad, F. H. A., and Abd El-Rahman, O. A. 1994. Polysaccharide production by *Aureobasidium pullulans*: factors affecting polysaccharide formation. *World J. Microbiol. Bacteriol.* 10: 423-426.

Banerjee, D. and Pati, B. R. 2007. Optimization of tannase production by *Aureobasidium pullulans* DBS66. *J. Microbiol. Biotechnol.* 17: 1049-1053.

Barnett, C., Smith, A., Scanlon, B., and Israilides, C. J. 1999. Pullulan production by *Aureobasidium pullulans* growing on hydrolysed potato starch waste. *Carbohydr. Polymers* 38: 203-209.

Bernier, B. 1958. The production of polysaccharides by fungi active in the decomposition of wood and forest litter. *Can. J. Microbiol.* 4: 195-204.

Bender, H., Lehmann, J., and Wallenfels, K. 1959. Pullulan, an extracellular glucan from *Pullularia pullulans. Biochim. Biophys. Acta* 36: 309-316.

Bender, H. and Wallenfels, K. 1961. Investigations on pullulan. II. Specific degradation by means of a bacterial enzyme. *Biochem. Zeitschrift* 334: 79-95.

Benesi A. J. and Brant D. A. 1985. Trends in molecular motion for a series of glucose oligomers and the corresponding polymer pullulan as measured by carbon-13 NMR relaxation. *Macromolecules* 18: 1109–1116.

Baum, D. A. and Shaw, K.L. 1995. Genealogical perspectives on the species problem. In: P.C. Hoch, A.G. Stephenson (eds.), *Experimental and Molecular Approaches to Plant Biosystematics,* pp. 289-303. Missouri Botanical Garden, St. Louis, USA.

Bencheqroun, S. K., Bajji, M., Massart, S., Bentata, F., Labhilili, M., Achbani, H., El Jaafari, S., and Jijakli, M. H. 2006. Biocontrol of blue mold on apple fruits by *Aureobasidium pullulans* (strain Ach 1-1): *in vitro* and in situ evidence for the possible involvement of competition for nutrients. *Commun. Agric. Appl. Biol. Sci.* 71: 1151-1157.

Bermejo, J. M., Dominguez, J. B., Goni, F. M., and Uruburu, F. 1981a. Influence of carbon and nitrogen sources on the transition from yeast-like cells to chlamydospores in *Aureobasidium pullulans. Antonie van Leeuwenhoek J. Microbiol.* 47: 107-119.

Bermejo, J. M., Dominguez, J. B., Goni, F. M., and Uruburu, F. 1981b. Influence of pH on the transition from yeast-like cells to chlamydospores in *Aureobasidium pullulans. Antonie van Leeuwenhoek J. Microbiol.* 47: 385-392.

Berry, D. R. 1988. Physiology of Industrial Fungi. pp. 146-149. Oxford London: Blackwell Scientific Publication.

Blackwell, M., Hibbett, D. S., Taylor, J. W., and Spatafora, J. W. 2006. Research coordination networks: a phylogeny for kingdom Fungi (Deep Hypha). *Mycologia* 98: 829-837.

Bolignano, G. and Criseo, G. J. 2003. Disseminated nosocomial fungal infection by *Aureobasidium pullulans* var. *melanigenum*: a case report. *Clin. Microbiol.* 41: 4483-4485.

Bouveng, H. O., Kiessling, H., Lindberg, B., and McKay, J. 1962. Polysaccharides elaborated by *Pullularia pullulans*. I. The neutral glucan synthesised from sucrose solutions. *Acta Chem. Scand.* 16: 615-622.

Brown, R. G., Hanic, L. A., and Hsiao, M. 1973. Structure and chemical composition of yeast chlamydospores of *Aureobasidium pullulans*. *Can. J. Microbiol.* 19: 163-168.

Bulmer, M. A., Catley, B. J., and Kelly, P. J. 1987. The effect of ammonium ions and pH on the elaboration of the fungal extracellular polysaccharide, pullulan, by *Aureobasidium pullulans*. *Appl. Microbiol. Biotechnol.* 25: 362-365.

Carolan, G., Catley, B. J., and McDougal, F. J. 1983. The location of tetrasaccharide units in pullulan. *Carbohydr. Res.* 114: 237-243.

Catley, B. J. 1970. Pullulan, a relationship between molecular weight and fine structure. *FEBS Lett.* 10: 190-193.

Catley, B. J. 1979. Pullulan synthesis by *Aureobasidium pullulans*, In: R. C. W. Berkeley, G. W. Gooday, and D. C. Ellwood (eds)., *Microbial Polysaccharides and Polysaccharases* , pp. 69-84. London: Academic Press.

Catley, B. J. and Whelan, W. J. 1971. Observations on the structure of pullulan. *Arch. Biochem. Biophys.* 143: 138-142.

Catley, B. J. and McDowell, W. 1982. Lipid-linked saccharides formed during pullulan biosynthesis in *Aureobasidium pullulans*. *Carbohydr. Res.* 103: 65-75.

Catley, B. J., Ramsay, A., and Servis, C. 1986. Observations on the structure of the fungal extracellular polysaccharide, pullulan. *Carbohydr. Res.* 153: 79-86.

Campbell, B. S., Siddique, A. B., McDougall, B. M., and Seviour, R. J. 2004. Which morphological forms of the fungus *Aureobasidium pullulans* are responsible for pullulan production? *FEMS Microbiol. Lett.* 232: 225-228.

Cernakova, M., and Kockova-Kratochvilova, A., Suty, L., Zemek, J., and Kuniak, L., 1980. Biochemical similarities among strains of *Aureobasidium pullulans* (de Bary) Arnaud. *Folia Microbiologica* 25: 68-73.

Chi, Z. M., Yan, K. R., Gao, L. M., Li, J., Wang, X. H., and Wang, L. 2008. Diversity of marine yeasts with high protein content and evaluation of their nutritive compositions. *J. Mar. Biol. Assoc. UK* 88: 1–6.

Chi, Z., Wang, F., Chi, Z., Yue, L., Liu, G., and Zhang, T. 2009. Bioproducts from *Aureobasidium pullulans*, a biotechnologically important yeast. *Appl. Microbiol. Biotechnol.* DOI 10.1007/s00253-009-1882-2. Published online: February 7.

Christov, L. P., Myburgh, J., O'Neill, F. H., Van Tonder, A., Prior, B.A. 1999. Modification of the carbohydrate composition of sulfite pulp by purified and characterized beta-xylanase and beta-xylosidase of *Aureobasidium pullulans. Biotechnol. Prog.* 15: 196-200.

Ci, S. X., Huynh, T. H., Louie, L. W., Yang, A., Beals, B. J., Ron, N., Tsang, W. G., Soon-Shiong, P., and Desai, N. P. 1999. Molecular mass distribution of sodium alginate by high-performance size-exclusion chromatography. *J. Chromatogr. A.* 864: 199-210.

Cooke, W. B. 1959. An ecological life history of *Aureobasidium pullulans* (de Bary) Arnaud. *Mycopathologia et Mycologia Applicata* 12: 1-45.

Cooke W. B. 1962. A taxonomic study in the "black yeasts." *Mycopathol. Mycol. Appl.* 17: 1-43.

Dais, P., Vlachou, S., and Taravel, F. R. 2001. ^{13}C nuclear magnetic relaxation study of segmental dynamics of the heteropolysaccharide pullulan in dilute solutions. *Biomacromolecules.* 2: 1137-1147.

de Hoog, G. S. and Yurlova, N. A. 1994. Conidiogenesis, nutritional physiology and taxonomy of *Aureobasidium* and *Hormonema. Antonie van Leeuwenhoek Int. J. Gen. Molec. Microbiol.* 65: 41-54.

de Hoog, G. S. 1998. A key to the anamorph genera of yeastlike archi- and euascomycetes, In: C. P. Kurtzman and J. W. Fell (Eds.), *The Yeasts, a Taxonomic Study.* pp.123-125. Amsterdam: Elsevier.

de Hoog, G. S., Zalar, P., Urzi, C., de Leo, F., Yulova, N. A., and Sterflinger, K. 1999. Relationships of dothideaceous black yeasts and meristematic fungi based on 5.8S and ITS2 sequence comparison. *Studies in Mycology*. 43: 31-37.

de Jager E. S., Wehner F. C., and Korsten L. 2001. Microbial Ecology of the Mango Phylloplane. *Microb. Ecol.* 42: 201–207.

Dennis, C. and Buhagiar, R. W. M. 1973. Comparative study of *Aureobasidium pullulans*, *A. prunorum* sp. nov., and *Trichosporon pullulans*. *Transactions of the British Mycological Society* 60: 567–575.

Dettman, F. R., Jacobson, D. J., and Taylor, J. W. 2006. Multilocus sequence data reveal extensive phylogenetic species diversity within the *Neurospora discreta* complex. *Mycologia* 98: 436–446.

Domsch, K. H., Gams, W., and Anderson, T. 1993. *Compendium of Soil Fungi*. New York: Academic Press.

Dos Santos, V. L., Monteiro Ade, S., Braga, D. T., and Santoro, M. M. 2009. Phenol degradation by *Aureobasidium pullulans* FE13 isolated from industrial effluents. *J.Hazard. Mater.* 161: 1413-1420.

Duan, X. H., Chi, Z. M., Wang, L., and Wang, X. H. 2008. Influence of different sugars on pullulan production and activities of α-phosphoglucose mutase, UDPG-pyrophosphorylase and glucosyltransferase involved in pullulan synthesis in *Aureobasidium pullulans* Y68. *Carbo. Hydr. Polym.* 73: 587–593.

Elinov., N. P., Glazova, N. V., Kravchenko, S. B., Potekhina, T. S., and Siluyanova, N. A. 1987. Method of producing aubasidan. *U. S. S. R. Patent* 1,339,129.

Finkelman, M. A. J. and Vardanis, A. 1982. Pullulan elaboration by *Aureobasidium pullulans* protoplasts. *Appl. Environ. Microbiol.* 44: 121-127.

Fukuhara H. 1995. Linear DNA plasmids of yeasts. *FEMS Microbiol. Lett.* 131:1-9.

Gibbs, P. A. and Seviour, R. J. 1996. Does the agitation rate and/or oxygen saturation influence exopolysaccharide production by *Aureobasidium pullulans* in batch culture? *Appl. Microbiol. Biotechnol.* 46: 503-510.

Gibson, L. H.and Coughlin, R. W. 2002. Optimization of high molecular weight pullulan production by *Aureobasidium pullulans* in batch fermentations. *Biotechnol. Prog.* 18: 675-678.

Glass, N. L. and Donaldson, G. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microbiol.* 61: 1323–1330.

Glinel, K, Sauvage, J. P., Oulyadi, H., and Huguet, J. 2000. Determination of substituents distribution in carboxymethylpullulans by NMR spectroscopy. *Carbohydr. Res.* 328: 343-354.

Gunde-Cimermana, N., Zalarb, P., de Hoog, G. S., and Plemenitasd, A. 2000. Hypersaline waters in salterns - natural ecological niches for halophilic black yeasts. *FEMS Microbiol. Ecol.* 32: 235-240.

Hashida-Okado, T., Ogawa, A., Endo, M., Yasumoto, R., Takesako, K., and Kato, I. 1996. AUR1, a novel gene conferring aureobasidin resistance on *Saccharomyces cerevisiae*: a study of defective morphologies in Aur1p-depleted cells. *Mol. Gen. Genet.* 251: 236–244.

Hayashi, S., Hinotani, T., Takasaki, Y., and Imada, K. 1994. The enzymatic reaction for the production of panose and isomaltose by glucosyltransferase from *Aureobasidium*. *Lett. Appl. Microbiol.* 19: 247-248.

Hermanides-Nijhof, E.J. 1977. *Aureobasidium* and allied genera. *Studies in Mycology* 15: 141-166.

Hibbett, S. A., et al. 2007. A higher-level phylogenetic classification of the Fungi. *Mycol. Res.* 111: 509-547.

Huelsenbeck, J. P. and Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.

Imshenetskii, A. A., Kondrat'eva, T. F., and Smut'ko, A. N. 1981. Influence of carbon and nitrogen sources on pullulan biosynthesis by polyploid strains of *Pullularia pullulans*. *Mikrobiol.* 50: 102-105.

Israilides, C., Bocking, M., Smith, A., and Scanlon, B. 1994. A novel rapid coupled enzyme assay for the estimation of pullulan. *Biotechol. Appl. Biochem.* 19: 285-291.

Jabaji-Hare, S. H., Burger, G., Forget, L., and Lang, B. F. 1994. Extrachromosomal plasmids in pathogenic fungus *Rhizoctona sonali*. *Curr.Genetics* 25: 423-431.

Kikuchi, Y., Taguchi, R., Sakano, Y., and Kobayashi, T. 1973. Comparison of extracellular polysaccharide produced by *Pullularia pullulans* with polysaccharides in the cells and cell wall. *Agric. Biol. Chem.* 37: 1751-1753.

Kimura, T. and Horikoshi, K. 1990. Characterization of pullulan-hydrolysing enzyme from an alkalopsychrotrophic *Micrococcus* sp. *Appl. Microbiol. Biotechnol.* 34: 52-56.

Klimek, J. and Ollis, D. F. 1980. Extracellular microbial polysaccharides: kinetics of *Pseudomonas* sp., *Azotobacter vinelandii*, and *Aureobasidium pullulans* batch fermentations. *Biotechnol. Bioeng.* 22: 2321-2342.

Kudanga T. and Mwenje, E. 2005. Extracellular cellulase production by tropical isolates of *Aureobasidium pullulans*. *Can. J. Microbiol.* 51: 773-776.

Kurosawa, T., Sakai, K., Nakahara, T., Oshima, Y., Tabuchi, T. 1994. Extracellular accumulation of the polyol lipids, 3,5-dihydroxydecanoyl and 5-hydroxy-2-decenoyl esters of arabitol and mannitol, by *Aureobasidium* sp. *Biosci. Biotech. Biochem.* 58: 2057-2060.

Kurtzman, C. P. 2007a. *Blastobotrys americana* sp. nov., *B. illinoisensis* sp. nov., *B. malaysiensis* sp. nov., *B. muscicola* sp. nov., *B. peoriensis* sp. nov. and *B. raffinofermentans* sp. nov., six anamorphic yeast species. *Int. J. System. Evol. Microbiol.* 57: 1154-1162.

Kurtzman, C. P. 2007b. New anamorphic yeast species: *Candida infanticola* sp. nov., *Candida polysorbophila* sp. nov., *Candida transvaalensis* sp. nov., and *Trigonopsis californica* sp. nov. *Antonie Van Leeuwenhoek* 92: 221-231.

Kurtzman, C. P. 2007c. Eleven new species of *Sugiyamaella* and *Candida* from forest habitats. *Fed. Euro. Microbiol. Soc. Yeast Res.* 7: 1046-1063.

Lazaridou, A., Biliaderis, C. G., Roukas, T., and Izydorczyk, M. 2002. Production and characterization of pullulan from beet molasses using a nonpigmented strain of *Aureobasidium pullulans* in batch culture. *Appl. Biochem. Biotechnol.* 97: 1-22.

Leathers, T. D. 1986. Color variants of *Aureobasidium pullulans* overproduce xylanase with extremely high specific activity. *Appl. Environ. Microbiol.* 52: 1026-1030.

Leathers, T. D. 1986. Color variants of *Aureobasidium pullulans* overproduce xylanase with extremely high specific activity. *Appl. Environ. Microbiol.* 52: 1026-1030.

Leathers, T. D. 1987. Host amylases and pullulan production, in: *First Materials Biotechnology Symposium.* Technical Report NATICK/TR-88/033 (Kaplan, D. L., Ed.), pp. 175-185. Natick: U. S. Army.

Leathers, T. D. 1989. Purification and properties of xylanase from *Aureobasidium. J. Ind. Microbiol.* 4: 341-348.

Leathers, T. D. 1993. Substrate regulation and specificity of amylases from *Aureobasidium* strain NRRL Y-12,974. *FEMS Microbiol. Lett.* 110: 217-222.

Leathers, T. D. 2002. Pullulan. In: E.J. Vandamme, S. De Baets, A. Steinbüchel (eds), *Biopolymers*, vol. 6. *Polysaccharides II: Polysaccharides from Eukaryotes.* pp. 1-35. Wiley-VCH, Weinheim.

Leathers, T. D. 2003. Biotechnological production and applications of pullulan. *Appl. Microbiol. Biotechnol.* 62: 468-473.

Leathers, T. D. and Gupta, S. C. 1994. Production of pullulan from fuel ethanol byproducts by *Aureobasidium* sp. strain NRRL Y-12,974. *Biotechnol. Lett.* 16: 1163-1166.

Leathers, T. D. Kurtzman C.P., and Detroy, R.W. 1984. Overproduction and regulation of xylanase in *Aureobasidium pullulans* and *Cryptococcus albidus. Biotechnology and Bioengineering Symposium* 14: 225-240.

Leathers, T. D., Nofsinger, G.W., Kurtzman, C.P., and Bothast, R.J. 1988. Pullulan production by color variant strains of *Aureobasidium pullulans. J. Ind. Microbiol.* 3: 231-239.

LeDuy, A. and Boa, J. M. 1983. Pullulan production from peat hydrolyzate. *Can. J. Microbiol.* 29: 143-146.

Lee, K. Y. and Yoo, Y. J. 1993. Optimization of pH for high molecular weight pullulan. *Biotechnol. Lett.* 15: 1021-1024.

Lee, J. W., Yeomans, W. G., Allen A. L., Deng F., Gross R. A., and Kaplan D. L. 1999. Biosynthesis of novel exopolymers by *Aureobasidium pullulans*. *Appl. Environ. Microbiol.* 65: 5265–5271.

Leite, R. S., Bocchini, D. A., Martins Eda, S., Silva, D., Gomes, E., Da Silva, R. 2007. Production of cellulolytic and hemicellulolytic enzymes from *Aureobasidium pulluans* on solid state fermentation. *Appl. Biochem. Biotechnol.* 137-140: 281-288.

Li, X-L., Zhang, Z. Q., Dean, J. F., Eriksson, K. E., and Ljungdahl, L. G. 1993. Purification and characterization of a new xylanase (APX-II) from the fungus *Aureobasidium pullulans* Y-2311-1. *Appl. Environ. Microbiol.* 59: 3212-3218.

Li, X-L. and Ljungdahl, L. G. 1994. Cloning, sequencing, and regulation of a xylanase gene from the fungus *Aureobasidium pullulans* Y-2311-1. *Appl. Environ. Microbiol.* 60: 3160-3166.

Li, X-L. and Ljungdahl L. G. 1996. Expression of *Aureobasidium pullulans* xynA in, and secretion of the xylanase from *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* 62: 209-213.

Li, S., Cullen, D., Hjort, M., Spear, R., and Andrews, J. H. 1996. Development of an oligonucleotide probe for *Aureobasidium pullulans* based on the small-subunit rRNA gene. *Appl. Environ. Microbiol.* 62:1514-1518.

Liu S. and Steinbuchel A., 1996. Investigation of poly(β-L- malic acid) production by strains of *Aureobasidium pullulans*. *Appl. Microbiol. Biotechnol.* 46:273-278.

Liu, Y. J., Whelen, S., and Hall, B. D. 1999. Phylogenetic relationships among *Ascomycetes*: evidence from an RNA polymerase II subunit. *Molec. Biol. Evol.* 16: 1799-1808.

Loncaric, I., Oberlerchner, J. T., Heissenberger, B., and Moosbeckhofer. R. 2009. Phenotypic and genotypic diversity among strains of *Aureobasidium pullulans* in comparison with related species. *Antonie Van Leeuwenhoek.* 95:165-178.

Lumbsch, H. T. and Lindemuth, R. 2001. Major lineages of *Dothideomycetes* (*Ascomycota*) inferred from SSU and LSU rDNA sequences. *Mycol. Res.* 105: 901-908.

Madi, N. S., McNeil, B., and Harvey, L. M. 1996. Influence of culture pH and aeration on ethanol production and pullulan molecular weight by *Aureobasidium pullulans*. *J. Chem. Technol. Biotechnol.* 66: 343-350.

McGrath, M. J. and Andrews, J. H. 2006. Temporal changes in microscale colonization of the phylloplane by *Aureobasidium pullulans*. *Appl. Environ. Microbiol.* 72: 6234-6241.

McGrath, M. J. and Andrews, J. H. 2007 Role of microbial immigration in the colonization of apple leaves by *Aureobasidium pullulans*. *Appl. Environ. Microbiol.* 73: 1277-1286.

McNeil, B. and Kristiansen, B. 1987. Influence of impeller speed upon the pullulan fermentation. *Biotechnol. Lett.* 9: 101-104.

McNeil, B. and Kristiansen, B. 1990. Temperature effects on polysaccharide formation by *Aureobasidium pullulans* in stirred tanks. *Enzyme Microb. Technol.* 12: 521-526.

McNeil, B., Kristiansen, B., and Seviour, R. J. 1989. Polysaccharide production and morphology of *Aureobasidium pullulans* in continuous culture. *Biotechnol. Bioeng.* 33: 1210-1212.

Miller, J. H. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31: 426-428.

Miura, Y., Irie, T., Fujio, Y., and Ueda, S. 1978. Effect of amylase inhibitor on pullulan production. *Hakkokogaku* 56: 136-138.

Mokrousov, I. V., and Bulat S. A. 1992. Distinguishing between the biological species of the fungus *Aureobasidium pullulans* (de Bary) by the universally primed polymerase chain. *Genetika*. 28: 31-38.

Mokrousov, I. V. 1995a. Intraspecific differentiation of *Aureobasidium* spp. using universally primed polymerase chain reaction. *Genetika* 31: 908-914.

Mokrousov, I. V. 1995b. Phylogenetic analysis and differentiation of species of *Aureobasidium pullulans* (De Bary) Fungus. *Genetika* 3:, 780-785.

Nagarajan, G., Nam, M. H., Song, J. Y., Yoo, S. J., and Kim H. G. 2004. Genetic variation in *Fusarium oxysporum* f. sp. *fragariae* populations based on RAPD and rDNA RFLP analyses. *Plant Patho. J.* 20: 264-270.

Nagata, N., Nakahara, T., and Tahuchi, T. 1993. Fermentative production of poly(β-L-malic acid), a polyelectronic biopolyester by *Aureobasidium* sp. *Biosci. Biotech. Biochem.* 51: 638-642.

Nakajima-Kambe, T., Hirotani, N., and Nakahara, T. 1996. Poly(β-Malic Acid) Production by the Non-Growing Cells of *Aureobasidium* sp. Strain A-91 *J Ferment. Bioeng.* 82: 411-413.

National Center for Biotechnology Information (NCBI). 2008. Blast (Basic Local Alignment Search Tool). Available from: [http://blast.ncbi.nlm.nih.gov/Blast.cgi, March 20, 2009].

Niedoszytko, M., Chełmińska, M., Jassem, E., and Czestochowska, E. 2007. Association between sensitization to *Aureobasidium pullulans* (*Pullularia* sp) and severity of asthma. *Ann. Allergy Asthma Immunol.* 98:153-156.

Ohta, K., Moriyama, S., Tanaka, H., Shige, T., and Akimoto, H. 2001. Purification and characterization of an acidophilic xylanase from *Aureobasidium pullulans* var. *melanigenum* and sequence analysis of the encoding gene. *J. Biosci. Bioeng.* 92: 262-270.

Panda, A., Das, H., Deb, M., Khanal, B., and Kumar, S. 2006. *Aureobasidium pullulans* keratitis. *Clin. Experiment Ophthalmol.* 34: 260-264.

Peterson, S. W. 2004. Multilocus DNA sequence analysis shows that *Penicillium biourgeianum* Zaleski is a distinct species closely related to *P. brevicompactum* and *P. olsonii. Mycol. Res.* 108: 434-440.

Peterson, S. W. 2008. Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. *Mycologia* 100: 191-212.

Peterson, S. W., Bayer, E. M., and Wicklow, D. T. 2004. *Penicillium thiersii*, *Penicillium angulare* and *Penicillium decaturense*, new species isolated from wood-decay fungi in North America and multilocus DNA sequence analysis. *Mycologia* 96: 1280-1293.

Peterson, S. W., Vega, F. E., Posada, F., and Nagai C. 2005. *Penicillium coffeae*, a new endophytic species isolated from a coffee plant and its phylogenetic relationship to *P. fellutanum*, *P. theirsi,* and *P. brocae* based on parsimony analysis of multilocus DNA sequences. *Mycologia* 97: 659-666.

Pollock, T. J., Thorne, L., and Armentrout, R. W. 1992. Isolation of new *Aureobasidium* strains that produce high-molecular-weight pullulan with reduced pigmentation. *Appl. Environ. Microbiol.* 58: 877-883.

Pouliot J. M., Walton I., Nolen-Parkhouse M., Abu-Lail L. I., and Camesano T. A. 2005. Adhesion of *Aureobasidium pullulans* is controlled by uronic acid based polymers and pullulan. *Biomacromolecules*. 6: 1122-1131.

Prasongsuk, S., Berhow, M. A., Dunlap, C. A., Weisleder, D., Leathers, T. D., Eveleigh, D. E., and Punnapayak, H. 2007. Pullulan production by tropical isolates of *Aureobasidium pullulans*. *J. Ind. Microbiol. Biotech.* 34: 55-61.

Prasongsuk, S., Sullivan, R. F., Kuhirun, M., Eveleigh, D. E., and Punnapayak, H. 2005. Thailand habitats as sources of pullulan-producing strains of *Aureobasidium pullulans*. *World J. Microbiol. Biotech.* 21: 393-398.

Punnapayak, H., Sudhasham, M., Prasongsuk, S., and Pichayangkura, S. 2003. Characterization of *Aureobasidium pullulans* isolated from airborne spores in Thailand. *J. Ind. Microbiol. Biotech.* 30: 89-94.

Radulovi, M. D., Cvetkovi, O. G., Nikoli, S. D., Dordevi, D. S, Jakovljevi, D. M., and Vrvi, M. M. 2008. Simultaneous production of pullulan and biosorption of metals by *Aureobasidium pullulans* strain CH-1 on peat hydrolysate. *Bioresour. Technol.* 99: 6673-6677.

Ramos S. and Garcia Acha, I. 1975. A vegetative cycle of *Pullularia pullulans*. *Trans Br. Mycol. Soc.* 64: 129-135.

Reed-Hamer, B. and West, T. P. 1994. Effect of complex nitrogen sources on pullulan production relative to carbon source. *Microbios* 80: 83-90.

Reeslev, M. and Jensen, B. 1995. Influence of Zn^{2+} and Fe^{3+} on polysaccharide production and mycelium/yeast dimorphism of *Aureobasidium pullulans* in batch cultivations. *Appl. Microbiol. Biotechnol.* 42: 910-915.

Reeslev, M., Jorgensen, B. B., and Jorgensen, B. B. 1993. Influence of Zn^{2+} on yeast-mycelium dimorphism and exopolysaccharide production by the fungus *Aureobasidium pullulans* grown in a defined medium in continuous culture. *J. Gen. Microbiol.* 139: 3065-3070.

Reeslev, M., Strom, T., Jensen, B., and Olsen, J. 1997. The ability of the yeast form of *Aureobasidium pullulans* to elaborate exopolysaccharide in chemostat culture at various pH values. *Mycol. Res.* 101: 650-652.

Rekha M. R. and Sharma C. P. 2007. Pullulan as a Promising Biomaterial for Biomedical Applications: A Perspective. *Trends Biomater. Artif. Organs.* 20: 116-121.

Ronquist, F. and Huelsenbeck, J. P. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

Roukas, T. and Liakopoulou-Kyriakides, M. 1999. Production of pullulan from beet molasses by *Aureobasidium pullulans* in a stirred tank fermentor. *J. Food Eng.* 40: 89-94.

Saha, B. C. 2003. Hemicellulose bioconversion. *J. Ind. Microbiol. Biotech.* 30: 279-291.

Saha, B.C., Silman R. W., and Bothast R. J. 1993. Amylolytic enzymes produced by a color variant strain of *Aureobasidium pullulans*. *Curr. Microbio.* 26: 267-273.

Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. *Molecular Cloning – A Laboratory Manual,* 2nd Eds. New York: Cold Spring Habour Laboratory Press.

Sasahara, H. and Izumori, K. 2005. Production of L-sorbitol from L-fructose by *Aureobasidium pullulans* LP23 isolated from soy sauce mash. *J. Biosci. Bioeng.* 100: 223-226.

Schena, L., Ippolito, A., Zahavi, T., Cohen, L., Nigro, F., and Droby, S. 1999. Genetic diversity and biocontrol activity of *Aureobasidium pullulans* isolates against postharvest rots. *Postharvest Biol. Tecnol.* 17: 189-199.

Schena, L., Nigro, F., Pentimone, I., Ligorio, A., and Ippolito, A. 2003. Control of postharvest rots of sweet cherries and table grapes with endophytic isolates of *Aureobasidium pullulans*. *Postharvest Biol. Technol.* 30: 209-220.

Schoch, C. L., Shoemaker, R. A., Seifert, K. A., Hambleton, S., Spatafora, J. W., and Crous, P. W. 2006. A multigene phylogeny of the *Dothideomycetes* using four nuclear loci. *Mycologia* 98: 1042-1053.

Seo, H. P., Son, C. W., Chung, C. H., Jung, D. I., Kim, S. K., Gross, R. A., Kaplan, D. L., and Lee, J. W. 2004. Production of high molecular weight pullulan by *Aureobasidium pullulans* HP-2001 with soybean pomace as a nitrogen source. *Bioresour. Technol.* 95: 293-299.

Sevilla, M. J., Isusi, P., Gutierrez, R., Egea, L., and Uruburu, F. 1977. Influence of carbon and nitrogen sources on the morphology of *Pullularia pullulans*. *Trans. Br. Mycol. Soc.* 68: 300-303.

Silman, R. W., Bryan, W. L., and Leathers, T. D. 1990. A comparison of polysaccharides from strains of *Aureobasidium pullulans*. *FEMS Microbiol. Lett.* 71: 65-70.

Simon, I., Caye-Vaugien, C., and Bouchonneau, M. 1993. Relation between pullulan production, morphological state and growth conditions in *Aureobasidium pullulans*: new observations. *J. Gen. Microbiol.* 139: 979-985.

Singh, R. S., Saini, G. K., and Kennedy, J. F. 2008. Pullulan: Microbial sources, production and applications. *Carbohydr. Polymers* 73: 515–531.

Shin, H. T., Baig, S. Y., Lee, S. W., Suh, D. S., Kwon, S. T., Lim, Y. B., and Lee, J. H. 2004. Production of fructo-oligosaccharides from molasses by *Aureobasidium pullulans* cells. *Bioresour. Technol.* 93: 59-62.

Shingel, K. I. 2004. Current knowledge on biosynthesis, biological activity, and chemical modification of the exopolysaccharide, pullulan. *Carbohydr. Res.* 339: 447–460

Slightom, J. L., Metzger, B. P., Luu, H. T., and Elhammer, A. P. 2009. Cloning and molecular characterization of the gene encoding the Aureobasidin A biosynthesis complex in *Aureobasidium pullulans* BP-1938. *Gene* 431: 67-79.

Softberry, Inc. 2008. FGENESH+ (Gene finding with similarity). Available from: [http://linux1.softberry.com, March 3, 2009].

Sowa, W., Blackwood, A. C., and Adams, G. A. 1963. Neutral extracellular glucan of *Pullularia pullulans* (de Bary) Berkhout. *Can. J. Chem.* 41: 2314-2319.

Sugita, T., Nakajima, M., Ikeda, R., Matsushima, T., and Shinoda, T. 2002. Sequence analysis of the ribosomal DNA intergenic spacer 1 regions of *Trichosporon* species. *J.Clin. Microbiol.* 40: 1826-1830.

Suryanarayanan T. S., Wittlinger S. K., and Faeth S. H. 2005. Endophytic fungi associated with cacti in Arizona. *Mycol. Res.* 109: 635-639.

Sutar, R., David, J. K., Ganesan, K., Ghosh, A. K., Singhi, S., Chakrabarti, A., and Bachhawat, A. K. 2004. Comparison of ITSand IGS1 regions for strain typing of clinical and non-clinical isolates of *Pichia anomala*. *J. Med. Microbiol.* 53: 199-123.

Swofford, D. L. 2003. PAUP*: phylogenetic analysis using parsimony, version 4.0b 10. Sinauer Associates, Sunderland, MA.

Tada, R., Tanioka, A., Iwasawa, H., Hatashima, K., Shoji, Y., Ishibashi, K., Adachi, Y., Yamazaki, M., Tsubaki, K., and Ohno, N. 2008. Structural characterisation and biological activities of a unique type beta-D-glucan obtained from *Aureobasidium pullulans*. *Glycoconj. J.* 25: 851-861.

Taguchi, R., Kikuchi, Y., Sakano, Y., and Kobayashi, T. 1973. Structural uniformity of pullulan produced by several strains of *Pullularia pullulans*. *Agric. Biol. Chem.* 37: 1583-1588.

Takesako, K., Ikai, K., Haruna, F., Endo, M., Shimanaka, K., Sono, E., Nakamura, T., Kato, I., and Yamaguchi, H. 1991. Aureobasidins, new antifungal antibiotics. Taxonomy, fermentation, isolation, and properties. *J. Antibiot.* (Tokyo) 44: 919-924.

Takesako, K., Kuroda, H., Inoue, T., Haruna, F., Yoshikawa, Y., Kato, I., Uchida, K., Hiratani, T., and Yamaguchi, H. 1993. Biological properties of aureobasidin A, a cyclic depsipeptide antifungal antibiotic. *J. Antibiot.* (Tokyo) 46: 1414-1420.

Tanaka, H, Muguruma, M., Ohta, K. 2006. Purification and properties of a family-10 xylanase from *Aureobasidium pullulans* ATCC 20524 and characterization of the encoding gene. *Appl. Microbiol. Biotechnol.* 70: 202-211.

Taylor, J. W., Jacobson, D. J., Kroken, S., Kasuga, T., Geiser, D. M., Hibbett, D. S., and Fisher, M. C. 2000. Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics Biol.* 31: 21-32.

Taylor, P. E., Esch, R., Flagan, R. C., House, J., Tran, L., and Glovsky, M. M. 2006. Identification and possible disease mechanisms of an under-recognized fungus, *Aureobasidium pullulans*. *Int. Arch. Allergy Immunol.* 139: 45-52.

Temprano, J., Becker, B. A., Hutcheson, P. S., Knutsen, A. P., Dixit, A., and Slavin, R. G. 2007. Hypersensitivity pneumonitis secondary to residential exposure to *Aureobasidium pullulans* in 2 siblings. *Ann. Allergy Asthma Immunol.* 99: 562-566.

The European Molecular Biology Laboratory (EMBL). 2008. Multiple sequence alignment. Available from: [http://www.ebi.ac.uk/Tools/clustalw/index.html, March 3, 2009].

Thompson, J. D., Higgins, D. G., and Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673–4680.

Thorne, L. P., Pollock, T. J., and Armentrout, R. W. 1993. High molecular weight pullulan. *U. S. Patent* 5,268,460.

Thorne, L. P., Pollock, T. J., and Armentrout, R. W. 2000. High molecular weight pullulan and method for its production. *U. S. Patent* 6,010,899.

Toda, K., Gotoh, Y., Asakura, T., Yabe, I., and Furuse, H. 2000. Fluid viscosity of *Aureobasidium pullulans* cultures obtained at two different initial pH values. *J. Biosci. Bioeng.* 89: 258-261.

Tsujisaka, Y. and Mitsuhashi, M. 1993. Pullulan, In: R. L. Whistler and J. N. BeMiller (Eds.), *Industrial Gums. Polysaccharides and their derivatives*. Third Edition, pp 447-460. San Diego: Academic Press.

Urzi, C., De Leo, F., Lo Passo, C., and Criseo, G. 1999. Intra-specific diversity of *Aureobasidium pullulans* strains isolated from rocks and other habitats assessed by physiological methods and by random amplified polymorphic DNA (RAPD). *J Microbiol. Methods.* 36:95–105.

Valente, P., Ramos, J. P., and Leoncini, O. 1999. Sequencing as a tool in yeast molecular taxonomy. *Can. J. Microbiol.* 45: 949-958.

Viel, S., Capitani, D., Mannina, L., and Segre, A. 2003. Diffusion-ordered NMR spectroscopy: a versatile tool for the molecular weight determination of uncharged polysaccharides. *Biomacromolecules.* 4: 1843-1847.

Wallenfels, K., Keilich, G., Bechtler, G., and Freudenberger, D. 1965. Investigations on pullulan. IV. Resolution of structural problems using physical, chemical and enzymatic methods. *Biochem. Zeitschrift* 341: 433-450.

Wang, W. L., Chi, Z. M., Chi, Z., Li, J., and Wang, X. H. 2009. Siderophore production by the marine-derived Aureobasidium pullulans and its antimicrobial activity. *Bioresour. Technol.* 100: 2639-2641.

Wecker, A. and Onken, U. 1991. Influence of dissolved oxygen concentration and shear rate on the production of pullulan by *Aureobasidium pullulans*. *Biotechnol. Lett.* 13: 155-160.

West, T. P. and Reed-Hamer, B. 1991. Ability of *Aureobasidium pullulans* to synthesize pullulan upon selected sources of carbon and nitrogen. *Microbios* 67: 117-124.

West, T. P. and Reed-Hamer, B. 1992. Influence of vitamins and mineral salts upon pullulan synthesis of *Aureobasidium pullulans*. *Microbios* 71: 115-123.

West, T. P. and Reed-Hamer, B. 1993. Polysaccharide production by a reduced pigmentation mutant of the fungus *Aureobasidium pullulans*. *FEMS Microbiol. Lett.* 113: 345-349.

West, T. P. and Strohfus, B. 1996. A pullulan-degrading enzyme activity of *Aureobasidium pullulans*. *J. Basic Microbiol.* 36: 377-380.

West, T. P. and Strohfus, B. 1997. Effect of manganese on polysaccharide production and cellular pigmentation in the fungus *Aureobasidium pullulans*. *World J. Microbiol. Biotechnol.* 13: 233-235.

West, T. P. and Strohfus, B. 2001. Polysaccharide production by a reduced pigmentation mutant of Aureobasidium pullulans NYS-1. *Lett. Appl. Microbiol.* 33:169-172.

White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (eds), *PCR Protocols: a guide to methods and applications*. pp. 315-322. New York: Academic Press.

Wickerham, L.J . and Kurtzman, C. P. 1975. Synergistic color variants of *Aureobasidium pullulans*. *Mycologia* 67: 342-361.

Wiley, B. J., Ball, D. H., Arcidiacono, S. M., Sousa, S., Mayer, J. M., and Kaplan, D. L. 1993. Control of molecular weight distribution of the biopolymer pullulan produced by *Aureobasidium pullulans*. *J. Environ. Polymer Degrad.* 1: 3-9.

Wu, S., Jin, Z., Tong, Q., and Chen, H. 2008. Sweet potato: A novel substrate for pullulan production by *Aureobasidium pullulans*. *Carbohydr. Polymers*. Article in Press. Doi:10.1016/j.carbpol.2008.11.034.

Wu, S., Jin, Z., Kim, J. M., Tongand, Q., and Chen, H. 2009. Downstream processing of pullulan from fermentation broth. *Carbohydr. Polymers*. Article in Press. Doi:10.1016/j.carbpol.2009.02.023.

Yamasaki, H., Lee, M-S., Tanaka, T., and Nakanishi, K. 1993. Characteristics of cross-flow filtration of pullulan broth. *Appl. Microbiol. Biotechnol.* 39 : 26-30.

Yoshikawa, J., Amachi, S., Shinoyama, H., and Fujii, T. 2008. Production of fructooligosaccharides by crude enzyme preparations of β-fructofuranosidase from Aureobasidium pullulans. *Biotechno.l Lett*. 30:535–539

Yurlova, N. A., Uijthof J. M., and de Hoog, G. S. 1996. Distinction of species in *Aureobasidium* and related genera by PCR-ribotyping. *Antonie Van Leeuwenhoek*. 69: 323-329.

Yurlova, N. A. and de Hoog, G. S. 1997. A new variety of *Aureobasidium pullulans* characterized by exopolysaccharide structure, nutritional physiology and molecular features. *Antonie van Leeuwenhoek Int. J. Gen. Molec. Microbiol.* 72: 141-147.

Yurlova, N. A., Mokrousov, I. V., and de Hoog, G. S. 1995. Intraspecific variability and exopolysaccharide production in *Aureobasidium pullulans*. *Antonie van Leeuwenhoek Int. J. Gen. Molec. Microbiol.* 68 : 57-63.

Zalar, P., Gostinčar C., de Hoog, G. S., Uršič, V., Sudhadham, M., and Gunde-Cimerman N. 2008. Redefinition of *Aureobasidium pullulans* and its varieties. *Studies in Mycology* 61: 21–38.

Zhang, L., and Chi, Z. M. 2007. Screening and identification of a cellulase producing marine yeast and medium and fermentation condition optimization for cellulase production. *J. Ocean. Univ. Chin. Sup.* II37:101–108.

APPENDICES

Appendix A

Culture media

1. Malt Extract Agar (MEA)

Malt extract	20.0	g
Peptone	1.0	g
Dextrose	20.0	g
Agar	25.0	g

Dissolved in distilled water to final volume 1 liter.

Note that: sterile dextrose should be prepared separately and added after autoclaving to prevent carmolization.

2. Yeast Malt Agar (YMA)

Yeast extract	3.0	g
Malt extract	3.0	g
Bacto-peptone	5.0	g
Dextrose	10.0	g
Agar	20.0	g

Dissolved in distilled water to final volume 1 liter.

3. Pullulan Production (PM) medium

Sucrose	50.0	g
Bacto-peptone	0.6	g
K_2HPO_4	5.0	g
$MgSO_4.7H_2O$	0.4	g
NaCl	1.0	g
Yeast extract	0.4	g

Dissolved in 800 ml of distilled water and adjusted pH to 6.5 with HCl.

Added distilled water to final volume 1 liter.

4. Starch Agar medium

Soluble starch	10.0	g
$NaNO_3$	2.0	g
$MgSO_4.7H_2O$	0.5	g
NaCl	0.5	g
$FeSO_4.7H_2O$	0.01	g
Yeast extract	0.4	g
Agar	20.0	g

Dissolved in distilled water to final volume 1 liter.

5. Crypto-Basol (CB) medium

Yeast nitrogenous base	6.7	g
Asparagine	2.0	g
KH2PO4	5.0	g
Oat spelt xylan	10.0	g

Dissolved in distilled water to final volume 1 liter.

6. LB (Luria-Bertani) medium

Bacto-tryptone	10.0	g
Yeast extract	5.0	g
NaCl	10.0	g
Agar	10.0	g

Dissolved in 800 ml of distilled water and adjusted pH to 7.5 with NaOH.

Added distilled water to final volume 1 liter.

Note that: added antibiotics to final concentration 50 µg/ml, if needed.

Appendix B

PCR reactions and condition

1. DNA Amplification of DNA walking in GenomeWalker library

Reaction

28 µl	sterile distilled water
10 µl	5 x amplifying buffer
2 µl	dNTPs (10 mM each)
2 µl	AP1 primer (10 µM)
2 µl	GSP primer (10 µM)
1 µl	Phusion Hot Start DNA polymerase (2 U/µl) (Finnzymes)
5 µl	DNA library
50 µl	Total volume

Condition

98°C	30 sec	
98°C	10 sec	35 cycles
55°C	30 sec	
72°C	1 min	
72°C	7 min	

2. DNA Amplification of DIG-labeling probe

Reaction (Probe 3:1 ratio (unlabeled/labeled))

1 µl	DNA (~0.1 ng)
6 µl	primer AP-GSP3f (3.2 pmol/µl)
6 µl	primer AP-GSP5r (3.2 pmol/µl)
5 µl	10 x amplifying buffer
1 µl	dATP (10 mM)
1 µl	dCTP (10 mM)
1µl	dGTP (10 mM)

0.75 µl dTTP (10 mM)

2.5 µl DIG UTP (1 mM)

23.5 µl sterile distilled water

2 µl *Taq* DNA polymerase (1 U/µl) (Roche)

50 µl Total volume

Condition

96°C 1 min

96°C 30 sec
55°C 1 min } 35 cycles
72°C 1 min

72°C 7 min

3. DNA Amplification of α-amylase mRNA detection and large subunit (26S)

Reaction

5 µl 10 x amplifying buffer

2 µl Primer F (10 µM)

2 µl Primer R (10 µM)

5 µl dNTPs (2 mM each)

32 µl sterile distilled water

2 µl Red *Taq* DNA polymerase (1 U/µl) (Sigma)

2 µl DNA template

50 µl Total volume

Condition

96°C 1 min

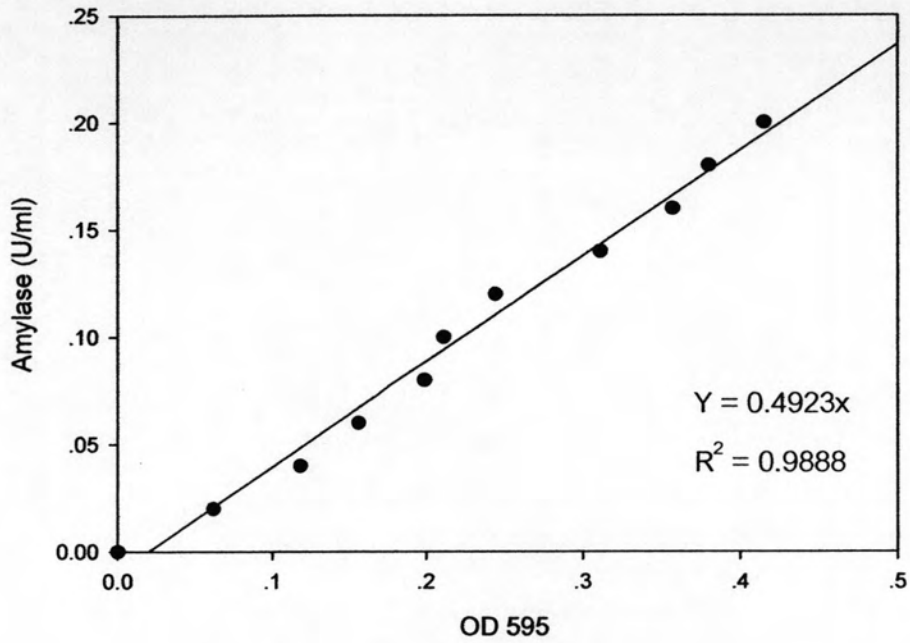
96°C 30 sec
52°C 1 min } 35 cycles
72°C 1 min

72°C 7 min

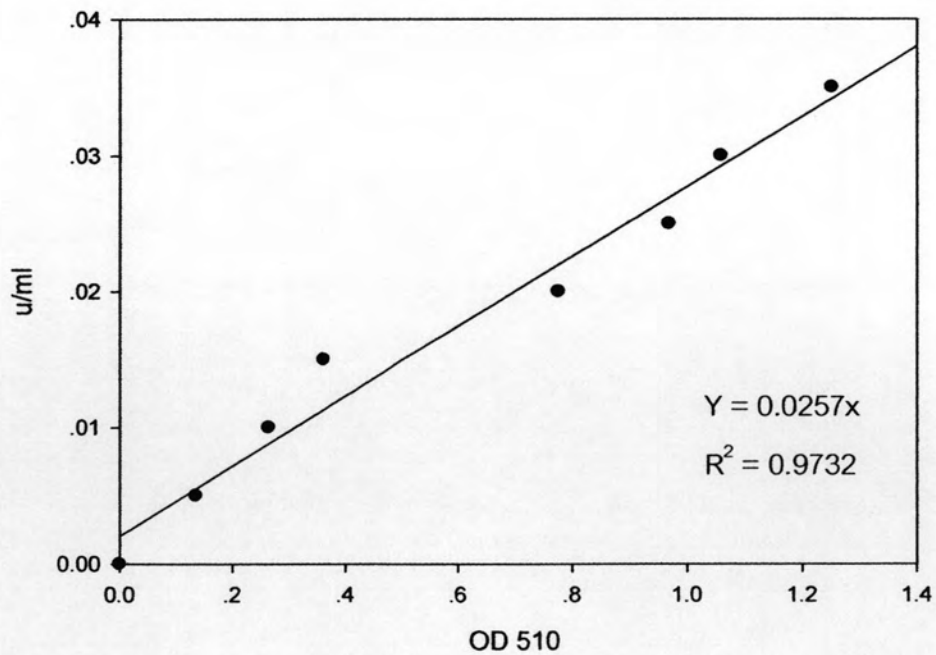
Appendix C

Standard curve

A. Standard curve for α-amylase



B. Standard curve for pullulanase



Appendix D

Data tables of Figure 4.15 – 4.26 and semi-log growth curves

Table 1 Cell growth (OD_{600}) of *A. pullulans* cultured in standard PM (5.0% sucrose and 0.1% N-sources) at day 0, 2, 4, 6, and 8 (Numbers were average ± standard error)

Day	CU 3	CU 20	CU 36	NRM2	NRRL Y-12974
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	1.9 ± 0.1	1.2 ± 0.0	1.9 ± 0.1	2.8 ± 0.3	2.1 ± 0.4
4	4.8 ± 1.0	2.7 ± 0.1	3.6 ± 1.0	3.3 ± 0.1	4.8 ± 0.8
6	5.4 ± 0.4	3.0 ± 0.1	4.1 ± 1.3	3.7 ± 0.3	4.3 ± 0.2
8	5.6 ± 0.5	3.6 ± 0.1	4.4 ± 0.1	4.1 ± 0.1	2.4 ± 0.2

Table 2 Cell growth (OD_{600}) of *A. pullulans* NRRL Y-12974 cultured in modified PM at day 0, 2, 4, 6, and 8 (Numbers were average ± standard error)

Day	5.0% Sucrose, 0.1% N-sources	5.0% Sucrose, 0.3% N-sources	5.0% Starch, 0.1% N-sources	5.0% Starch, 0.3% N-sources
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	2.1 ± 0.4	2.0 ± 0.1	2.6 ± 0.0	3.0 ± 0.1
4	4.8 ± 0.8	3.7 ± 0.4	4.0 ± 0.5	4.6 ± 0.6
6	4.3 ± 0.2	4.3 ± 0.5	4.3 ± 0.2	6.2 ± 1.2
8	2.4 ± 0.2	5.7 ± 0.7	4.5 ± 0.2	6.9 ± 0.4

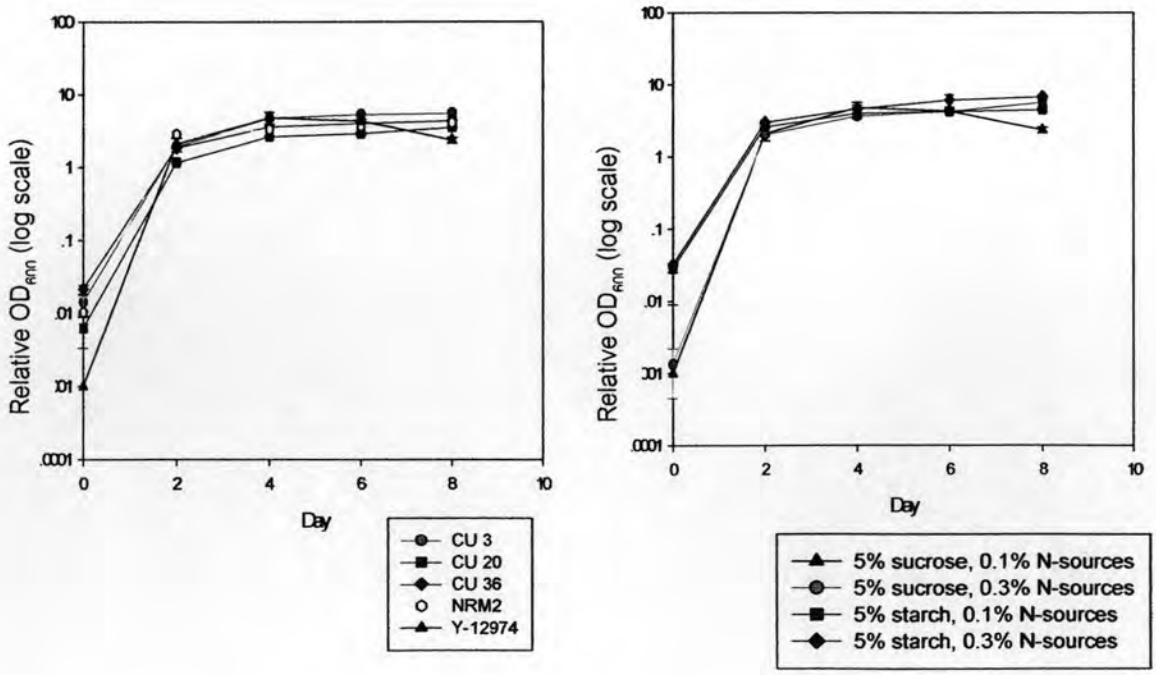


Table 3 pH measurement of culture supernatants of *A. pullulans* grown in standard PM (5.0% sucrose and 0.1% N-sources) at day 0, 2, 4, 6, and 8 (Numbers were average ± standard error)

Day	CU 3	CU 20	CU 36	NRM2	NRRL Y-12974
0	6.5 ± 0.0	6.5 ± 0.0	6.5 ± 0.0	6.5 ± 0.0	6.5 ± 0.0
2	4.0 ± 0.0	4.4 ± 0.0	4.8 ± 0.1	3.9 ± 0.0	5.5 ± 0.4
4	3.6 ± 0.0	3.7 ± 0.1	3.9 ± 0.0	3.7 ± 0.0	4.3 ± 0.1
6	3.5 ± 0.0	3.7 ± 0.0	3.7 ± 0.1	3.6 ± 0.0	4.2 ± 0.0
8	3.5 ± 0.0	3.7 ± 0.1	3.5 ± 0.0	3.6 ± 0.0	4.2 ± 0.0

Table 4 pH measurement of culture supernatants of *A. pullulans* NRRL Y-12974 grown in modified PM at day 0, 2, 4, 6, and 8 (Numbers were average ± standard error)

Day	5.0% Sucrose, 0.1% N-sources	5.0% Sucrose, 0.3% N-sources	5.0% Starch, 0.1% N-sources	5.0% Starch, 0.3% N-sources
0	6.5 ± 0.0	6.5 ± 0.0	6.5 ± 0.0	6.5 ± 0.0
2	5.5 ± 0.4	5.8 ± 0.0	6.0 ± 0.1	6.0 ± 0.0
4	4.3 ± 0.1	5.2 ± 0.0	5.4 ± 0.0	5.5 ± 0.0
6	4.2 ± 0.0	5.0 ± 0.0	5.3 ± 0.1	5.5 ± 0.0
8	4.2 ± 0.0	5.6 ± 0.4	5.8 ± 0.0	5.7 ± 0.0

Table 5 EPS yield (g/L) of *A. pullulans* cultured in standard PM (5.0% sucrose and 0.1% N-sources) at day 2, 4, 6, and 8 (Numbers were average ± standard error)

Day	CU 3	CU 20	CU 36	NRM2	NRRL Y-12974
2	9.8 ± 0.1	3.8 ± 0.1	3.1 ± 0.1	5.8 ± 0.0	12.0 ± 0.1
4	17.6 ± 0.2	14.1 ± 1.0	6.2 ± 0.1	10.0 ± 0.1	31.3 ± 1.1
6	21.6 ± 0.8	22.2 ± 0.8	7.1 ± 1.0	11.6 ± 0.2	39.0 ± 0.1
8	24.6 ± 0.4	29.0 ± 1.4	6.7 ± 0.3	17.0 ± 0.3	39.8 ± 0.2

Table 6 EPS yield (g/L) of *A. pullulans* NRRL Y-12974 cultured in modified PM at day 2, 4, 6, and 8 (Numbers were average ± standard error)

Day	5.0% Sucrose, 0.1% N-sources	5.0% Sucrose, 0.3% N-sources	5.0% Starch, 0.1% N-sources	5.0% Starch, 0.3% N-sources
2	12.0 ± 0.1	6.3 ± 0.1	28.6 ± 0.6	30.3 ± 0.9
4	31.3 ± 1.1	15.0 ± 1.9	31.6 ± 1.0	36.0 ± 0.4
6	39.0 ± 0.1	23.8 ± 0.9	30.8 ± 0.6	22.5 ± 0.7
8	39.8 ± 0.2	16.8 ± 1.0	29.6 ± 1.3	18.0 ± 2.0

Table 7 EPS yield (g EPS dry weight / g cell dry weight) of *A. pullulans* cultured in standard PM (5.0% sucrose and 0.1% N-sources) at day 2, 4, 6, and 8 (Numbers were average ± standard error)

Day	CU 3	CU 20	CU 36	NRM2	NRRL Y-12974
2	3.2	1.9	1.2	1.7	3.2
4	3.4	4.5	1.0	2.3	5.5
6	2.9	5.8	0.9	2.3	6.7
8	3.1	7.1	0.7	2.9	6.5

Table 8 EPS yield (g EPS dry weight / g cell dry weight) of *A. pullulans* NRRL Y-12974 cultured in modified PM at day 2, 4, 6, and 8 (Numbers were average ± standard error)

Day	5.0% Sucrose, 0.1% N-sources	5.0% Sucrose, 0.3% N-sources	5.0% Starch, 0.1% N-sources	5.0% Starch, 0.3% N-sources
2	3.2	1.7	7.7	8.1
4	5.5	2.3	7.3	4.0
6	6.7	2.9	5.9	2.6
8	6.5	2.1	4.7	1.7

Table 9 Molecular weight (kD) of EPS precipitated from culture supernatants of *A. pullulans* grown in standard PM (5.0% sucrose and 0.1% N-sources) at day 2, 4, 6, and 8 (Numbers were average ± standard error)

Day	CU 3	CU 20	CU 36	NRM2	NRRL Y-12974
2	722 ± 45	1,070 ± 90	664 ± 97	512 ± 18	128 ± 118
4	327 ± 30	302 ± 71	415 ± 131	89 ± 11	170 ± 40
6	500 ± 51	114 ± 8	186 ± 15	33 ± 3	52 ± 3
8	459 ± 9	43 ± 5	76 ± 11	18 ± 4	26 ± 1

Table 10 Molecular weight (kD) of EPS precipitated from culture supernatants of *A. pullulans* NRRL Y-12974 grown in modified PM at day 2, 4, 6, and 8 (Numbers were average ± standard error)

Day	5.0% Sucrose, 0.1% N-sources	5.0% Sucrose, 0.3% N-sources	5.0% Starch, 0.1% N-sources	5.0% Starch, 0.3% N-sources
2	128 ± 118	857 ± 67	2 ± 0	2 ± 0
4	170 ± 40	425 ± 56	3 ± 0	3 ± 0
6	52 ± 3	223 ± 60	3 ± 0	3 ± 0
8	26 ± 1	356 ± 23	3 ± 0	2 ± 0

Table 11 Viscosity (cP) of EPS precipitated from culture supernatants of *A. pullulans* grown in standard PM (5.0% sucrose and 0.1% N-sources) at day 2, 4, 6, and 8. Measured at 25°C with a rotation of 30 rpm (shear rate of 39.6 1/S) (Numbers were average ± standard error)

Day	CU 3	CU 20	CU 36	NRM2	NRRL Y-12974
2	4.3 ± 01	9.9 ± 0.5	5.8 ± 1.1	3.7 ± 0.3	6.4 ± 1.0
4	5.0 ± 0.1	7.0 ± 0.2	32.1 ± 2.5	2.5 ± 0.2	3.4 ± 0.1
6	8.3 ± 0.8	5.6 ± 0.4	38.5 ± 5.0	2.1± 0.0	2.5 ± 0.1
8	8.5 ± 0.7	3.4 ± 0.4	66.1 ± 3.9	2.4 ± 0.1	2.3 ± 0.2

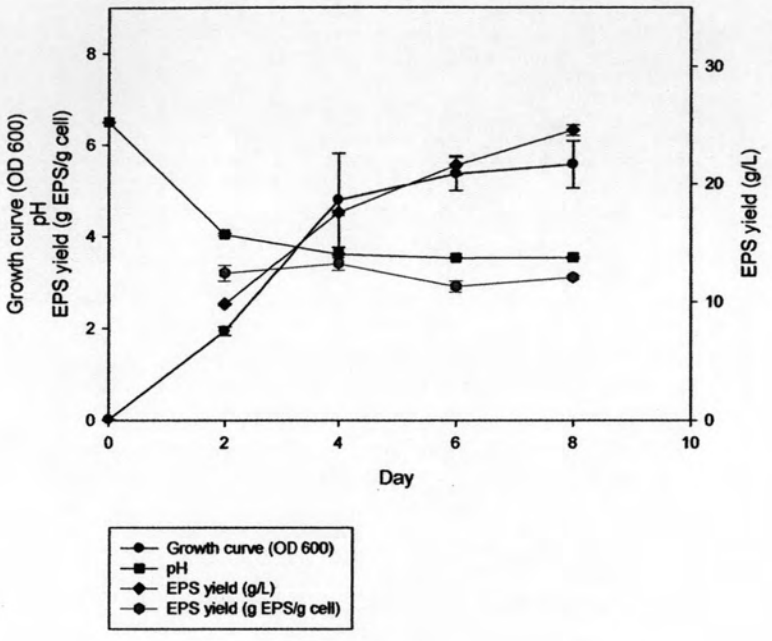
Table 12 Viscosity (cP) of EPS precipitated from culture supernatants of *A. pullulans* NRRL Y-12974 grown in modified PM at day 2, 4, 6, and 8. Measured at 25°C with a rotation of 30 rpm (shear rate of 39.6 1/S) (Numbers were average ± standard error)

Day	5.0% Sucrose, 0.1% N-sources	5.0% Sucrose, 0.3% N-sources	5.0% Starch, 0.1% N-sources	5.0% Starch, 0.3% N-sources
2	6.4 ± 1.0	6.0 ± 0.6	2.4 ± 0.3	1.5 ± 0.1
4	3.4 ± 0.1	7.5 ± 0.9	2.5 ± 0.1	2.1 ± 0.1
6	2.5 ± 0.1	5.0 ± 1.5	2.1 ± 0.0	1.7 ± 0.1
8	2.3 ± 0.2	5.8 ± 0.2	2.4 ± 0.2	3.3 ± 0.3

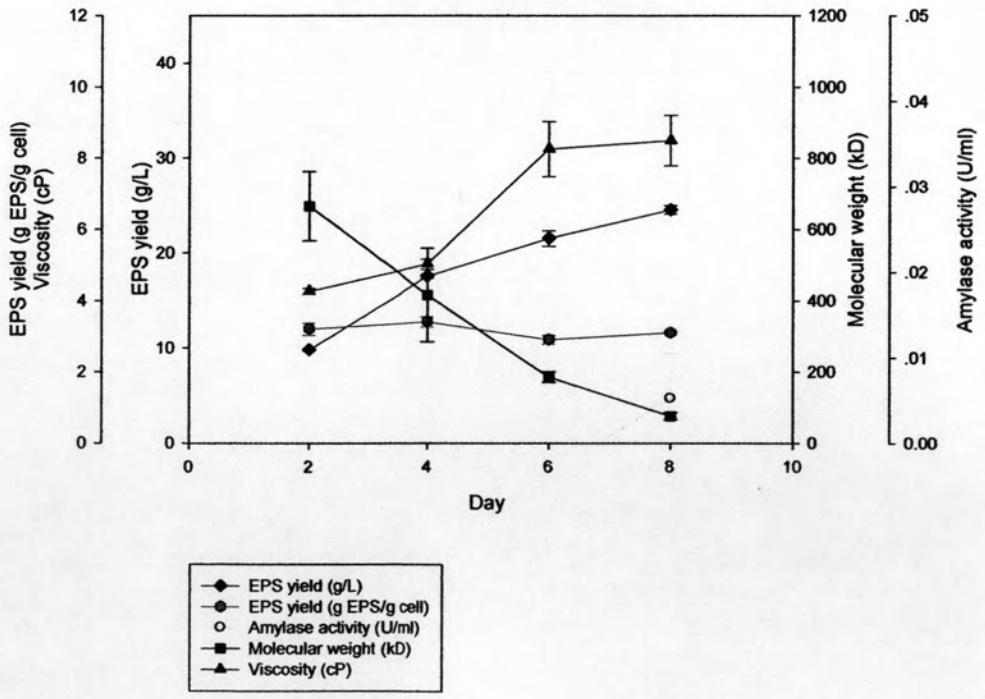
Appendix E

Graphs of combine data of each strain from Result 4.2.1

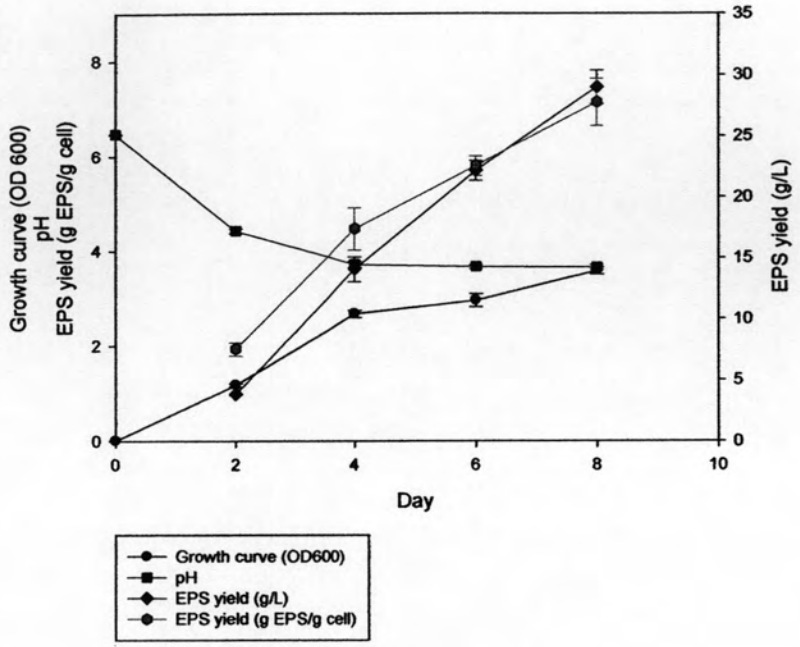
CU 3



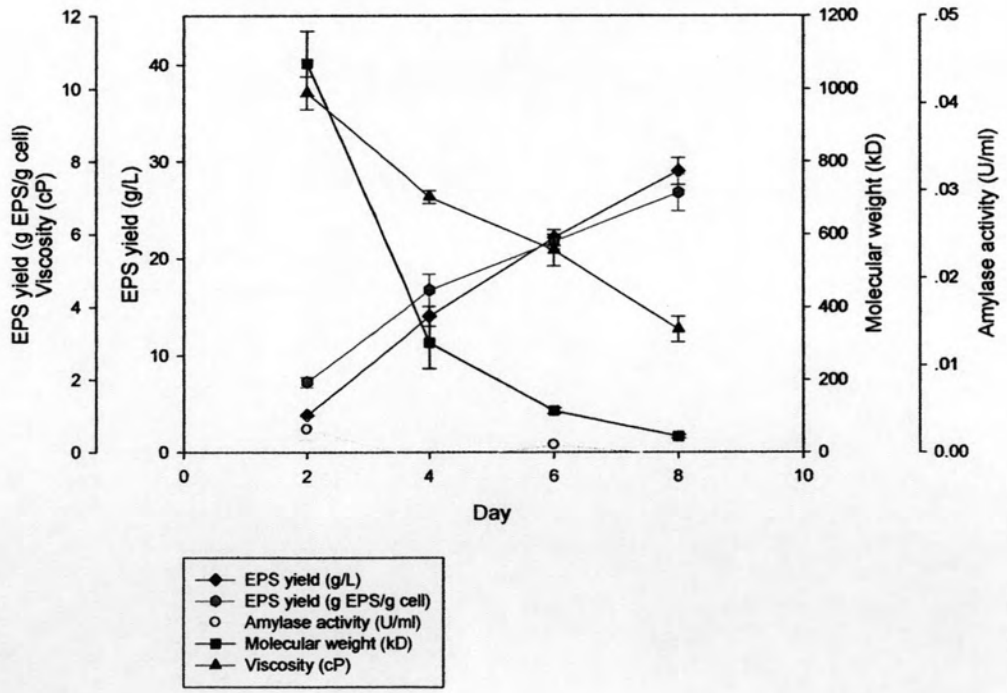
CU 3



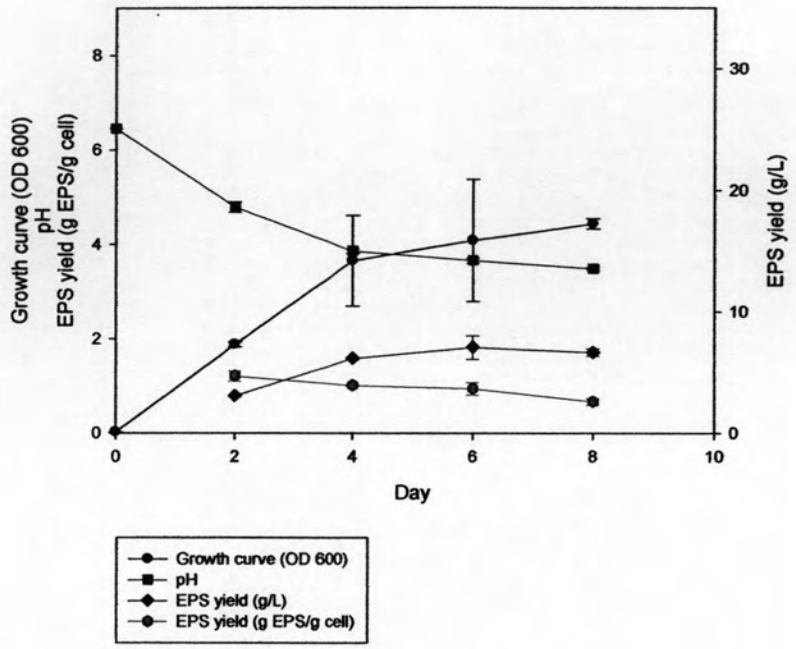
CU 20


Growth curve (OD 600)
pH
EPS yield (g EPS/g cell)
EPS yield (g/L)
Day
Growth curve (OD600)
pH
EPS yield (g/L)
EPS yield (g EPS/g cell)

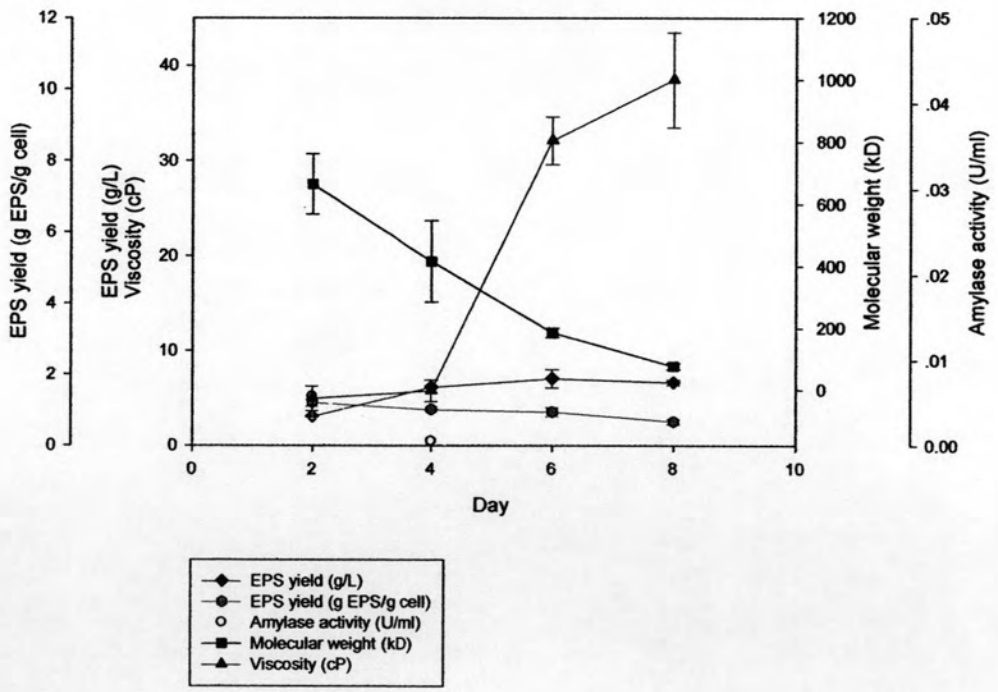
CU 20


EPS yield (g EPS/g cell)
Viscosity (cP)
EPS yield (g/L)
Molecular weight (kD)
Amylase activity (U/ml)
Day
EPS yield (g/L)
EPS yield (g EPS/g cell)
Amylase activity (U/ml)
Molecular weight (kD)
Viscosity (cP)

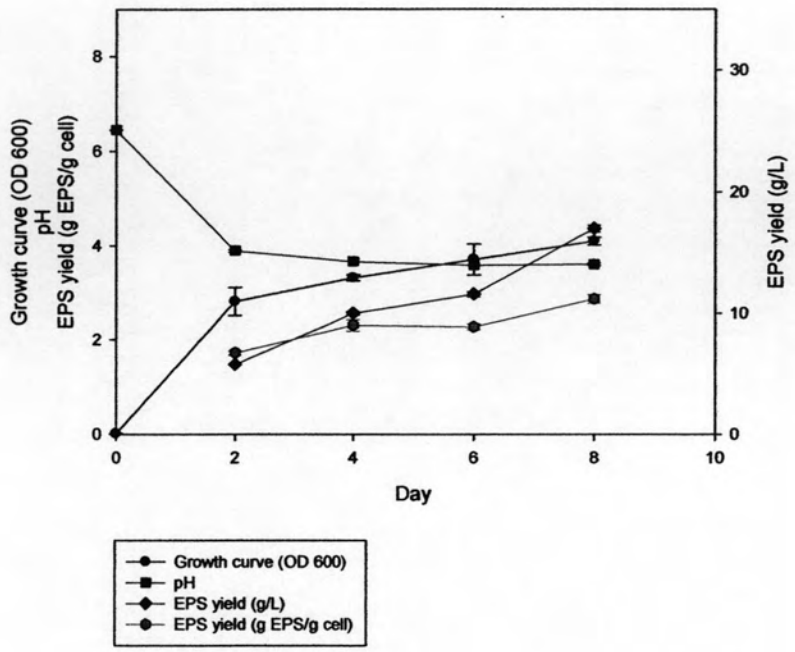
CU 36


Growth curve (OD 600)
pH
EPS yield (g EPS/g cell)
EPS yield (g/L)
Day
Growth curve (OD 600)
pH
EPS yield (g/L)
EPS yield (g EPS/g cell)

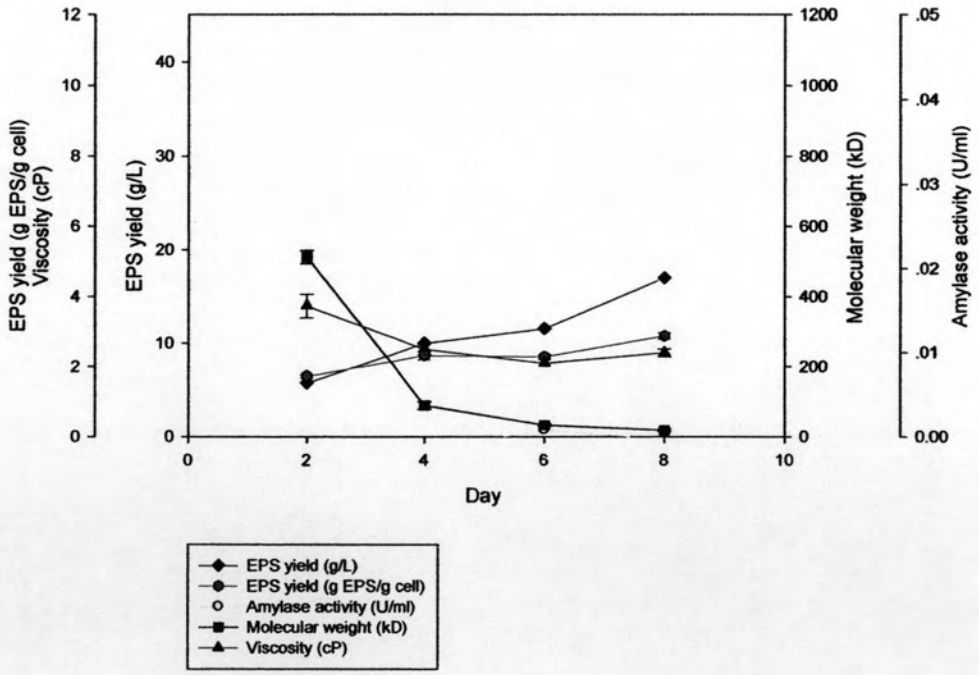
CU 36


EPS yield (g EPS/g cell)
EPS yield (g/L)
Viscosity (cP)
Molecular weight (kD)
Amylase activity (U/ml)
Day
EPS yield (g/L)
EPS yield (g EPS/g cell)
Amylase activity (U/ml)
Molecular weight (kD)
Viscosity (cP)

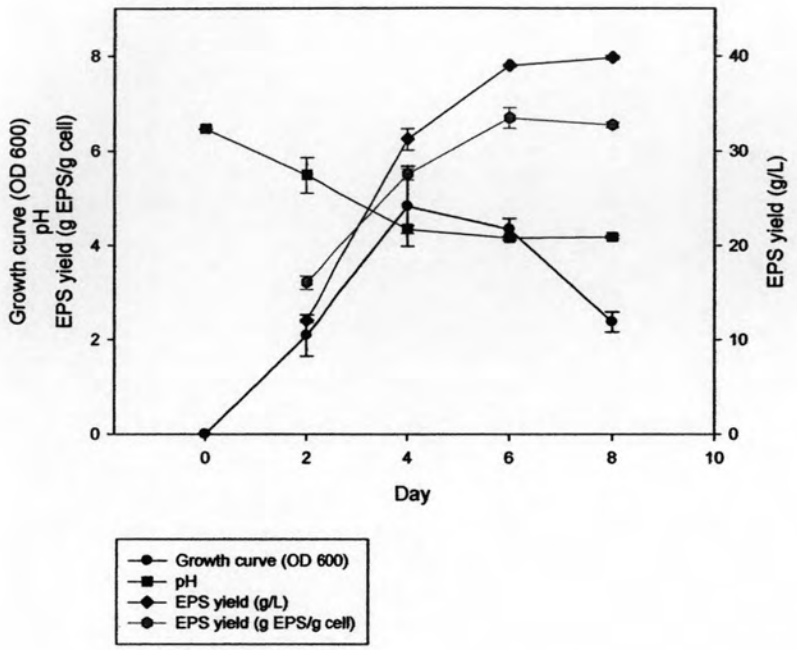
NRM2


Growth curve (OD 600)
pH
EPS yield (g EPS/g cell)
EPS yield (g/L)
Day
Growth curve (OD 600)
pH
EPS yield (g/L)
EPS yield (g EPS/g cell)

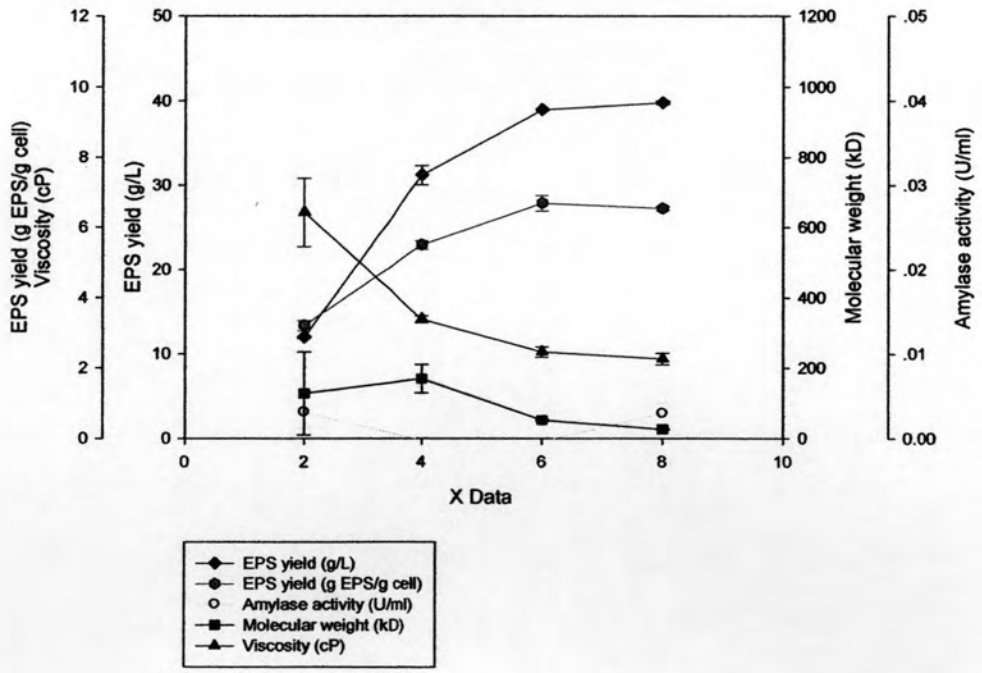
NRM2


EPS yield (g EPS/g cell)
Viscosity (cP)
EPS yield (g/L)
Molecular weight (kD)
Amylase activity (U/ml)
Day
EPS yield (g/L)
EPS yield (g EPS/g cell)
Amylase activity (U/ml)
Molecular weight (kD)
Viscosity (cP)

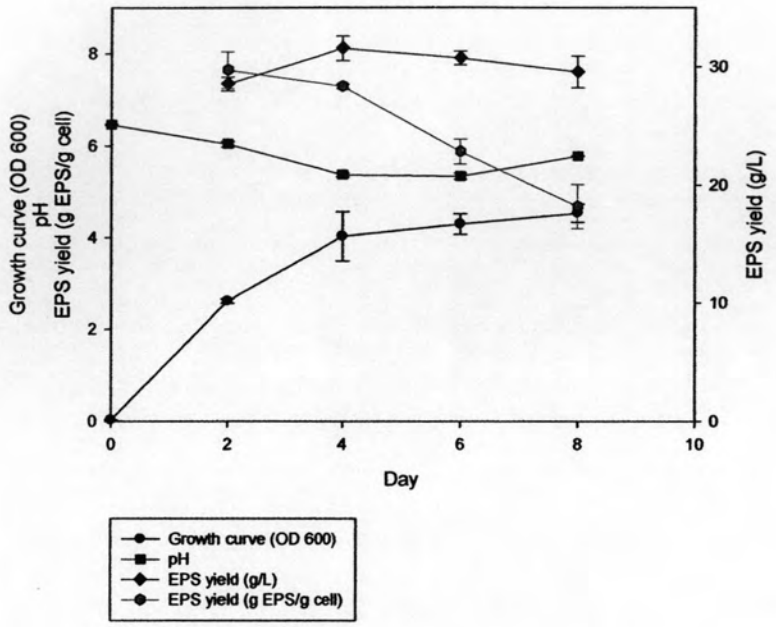
Y-12974 (PM; 5% sucrose, 0.1% N-sources)



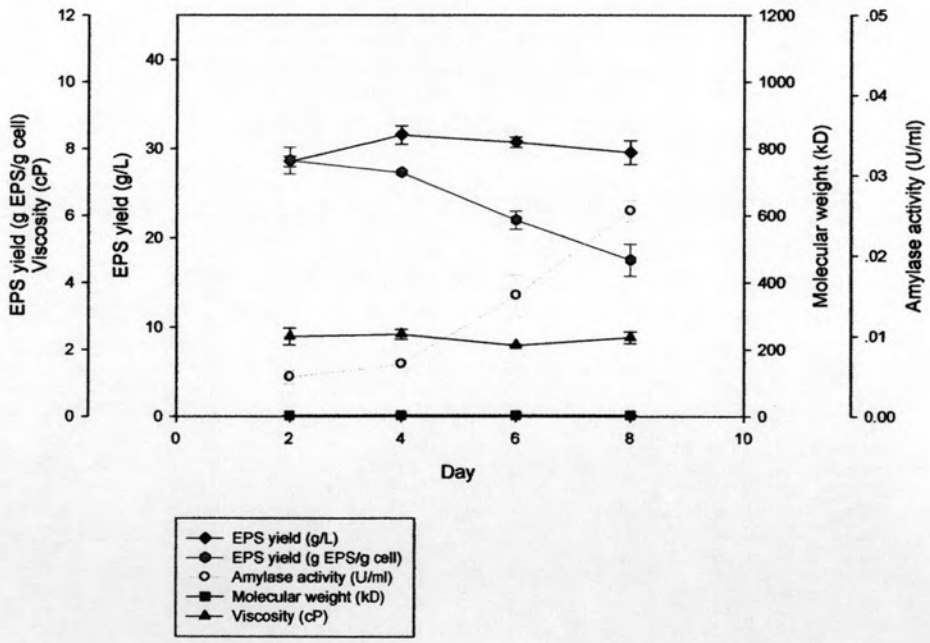
Y-12974 (PM; 5% sucrose, 0.1% N-sources)



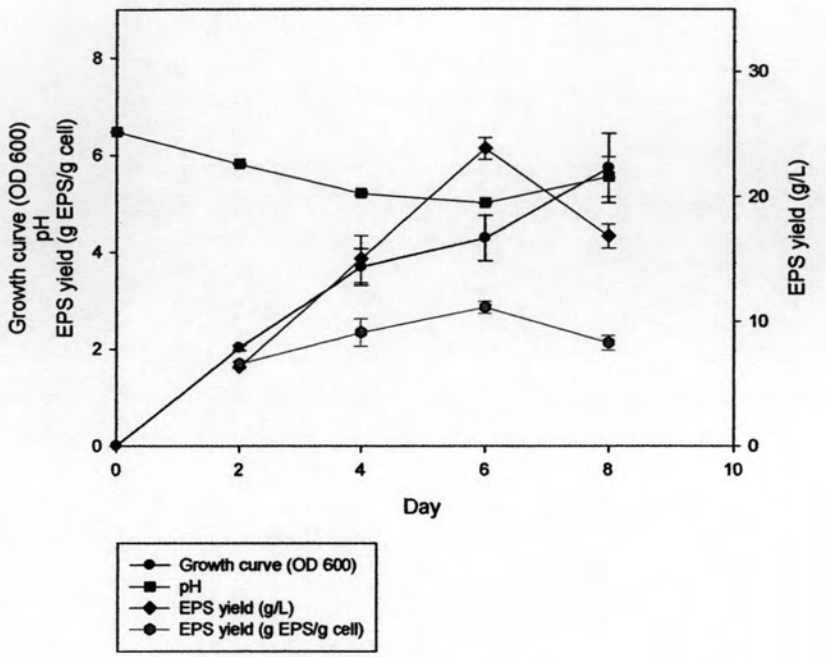
Y-12974 (PM; 5% starch, 0.1% N-sources)



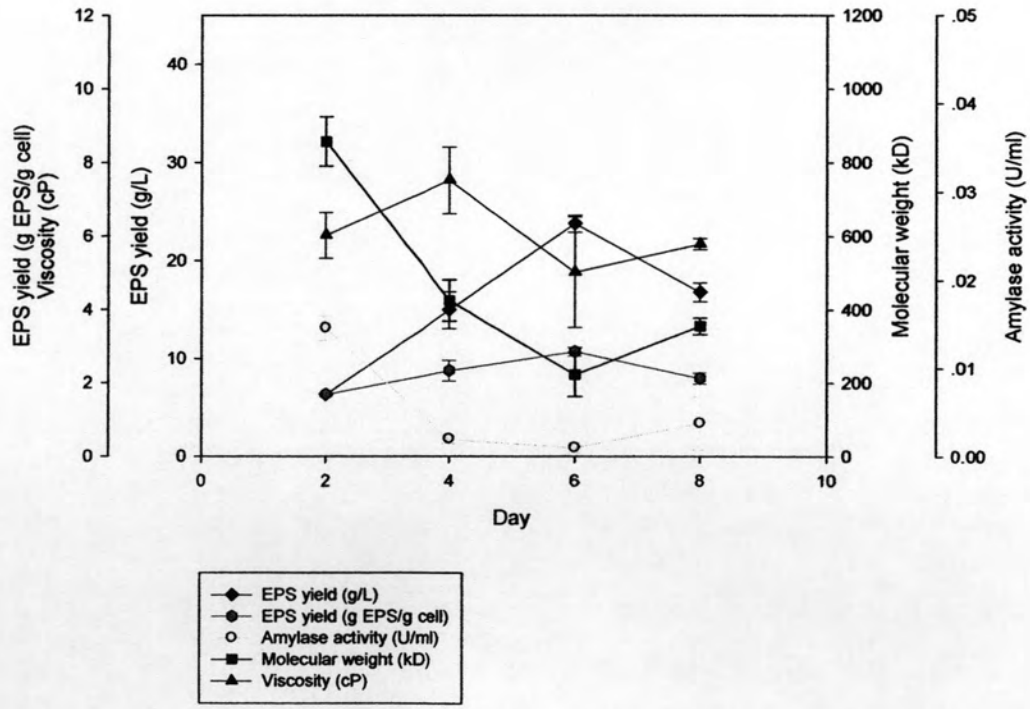
Y-12974 (PM; 5% starch, 0.1% N-sources)



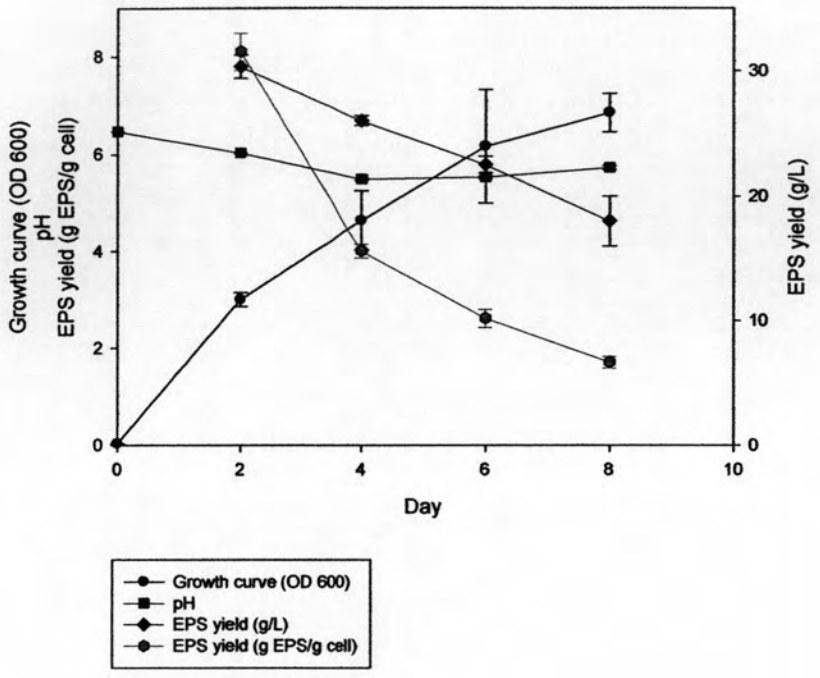
Y-12974 (PM; 5% sucrose, 0.3% N-sources)



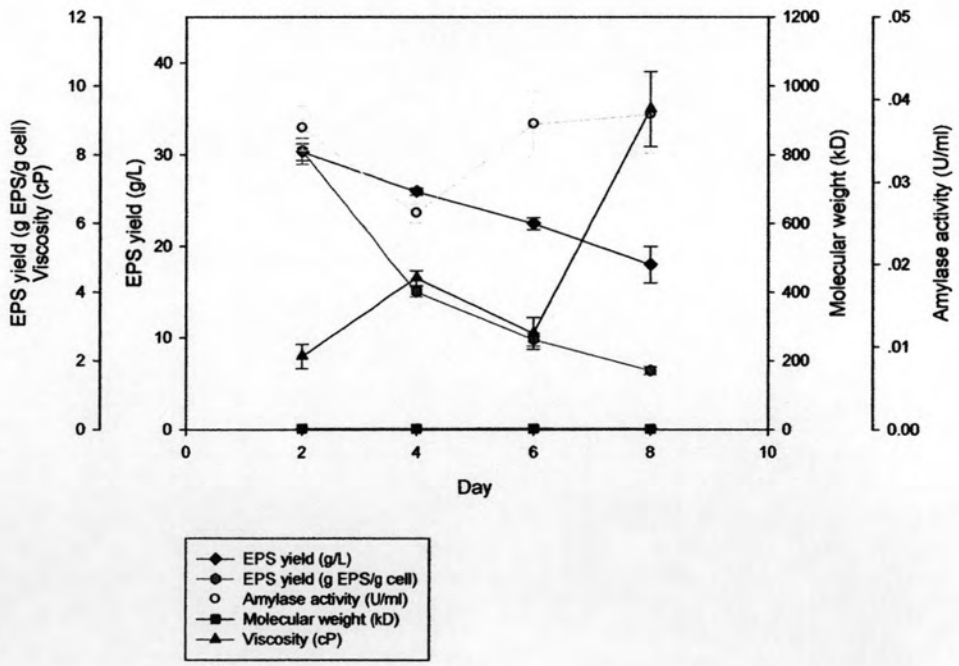
Y-12974 (PM; 5% sucrose, 0.3% N-sources)



Y-12974 (PM; 5% starch, 0.3% N-sources)



Y-12974 (PM; 5% starch, 0.3% N-sources)

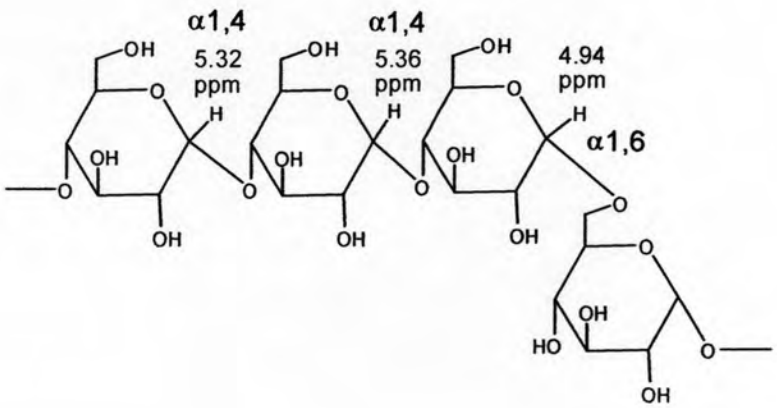


Appendix F

NMR

Pullulan Repeat Unit: Proton NMR

Pullulan has a repeat unit of maltotriose (Glc-α-1,4-Glc- α-1,4-Glc) connected by an α-1,6-linkage. The NMR shows two α-1,4 proton signals (5.32 ppm and 5.36 ppm) and one α-1,6 proton (4.94 ppm). Equal intensity of three signal indicates a 2:1 ratio of α-1,4 : α-1,6. Chemical shifts of protons around the glucose rings (H2, H3, H4, H5, and H6) are shown in table 1 (courtesy of Neil P.J. Price, USDA, Peoria, IL, USA).



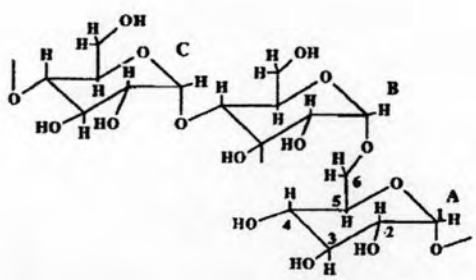
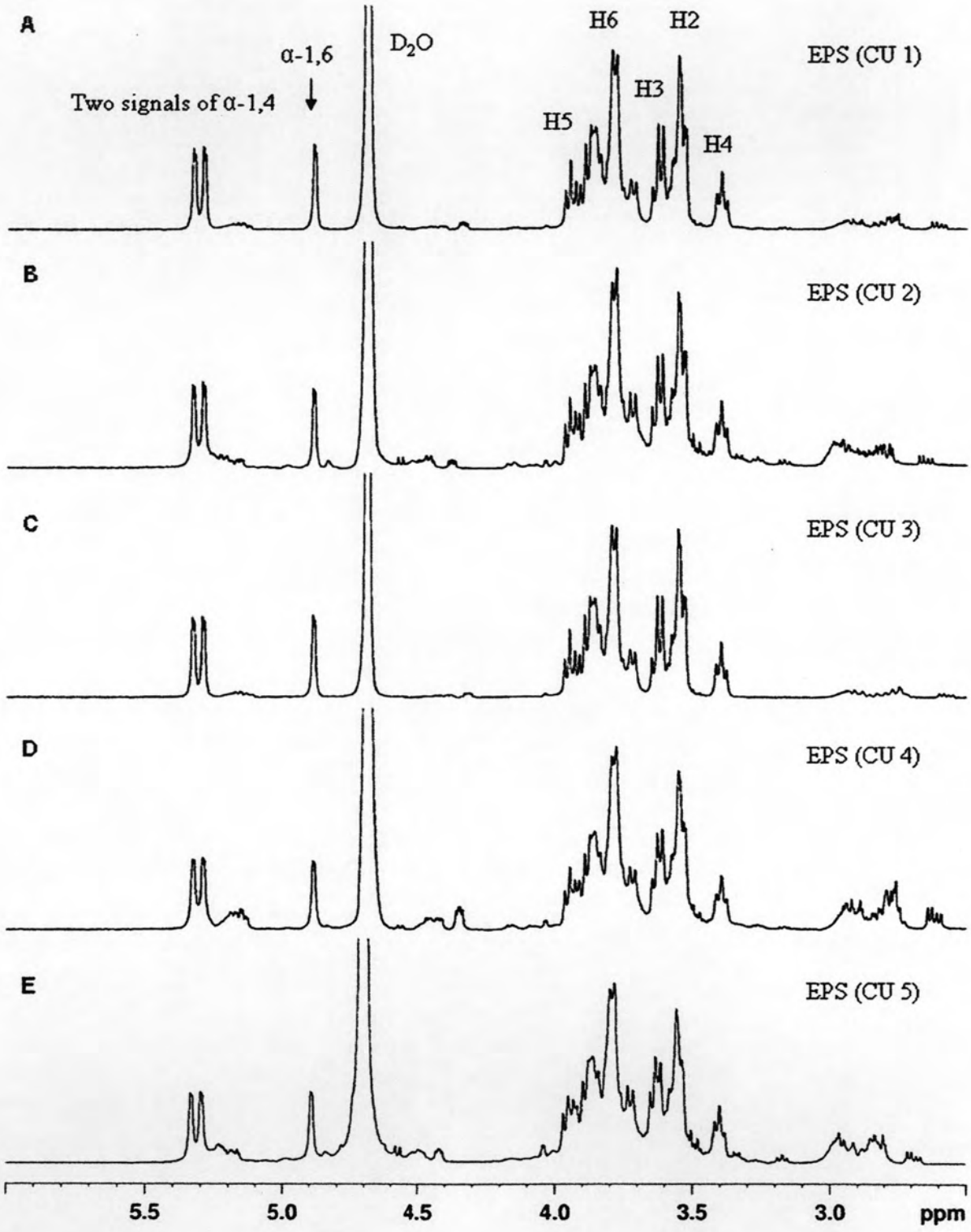
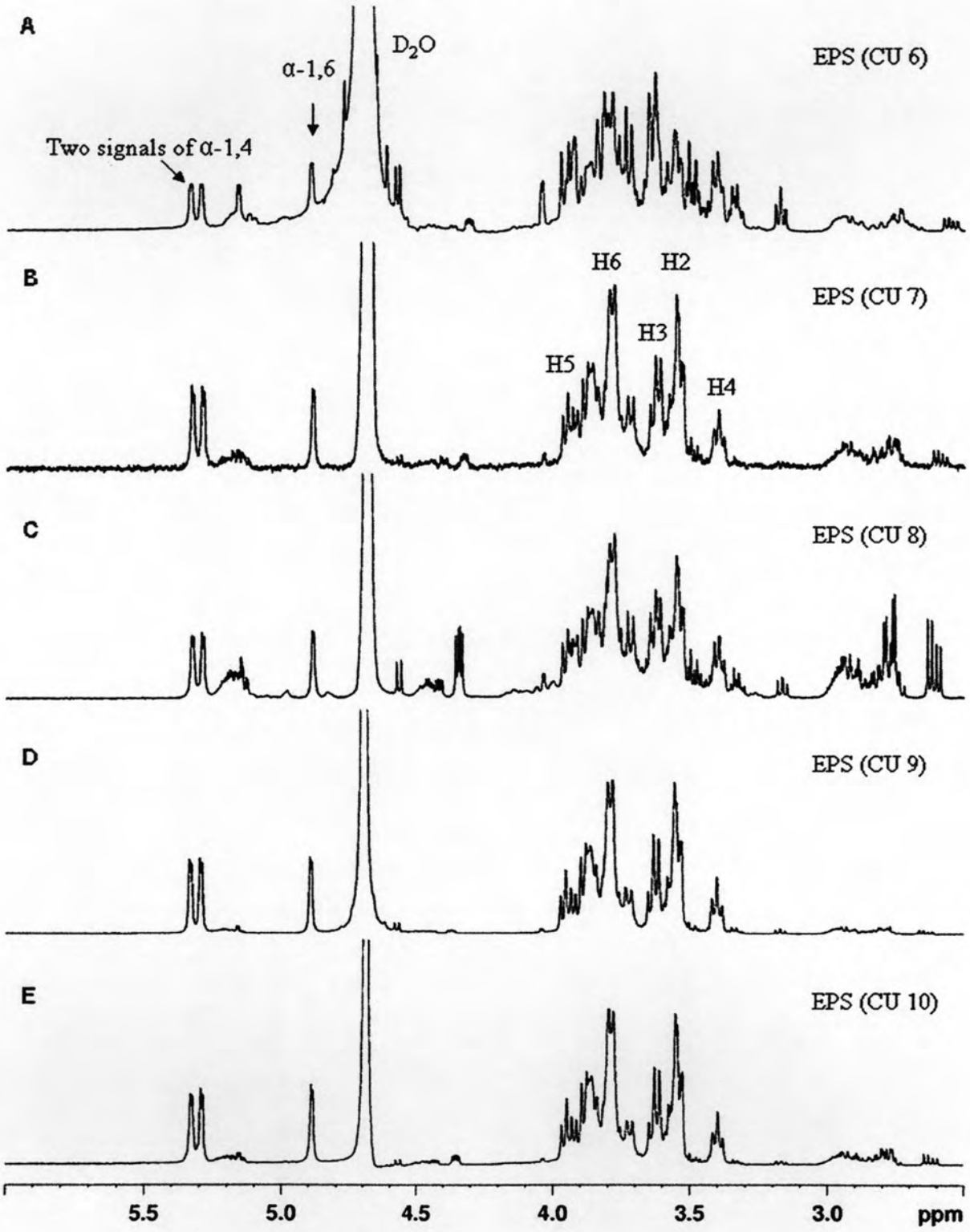


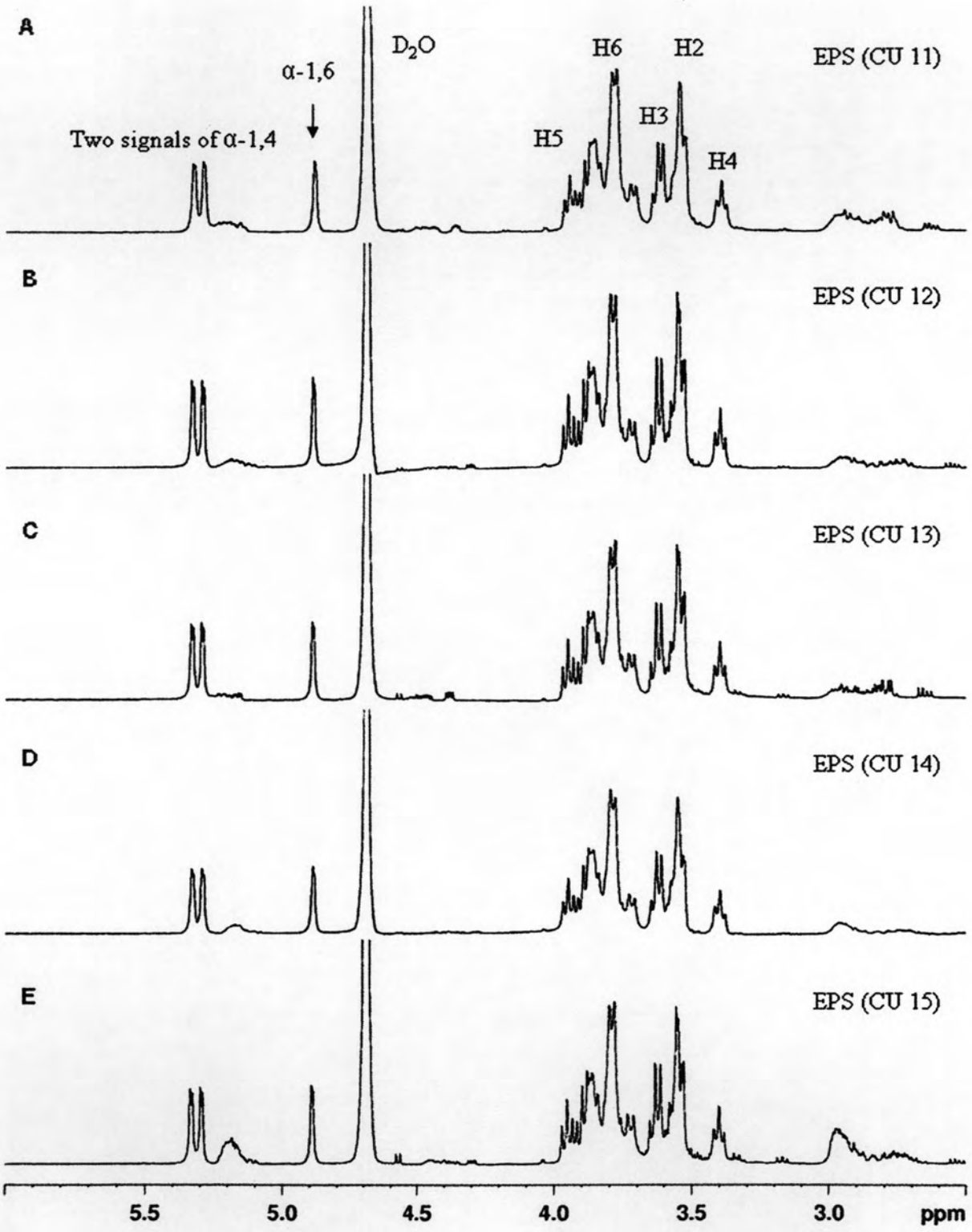
Table 1.
Chemical Shifts (ppm) of Pullulan Protons at 55°C (DSS reference).

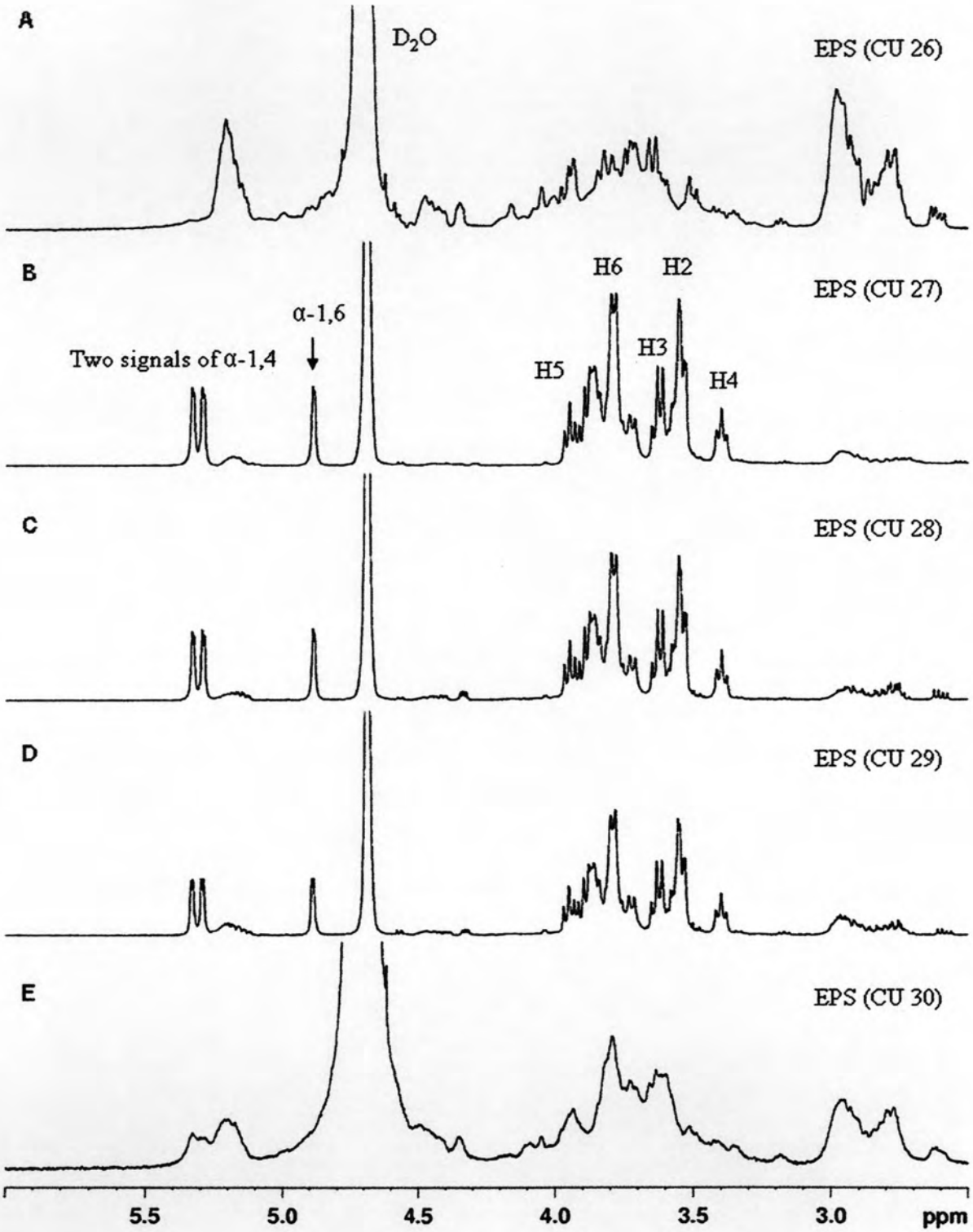
Sigma, Pfanstiehl	H-1	H-2	H-3	H-4	H-5	H-6
(1→4)-(1→6)-(1→4) Glc	4.94	3.59	3.99	3.65	3.84	3.82, 3.91
(1→4)-(1→4)-(1→6) Glc	5.36	3.63	3.94	3.58	3.91	3.82, 3.84
(1→6)-(1→4)-(1→4) Glc	5.32	3.60	3.69	3.45	3.91	3.79, 3.91
AVEBE						
(1→4)-(1→6)-(1→4) Glc	4.94	3.59	3.99	3.65	3.84	3.82, 3.91
(1→4)-(1→4)-(1→6) Glc	5.36	3.63	3.94	3.58	3.91	3.82, 3.84
(1→6)-(1→4)-(1→4) Glc	5.32	3.60	3.69	3.45	3.91	3.79, 3.91
(1→4)-(1→4)-(1→4) Glc	5.31	3.62	3.98	3.65	3.91	n.d.
α Reducing Glc	5.23	3.56	3.97	3.65	n.d.	n.d.
β Reducing Glc	4.63	3.27	3.77	3.65	n.d.	n.d.

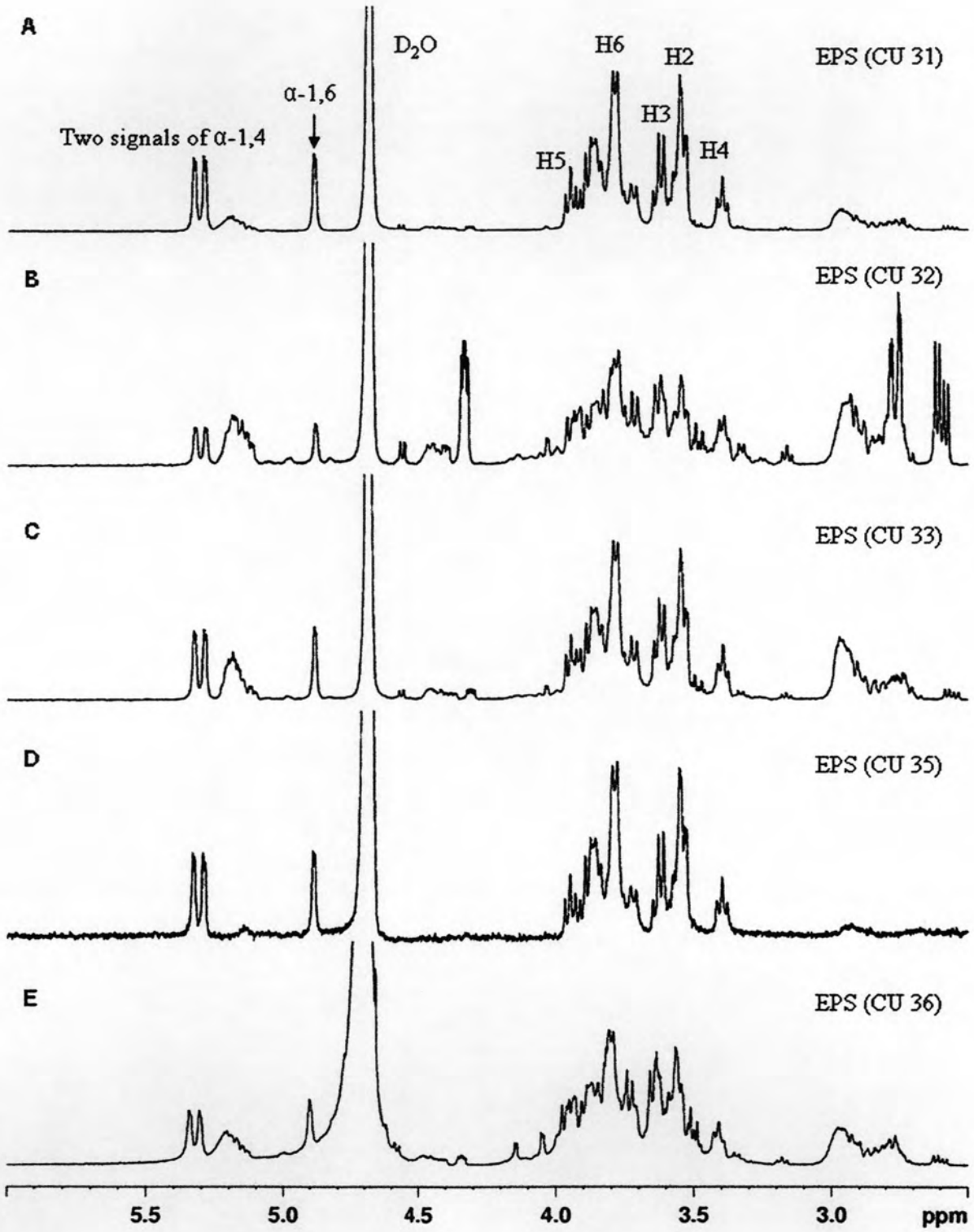
n.d. = not determined.

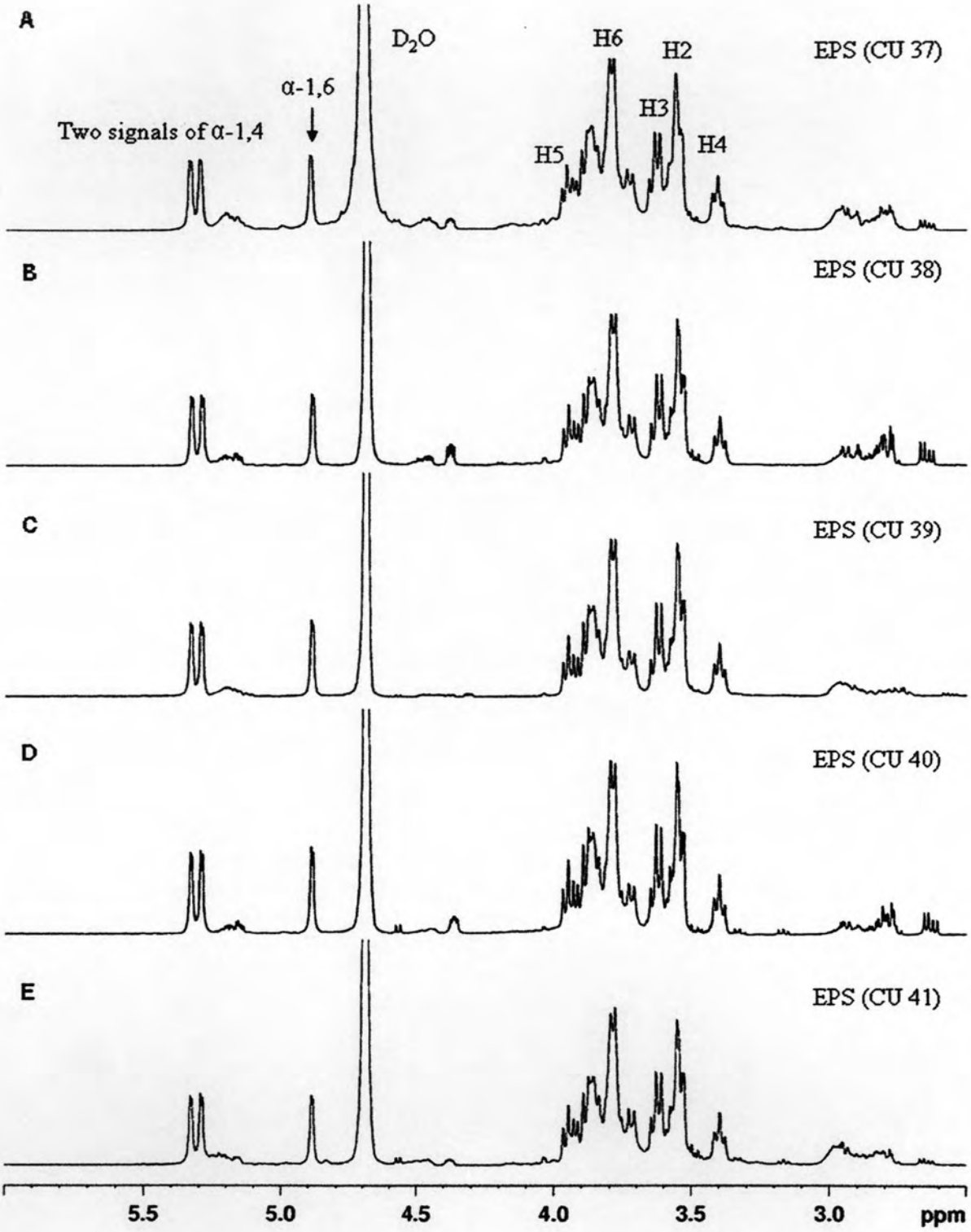

A
α-1,6
D2O
H6
H2
EPS (CU 1)
Two signals of α-1,4
H5
H3
H4
B
EPS (CU 2)
C
EPS (CU 3)
D
EPS (CU 4)
E
EPS (CU 5)
5.5
5.0
4.5
4.0
3.5
3.0
ppm

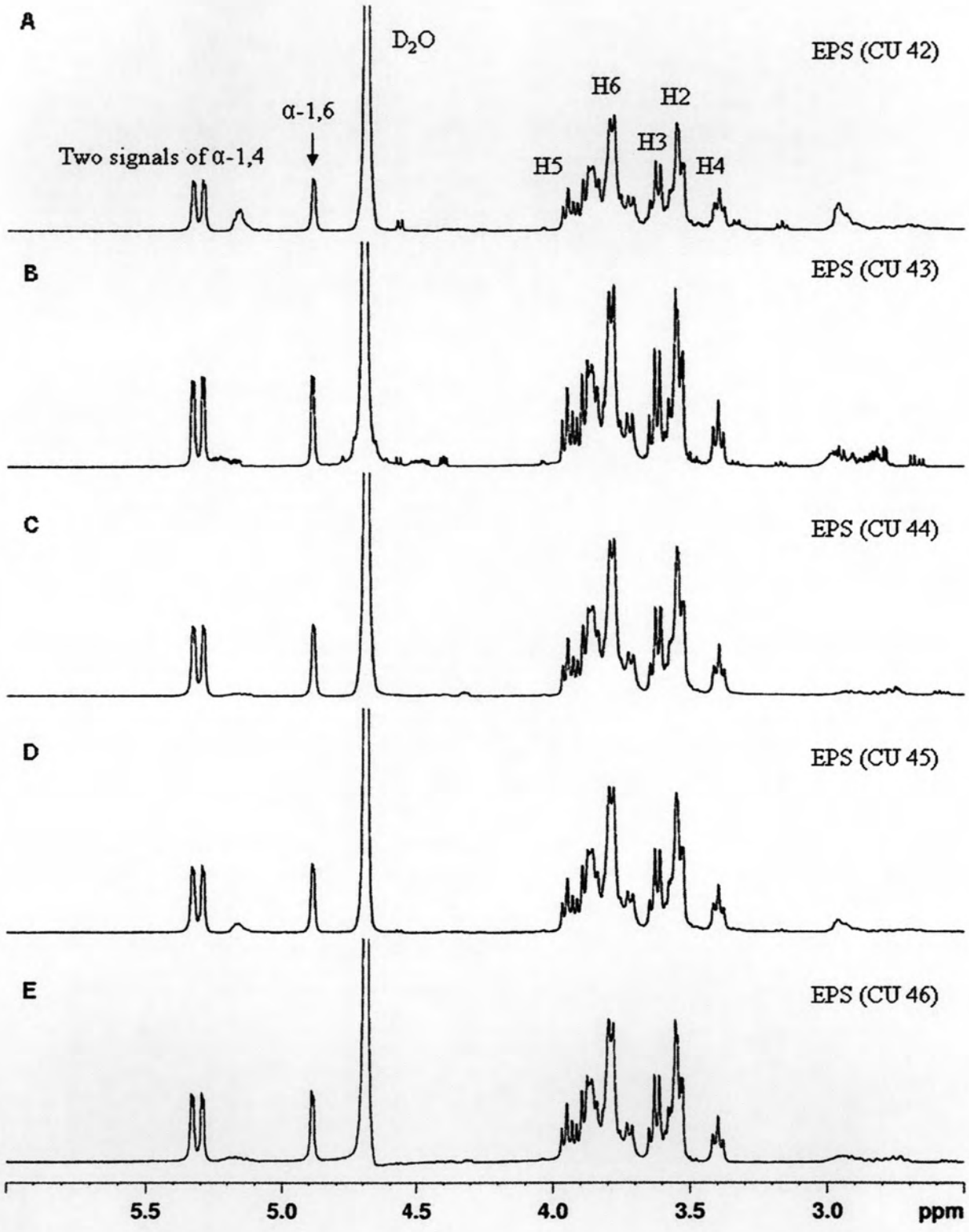

A
α-1,6
D_2O
EPS (CU 6)
Two signals of α-1,4
B
H6
H2
EPS (CU 7)
H3
H5
H4
C
EPS (CU 8)
D
EPS (CU 9)
E
EPS (CU 10)
5.5
5.0
4.5
4.0
3.5
3.0
ppm

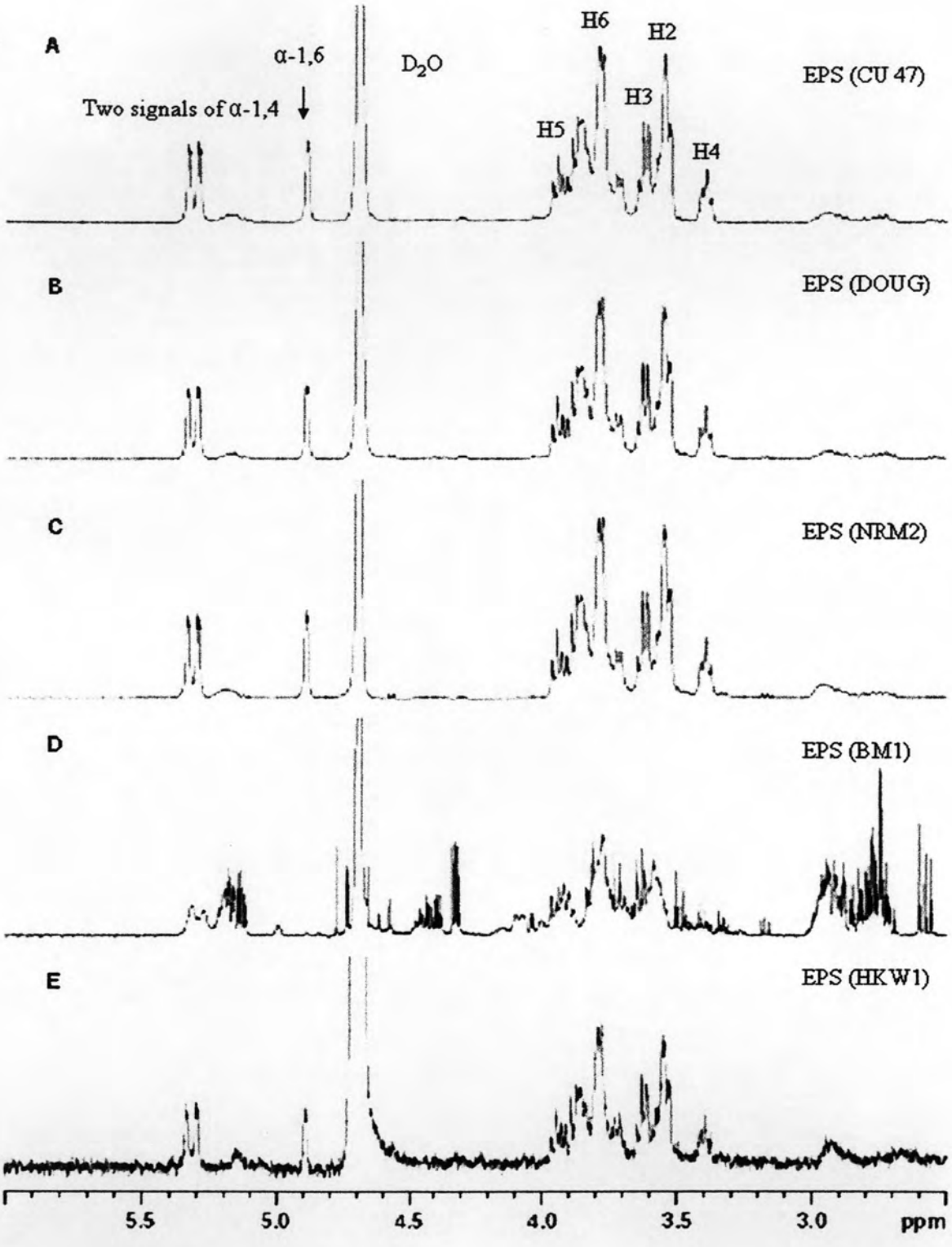

A
α-1,6
D2O
H6
H2
EPS (CU 11)
H3
Two signals of α-1,4
H5
H4
B
EPS (CU 12)
C
EPS (CU 13)
D
EPS (CU 14)
E
EPS (CU 15)
5.5
5.0
4.5
4.0
3.5
3.0
ppm


A
D_2O
EPS (CU 26)
B
H6
H2
EPS (CU 27)
α-1,6
Two signals of α-1,4
H5
H3
H4
C
EPS (CU 28)
D
EPS (CU 29)
E
EPS (CU 30)
5.5
5.0
4.5
4.0
3.5
3.0
ppm

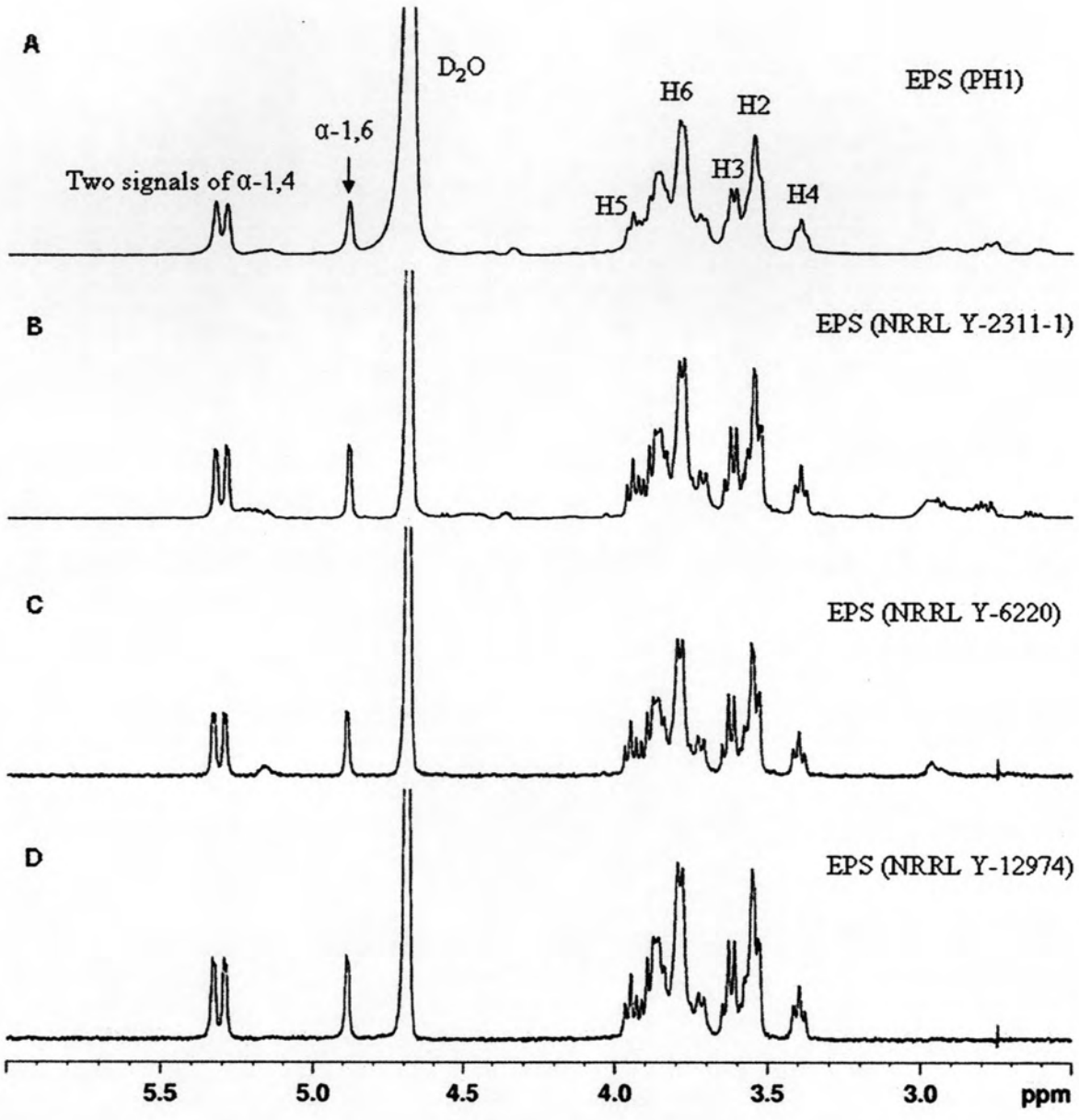

A
Two signals of α-1,4
α-1,6
D2O
H5
H6
H3
H2
H4
EPS (CU 31)
B
EPS (CU 32)
C
EPS (CU 33)
D
EPS (CU 35)
E
EPS (CU 36)
5.5
5.0
4.5
4.0
3.5
3.0
ppm

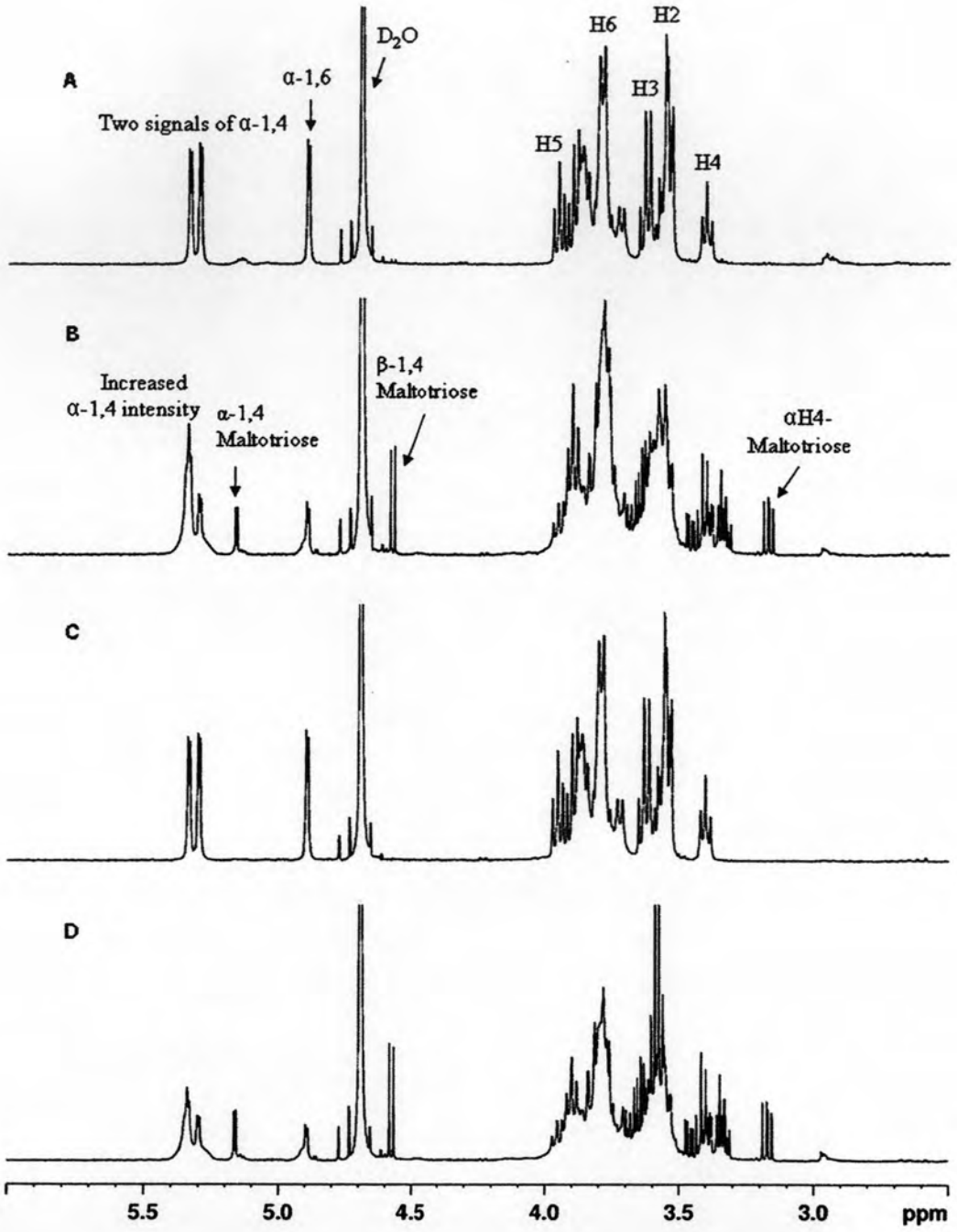

A
Two signals of α-1,4
α-1,6
D2O
H5
H6
H3
H2
H4
EPS (CU 37)
B
EPS (CU 38)
C
EPS (CU 39)
D
EPS (CU 40)
E
EPS (CU 41)
5.5
5.0
4.5
4.0
3.5
3.0
ppm


A
D_2O
EPS (CU 42)
H6
H2
α-1,6
H3
Two signals of α-1,4
H5
H4
B
EPS (CU 43)
C
EPS (CU 44)
D
EPS (CU 45)
E
EPS (CU 46)
5.5
5.0
4.5
4.0
3.5
3.0
ppm



Note that: Since the EPS of BM1 was hardly dissolved, It contained dark pigment and other insoluble polymers, therefore the NMR spectra shows many unknown peaks and no peak of α-1,6 EPS.

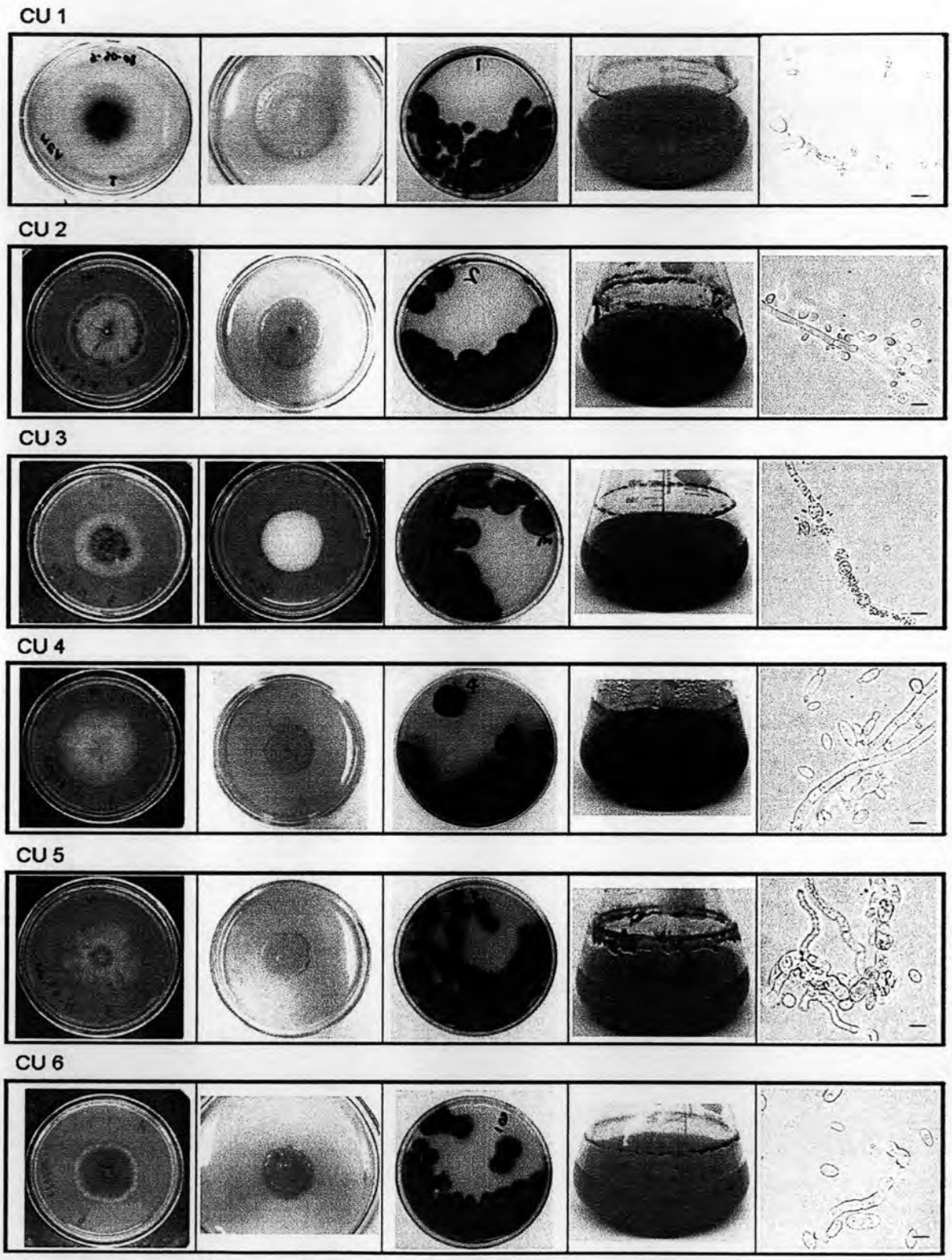

A
D2O
EPS (PH1)
H6
H2
α-1,6
H3
Two signals of α-1,4
H5
H4
B
EPS (NRRL Y-2311-1)
C
EPS (NRRL Y-6220)
D
EPS (NRRL Y-12974)
5.5
5.0
4.5
4.0
3.5
3.0
ppm



^{1}H-NMR spectra of exopolysaccharides (EPS) of *Aureobasidium pullulans* NRRL Y-12974 precipitated from pullulan production medium containing (A) 5.0% sucrose, 0.1% N-sources (B) 5.0% starch, 0.1% N-sources (C) 5.0% sucrose, 0.3% N-sources (D) 5.0% starch, 0.3% N-sources.

Appendix G

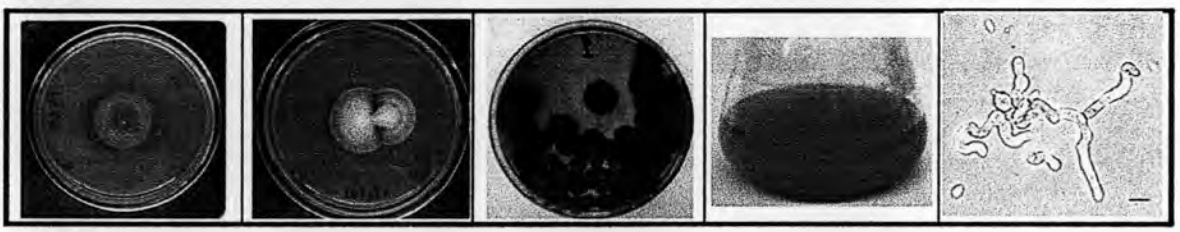
Morphological studies of each isolates



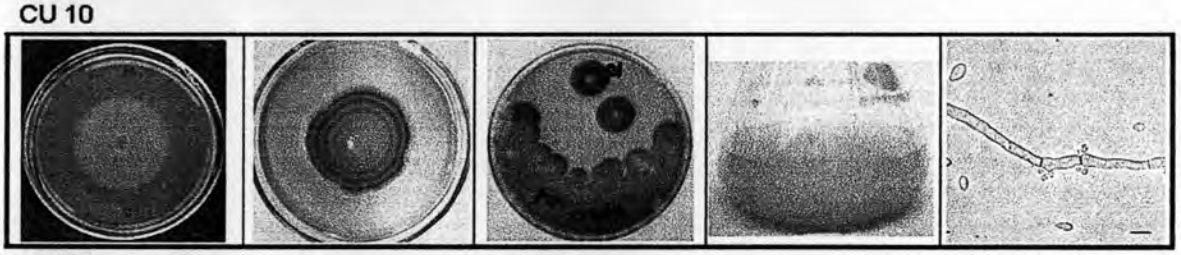
CU 7

CU 8

CU 9

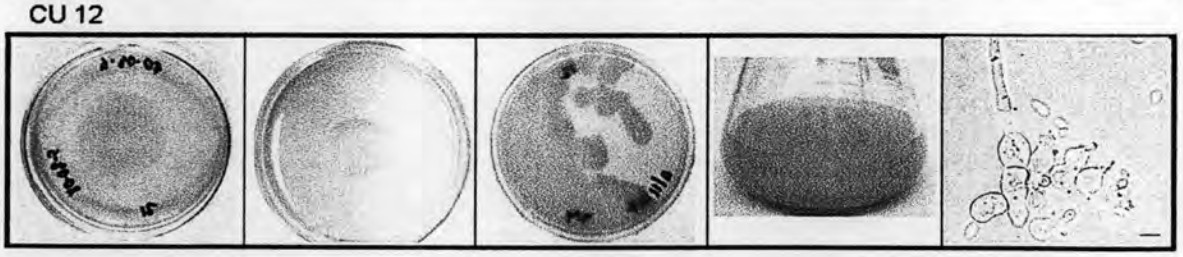


CU 10



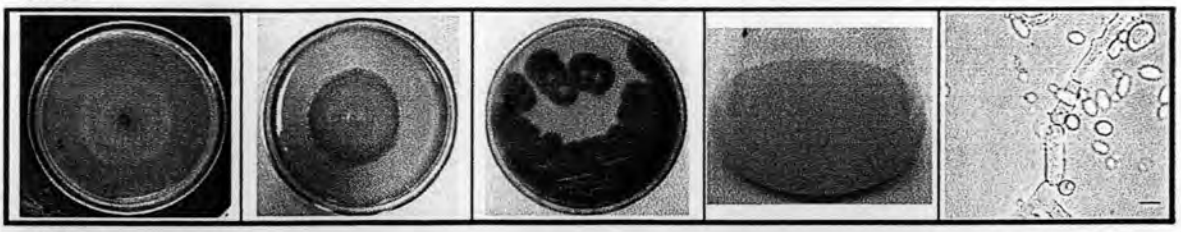
CU 11

CU 12

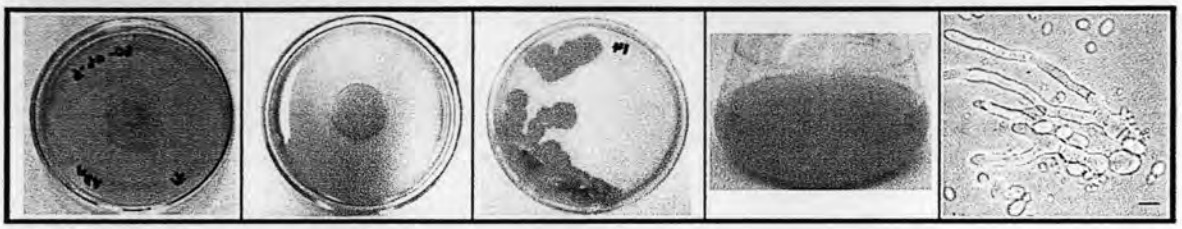


MEA (day 7)	YMA (Day 7)	YMA (after 2 months)	PM (Day 7)	Microscopy (YMB, day 1-3) (bar = 10 µm)

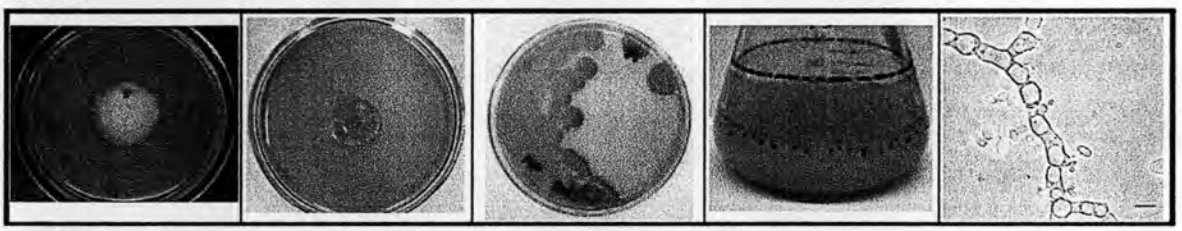
CU 13



CU 14



CU 15



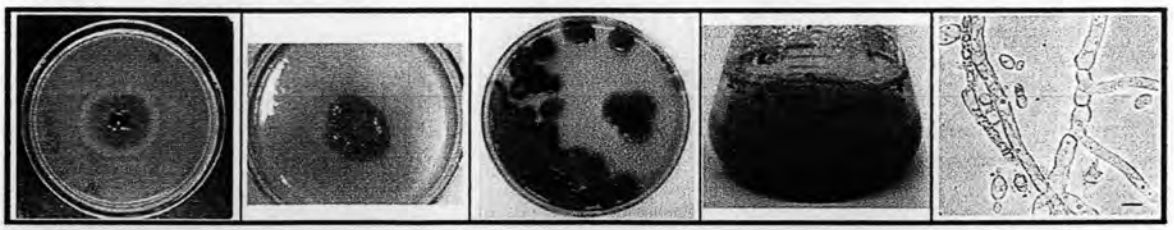
CU 16

CU 17

CU 18

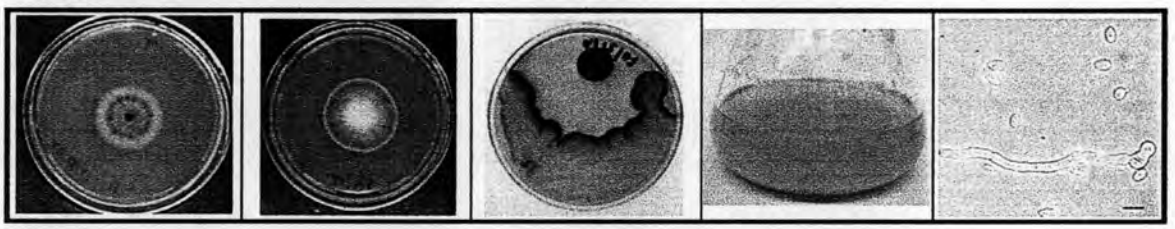
MEA (day 7)	YMA (Day 7)	YMA (after 2 months)	PM (Day 7)	Microscopy (YMB, day 1-3) (bar = 10 µm)

CU 19

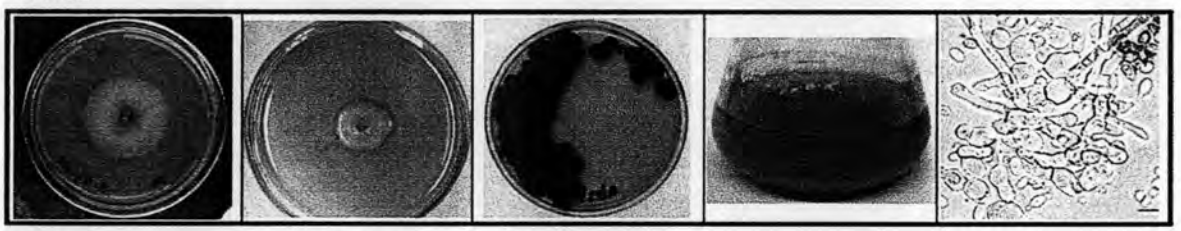


CU 20

CU 21



CU 22



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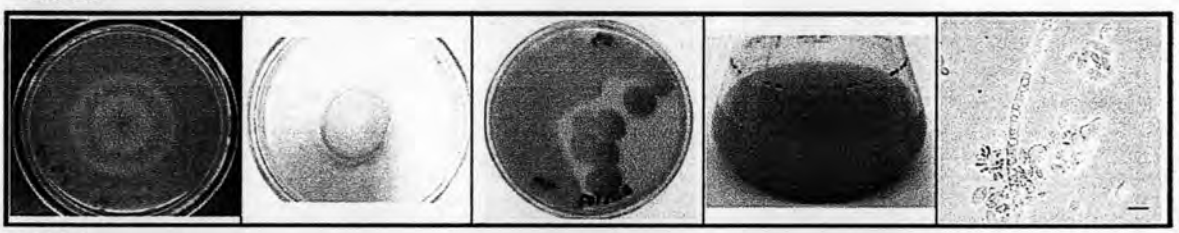
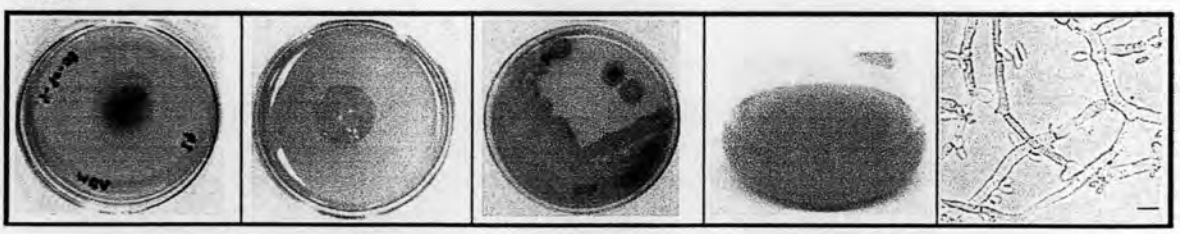


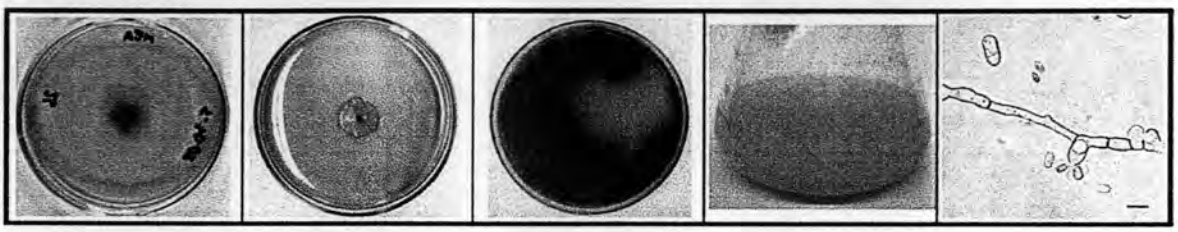
CU 24



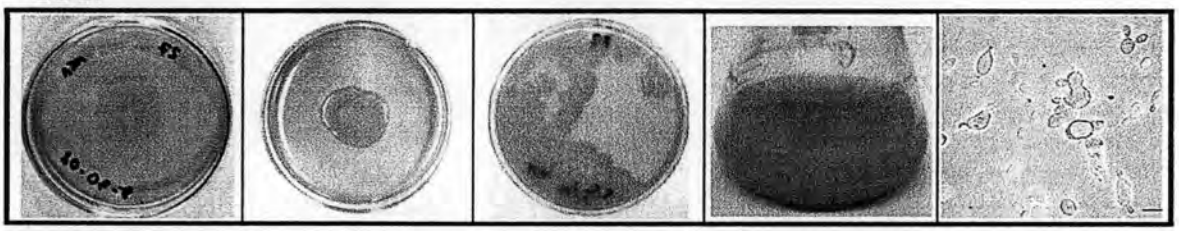
MEA (day 7)	YMA (Day 7)	YMA (after 2 months)	PM (Day 7)	Microscopy (YMB, day 1-3) (bar = 10 µm)

CU 25

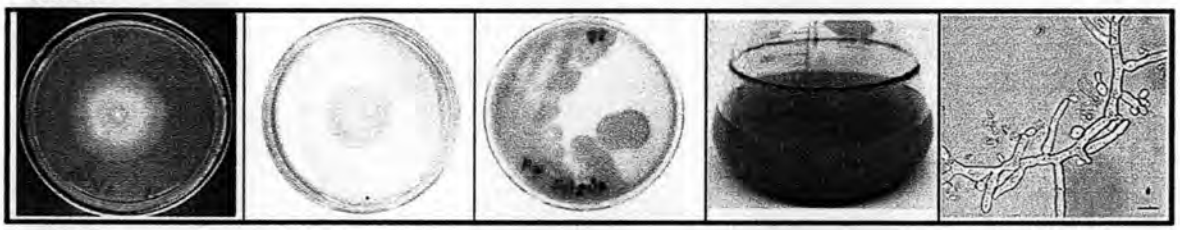
CU 26



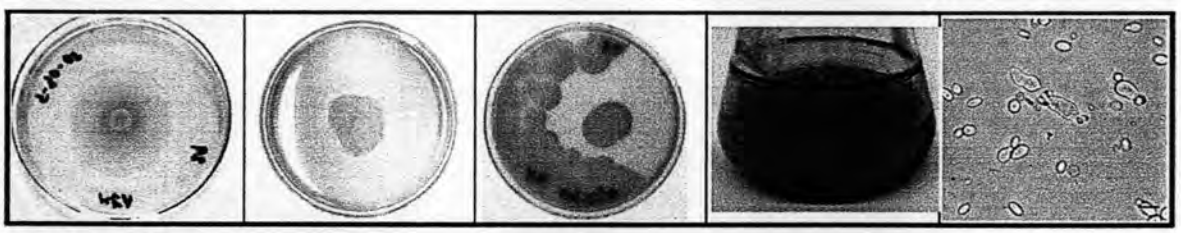
CU 27



CU 28



CU 29

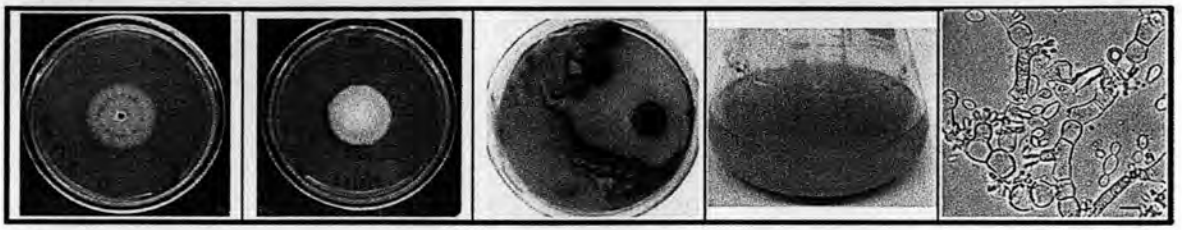


CU 30

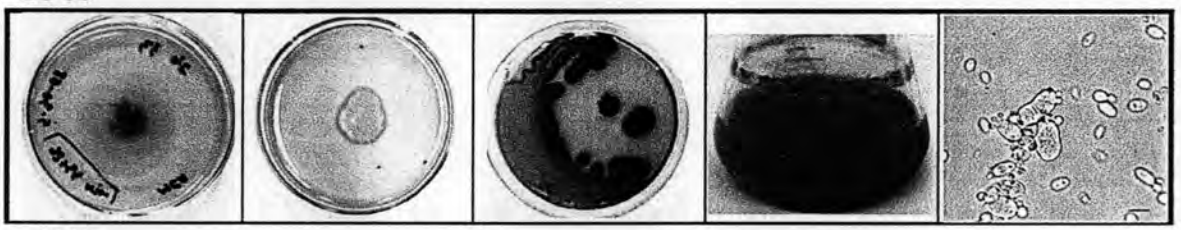
MEA (day 7)	YMA (Day 7)	YMA (after 2 months)	PM (Day 7)	Microscopy (YMB, day 1-3) (bar = 10 µm)

CU 31

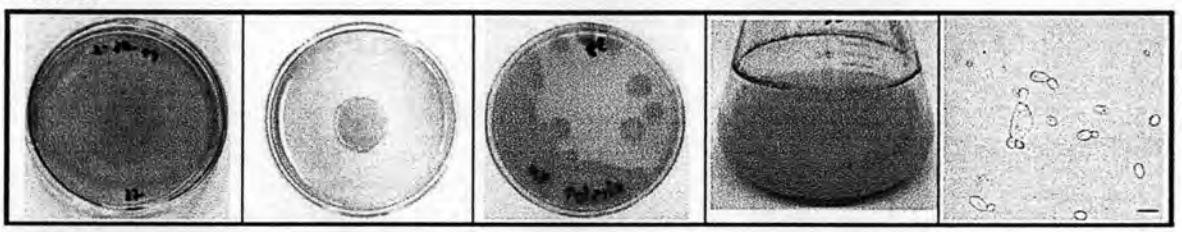
CU 32



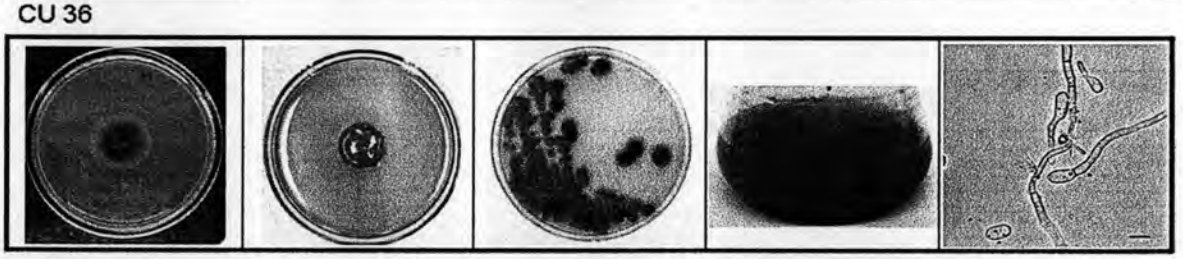
CU 33



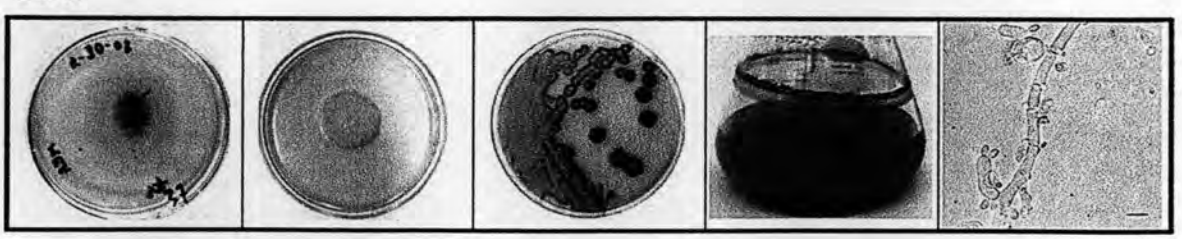
CU 35



CU 36

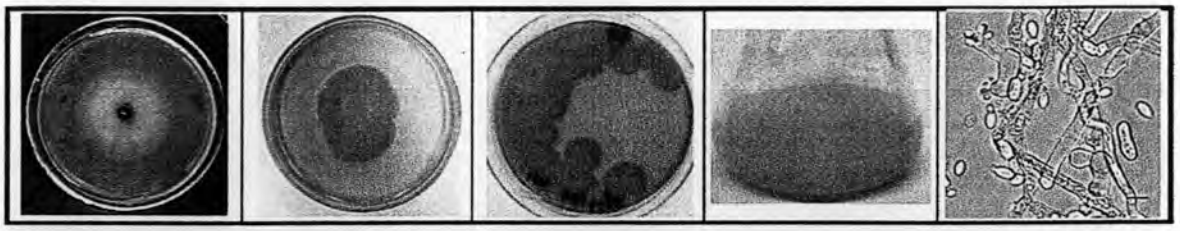


CU 37

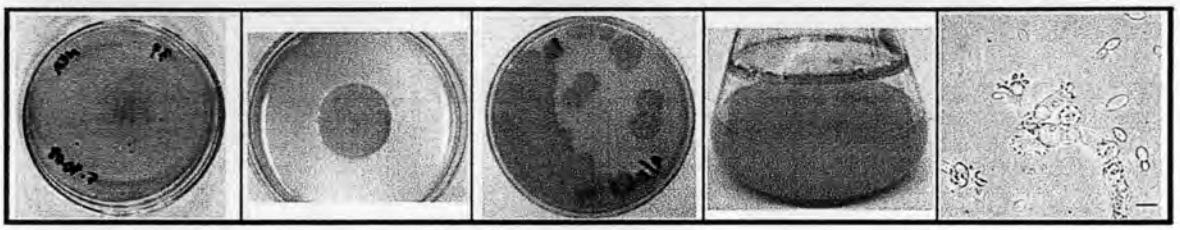


MEA (day 7)	YMA (Day 7)	YMA (after 2 months)	PM (Day 7)	Microscopy (YMB, day 1-3) (bar = 10 µm)

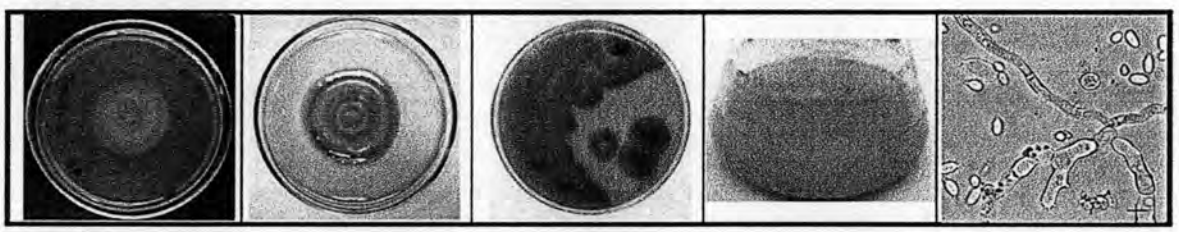
CU 38



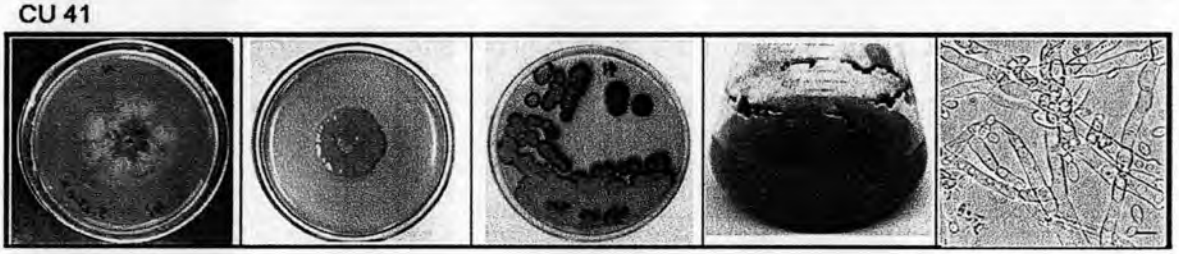
CU 39



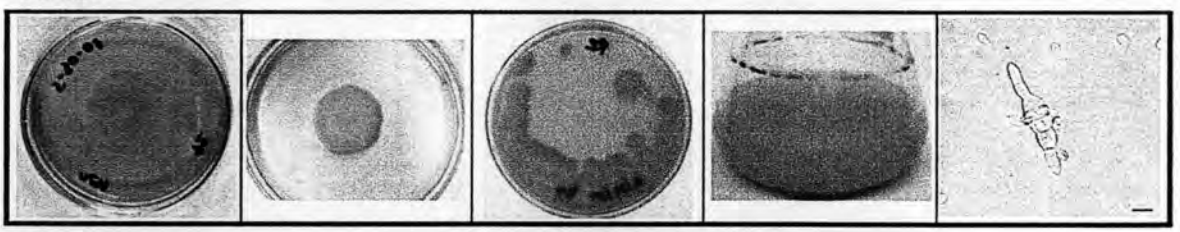
CU 40



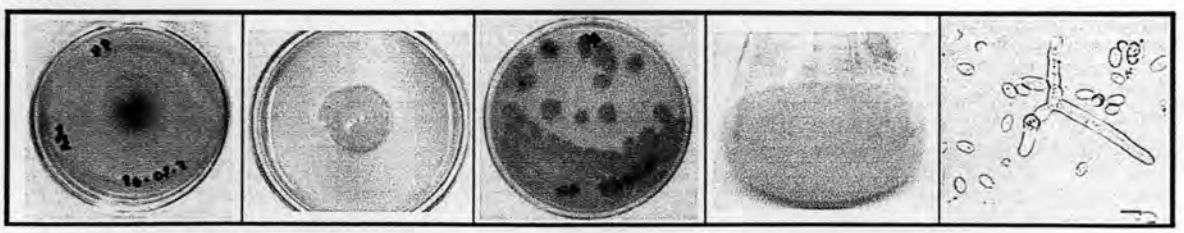
CU 41



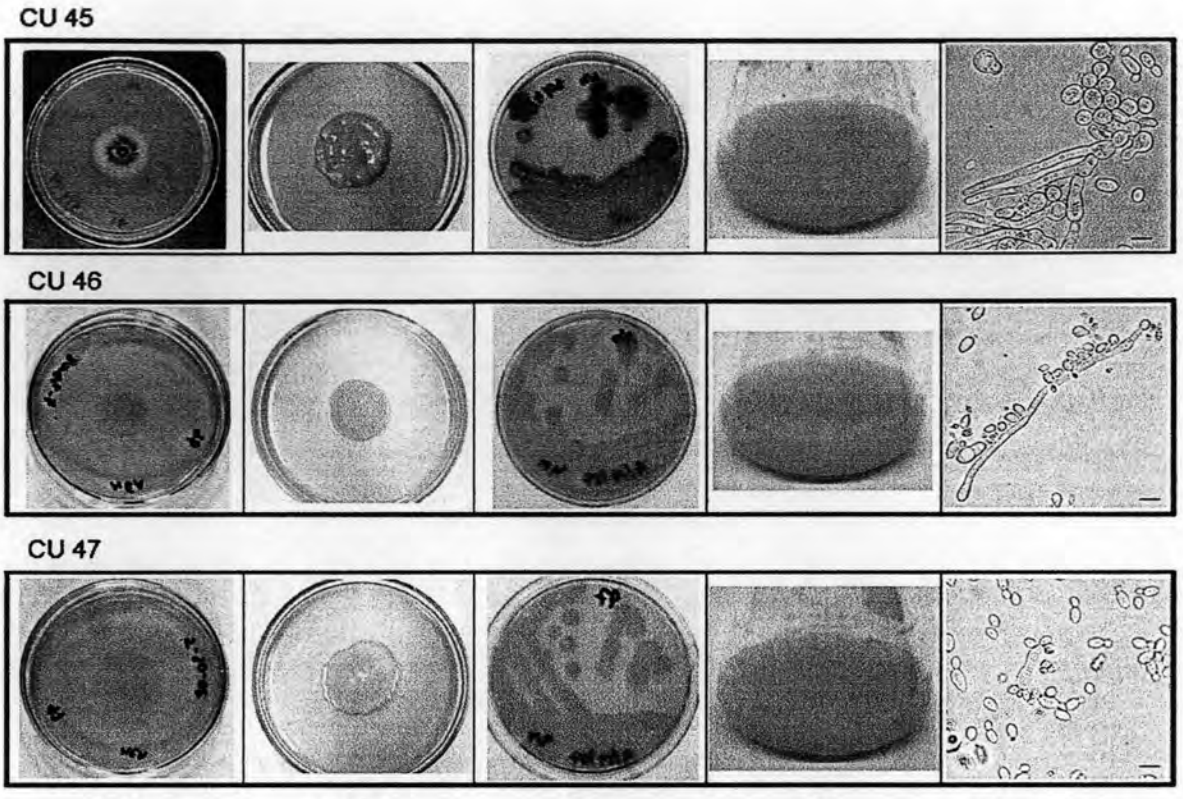
CU 42



CU 44

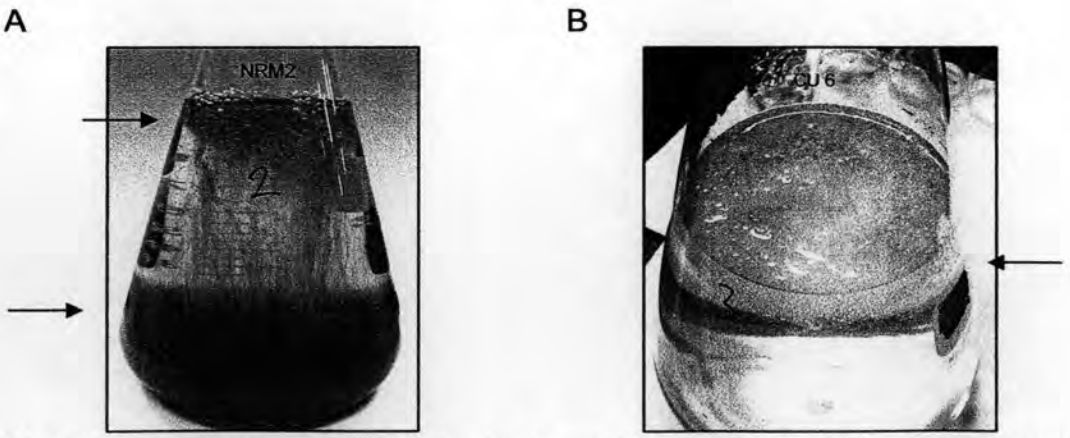


MEA (day 7)	YMA (Day 7)	YMA (after 2 months)	PM (Day 7)	Microscopy (YMB, day 1-3) (bar = 10 µm)



Appendix H

Two fractions of EPS obtained after precipitation by Ethyl alcohol



(A) Two distinct fractions obtained after ethanol precipitation of NRM2 culture supernatant (B) Single fraction of ethanol precipitate from CU 6. Both were cultured in standard PM and harvested at day 7.

Appendix I

Accession number of isolates in this study, deposited in Fungal Section, Professor Kasin Suvatabhandhu Herbarium, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.

Isolate	Accession number	Isolate	Accession number
CU 1	Pennapa 1 (BCU)	CU 26	Pennapa 26 (BCU)
CU 2	Pennapa 2 (BCU)	CU 27	Pennapa 27 (BCU)
CU 3	Pennapa 3 (BCU)	CU 28	Pennapa 28 (BCU)
CU 4	Pennapa 4 (BCU)	CU 29	Pennapa 29 (BCU)
CU 5	Pennapa 5 (BCU)	CU 30	Pennapa 30 (BCU)
CU 6	Pennapa 6 (BCU)	CU 31	Pennapa 31 (BCU)
CU 7	Pennapa 7 (BCU)	CU 32	Pennapa 32 (BCU)
CU 8	Pennapa 8 (BCU)	CU 33	Pennapa 33 (BCU)
CU 9	Pennapa 9 (BCU)	CU 35	Pennapa 34 (BCU)
CU 10	Pennapa 10 (BCU)	CU 36	Pennapa 35 (BCU)
CU 11	Pennapa 11 (BCU)	CU 37	Pennapa 36 (BCU)
CU 12	Pennapa 12 (BCU)	CU 38	Pennapa 37 (BCU)
CU 13	Pennapa 13 (BCU)	CU 39	Pennapa 38 (BCU)
CU 14	Pennapa 14 (BCU)	CU 40	Pennapa 39 (BCU)
CU 15	Pennapa 15 (BCU)	CU 41	Pennapa 40 (BCU)
CU 16	Pennapa 16 (BCU)	CU 42	Pennapa 41 (BCU)
CU 17	Pennapa 17 (BCU)	CU 44	Pennapa 43 (BCU)
CU 18	Pennapa 18 (BCU)	CU 45	Pennapa 44 (BCU)
CU 19	Pennapa 19 (BCU)	CU 46	Pennapa 45 (BCU)
CU 20	Pennapa 20 (BCU)	CU 47	Pennapa 46 (BCU)
CU 21	Pennapa 21 (BCU)	DOUG	Eveleigh 1 (BCU)
CU 22	Pennapa 22 (BCU)	NRM2	Sehanat 1 (BCU)
CU 23	Pennapa 23 (BCU)	BM1	Patcharawan 1 (BCU)
CU 24	Pennapa 24 (BCU)	HKW1	Patcharawan 2 (BCU)
CU 25	Pennapa 25 (BCU)	PH1	Patcharawan 3 (BCU)

VITA

Miss Pennapa Manitchotpisit was born on May 28th, 1972 in Bangkok, Thailand. She graduated from Satrividhaya School in March 1990 and received the Bachelor of Science degree with a major in Genetics from Chulalongkorn University in April 1995. She continued to study for the Master degree of Science in Molecular Genetics and Genetic Engineering, at Institute of Molecular Biology and Genetics, Salaya, Mahidol University and graduated in October 1999. After that, she worked as a research assistant at Institute of Molecular Biology and Genetics, Salaya, Mahidol University until October 2000 and then she had become the faculty member of Department of Medical Science, Faculty of Science, Rangsit University. Since November 2004, she has studied the degree of Philosophy of Science in Biological Science s Program, at the Faculty of Science, Chulalongkorn University.