

松杉靈芝 -葡聚糖? * 瞻 峴w性之研究

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摘要

靈芝之主要生理活性成份是一群具有 β -1,3鍵結之聚葡萄糖主鏈之高分子多醣體。本研究主要是以靈芝屬中之松杉靈芝 *Ganoderma tsugae* 建立起 β -葡聚糖?之分離、純化方法並探討其物化特性。松杉靈芝中之 β -1,3葡聚糖?利用硫酸銨分劃、Sephadex G-50及DEAE Spharose CL-6B管柱層析等步驟純化後，其回收率為4.2%， β -1,3葡聚糖?分別被純化15.1倍。其最適作用pH值為5.0，此酵素在pH值6.0時最為穩定。 β -1,3葡聚糖?之最適作用溫度為50℃，而當溫度在60℃或60℃以上時，此酵素會因變性而失去活性。在受質特異性方面，當以laminarin作為受質時，可得到最大之酵素活性，而以curdlan、lichenan及zymosan為受質時，其相對水解活性分別為16.1、43.7及26.3%。在金屬離子方面，10 mM Cu^{+2} 與酵素作用之後，其活性完全受到抑制，而當以 Fe^{+2} 、 Zn^{+2} 、 Mn^{+2} 、 Mg^{+2} 、 Na^{+2} 及 Ca^{+2} 與酵素作用之後，其抑制率介於30.0-85.2%，而 Co^{+2} 對 β -1,3葡聚糖?之活性影響最小。在抑制劑方面，常見之抑制劑皆能對 β -1,3葡聚糖?活性加以抑制，當L-抗壞血酸濃度達10 mM時，幾乎能完全的抑制 β -1,3葡聚糖?活性。在酵素動力學方面，當以laminarin最為受質時所求得之 K_m 值為5.99 mg/ml，而 V_{max} 則為129.87 OD/min.ml。 β -1,6葡聚糖?利用硫酸銨分劃、Sephadex G-50及DEAE Spharose CL-6B管柱層析等步驟純化後，其回收率為3.2%， β -1,6葡聚糖?分別被純化15.6倍。 β -1,6葡聚糖?顯示其具有較廣範圍之最適作用pH值；在pH 5.0時其活性最大，而此酵素在pH 6.0時最為穩定。其最適作用溫度為60℃，但當此酵素在50℃或50℃以上的溫度下時則很容易快速變性失活。在受質特異性方面，當以pustulan作為受質時，其具有最大之酵素活性，而zymosan也有48.3%的相對活性。在金屬離子方面，當以10 mM Ca^{+2} 與酵素作用之後，其活性完全受到抑制，而當以 Fe^{+2} 、 Zn^{+2} 、 Mn^{+2} 、 Mg^{+2} 、 Na^{+2} 及 Cu^{+2} 與酵素作用之後，其抑制率介於41.0- 84.6%。而 Co^{+2} 對 β -1,6葡聚糖?之活性影響不大。在抑制劑方面，常見之抑制劑皆能對 β -1,6葡聚糖?活性加以抑制，當sodium metabisulfite濃度達10 mM時幾乎能完全的抑制 β -1,6葡聚糖?活性。在酵素動力學方面，當以pustulan最為受質時所求得之 K_m 值為1.88 mg/ml，而 V_{max} 則為75.19 OD/min.ml。綜合以上結果可知， β -1,3葡聚糖?及 β -1,6葡聚糖?在受質特異性方面，僅對 β -1,3及 β -1,6鍵結之多醣體具有活性，對其他方式鍵結之多醣體沒有任何活性存在。因此，利用酵素分析方法來測定市售靈芝產品中之多醣體，應具有其之可行性。

關鍵詞：靈芝、生理活性、多醣體、 β -葡聚糖?、酵素分析

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參考文獻

1.丁懷謙 (2000)。食藥用菇多醣體之免疫生理活性。食品工業32 (5) 28-42。 2.王伯徹、邱世浩、黃仁彰 (1998)。食用菇保健食品特輯。食品工業30 (5) 1-36。 3.王伯徹、林錦堂、華傑 (1991)。靈芝菌種之長期保存。中國農業化學會誌28 (1) 86-93。 4.水野 卓及川合正允 (1992)。菇類的化學生化學。國立編譯館。台北。 5.唐瑞菁、程梅萍 (1992)。靈芝培養基的探討-酵母抽出物的取代。國立雲林技術學院學報4 (1) 145-156。 6.陳國城 (1999)。微生物酵素工程。P.33。 7.梁志欽 (1992)。松杉靈芝浸漬培養產生

的1,3-BETA-GLUCANASE對菌體外多醣的影響。國立台灣大學農業化學研究所碩士論文。8.莊榮輝、蘇仲卿(1986)。蛋白質膠體電泳檢定法。P.69-85。9.許瑞祥(1990)。靈芝屬菌株鑑定系統之研究。國立台灣大學農業化學研究所博士論文。10.許瑞祥(1995)。靈芝的研究現況與展望。生物產業(BIOINDUSTRY),6(4),289-296。11.曾浩洋(1996)。飼料用酵素及乳酸菌之生化評估及相關乳酸菌之分子分類。行政院國科會PB8211-2080。12.傅偉光(1999)。靈芝中機能性成分分析方法之簡介。健康靈芝(冬季號),15-18。13.趙東旭、楊新林、王幫武、朱鶴孫(1999)靈芝研究的若干進展。食用菌學報6(3)59-64。14.蘇遠志、黃世佑(1994)。微生物化學工程。P.594。15.AINSWORTH, G. C., SPARROW, F. K., AND SUSSMAN, A. S. (1973). THE FUNGI. VOL. IVA. A TAXONOMIC REVIEW WITH KEY: ASCOMYCETES AND FUNGI IMPERFECTI. ACADEMIC PRESS, INC. NEW YORK. 621PP. 16.AUTIO, K., SIMOINEN, T., SUORTTI, T., SALMENKALLIO, M. M., LASSILA, K.AND WILHELMSON,A. (2001). STRUCTURAL AND ENZYMIC CHANGES IN GERMINATED BARLEY AND RYE.JOURNAL-OF-THE-INST ITUTE-OF-BREWING, 107 (1) 19-25, 32 REF. 17.BARRAS, D. R.(1972) A GLUCAN ENDO-HYDROLASE FROM SCHIZOSACCHAR- OMYCES POMBE AND ITS RO -LE IN CELL WALL GROWTH. ANTON. LEEUWEN, 38(1):65-80. 18.BENGTSSON, S. (1992) STUDIES ON STRUCTURES AND PROPERTIES OF SOLUBLE CELL-WALL POLYSACC HARIDES IN RYE AND BARLEY. DISSERTATION-ABSTRACTS INTERNATIONAL, 53 (4) 649. 19.BRADFORD, M. (1976) A RAPID AND SENSITIVE METHOD FOR THE QUANTITATION OF MICROGRAM QUAN -TITIES OF PROTEIN UTILIZING THE PRINCIPLE OF PROTEIN-DYE BINDING. ANALYTICAL BIOCHEMIS -TRY, 72:248-254. 20.BODENMANN, J., HEINIGER, U. AND HOHL, H. R. (1985) EXTRACELLULAR ENZYME OF PHYTOPHTHORA INFESTANS:ENDO-CELLULASE, BETA- GLUCOSIDASES AND 1,3-BETA-GLUCANASE. CAN. J. MICROBIOL, 31(1)75-81. 21.CAI, Y. J., BUSWELL, J. A., AND CHANG, S. T. (1998). -GLUCANASE COMPONENTS OF THE CEL -LULOLYTIC SYSTEM OF THE EDIBLE STRAW MUSHROOM, VOLVARIELLA VOLVACEA. ENZYME AND MICR -OBIAL TECHNOLOGY, 22:122-129. 22.CARDER, J. H. (1986) DETECTION AND QUANTIFICATION OF CELLULASE BY CONGO RED STAINING OF SUBSTRATES IN A CUP-PLATE DIFFUSION ASSAY. ANAL. BIOCHEM, 153:75-82. 23.CHANG, R. (1994) EFFECTIVE DOSE OF GANODERMA IN HUMANS. IN PROC. CONTRIBUTED SYMPOSIUM 59A,B. 5TH INTL. MYCOL. CONGR., BUCHANAN PK, HSEU RS AND MONCALVO JM(EDS), TAIPEI,P.101 -13. 24.CHANG, R. (1996) THE CENTRAL IMPORTANCE OF THE 7-GLUCAN RECEPTOR AS THE BASIS OF IMMUNO -LOGIC BIOACTIVITY OF GANODERMA POLYSACCHARIDE. IN REISHI, MIZUNO T, KIM BK(EDS),II YAN -G PRESS, SEOUL, P.153-159. 25.CHASTERS, C. G. C., AND BULL. A. T.(1963) THE ENZYMIC DEGRADATION OF LAMINARIN. 1. THE DISTRIBUTION OF LAMINARINASE AMONG MICROORGANISMS. BIOCHEM. J, 86:28-31. 26.CHASTERS, C. G. C., AND BULL. A. T.(1963) THE ENZYMIC DEGRADATION OF LAMINARIN.. 2.THE MULTICOMPONENT NATURE OF FUNGAL LAMINARINASES. BIOCHEM. J, 86:31-38. 27.CHASTERS, C. G. C., AND BULL. A. T.(1963) THE ENZYMIC DEGRADATION OF LAMINARIN.3. SOME EFFECTS OF TEMPERATURE, PH AND VARIOUS CHEMICAL REAGENTS ON FUNGAL LAMINARINASES.BIOCH -EM. J, 86:38-45. 28.DAVIS, B. J. (1964) DISC ELECTROPHORESIS. METHOD AND APPLICATION TO HUMAN SERUM PROTEI N. ANN. N. Y. ACAD. SCI, 212(2):404-427. 29.DILL, I AND G. KRAEPELIN. (1986) MODEL FOR EXTENSIVE DELIGNIFICATION OF WOOD BY GANODE -RMA APPLANATUM. APPLIED-AND-ENVIRONMENT- MICROBIOLOGY, 52: 1305-1312. 30.DINGLE, J., REID, W. W. AND SOLOMONS, E. L. (1953) THE ENZYMIC DEGRADATION OF PECTIN AND OTHER POLYSACCHARIDES. APPLICATION OF THE CUP PLATE ASSAY TO THE ESTIMATION OF EN -ZYME. J. SCI. FOOD. AGRIC, 4:149-154. 31.DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. AND SMITH, F. (1956).COLORIM -ETRIC METHOD FOR DETERMINATION OF SUGARS AND RELATED SUBSTANCES. ANAL. CHEM, 28: 350- 356. 32.DOBOURDIEU, D., DESPLANQUES, C., VILLETZAZ, J. AND RIBEREAU-GAYON, P. (1985) INVESTIGA -TIONS OF AN INDUSTRIAL BETA-D-GLUCANASE FROM TRICHODERMA HARZIANUM. CARBOHYDR. RES, 144(2): 277-287. 33.ELWINGER, K. AND SATERBY, B. (1987) THE USE OF BETA-GLUCANASE IN PRACTICAL BROILER DIE -TS CONTAINING BARLY OR OAT. SWED. J. AGRIC. RES, 17:133-137. 34.FLEET, G. H. AND PHAFF, H. (1981) IN ENCYCLOPEDIA OF PLANT PHYSIOLOGY 13B (TANNER, W. AND LOEWOUS, F. A., EDS) SPRINGER-VERLAG, BERLIN, PP. 416-440. 35.FLEET, G. H. AND PHAFF, H. (1975) GLUCANASE IN SCHIZOSACCHAROMYCES. ISOLATION AND PROP -ERTIES OF AN EXO-BETA-GLUCANASE FROM THE CELL EXTRACT AND CULTURE FLUID OF SCHIZOSACC -HAROMYCES JAPONICUS VAR. VERSATILIS. BIOCHEM. BIOPHYS. ACTA, 410(2):318-332. 36.FLEET, G. H. AND PHAFF, H. (1974) GLUCANASE IN SCHIZOSACCHAROMYCES. ISOLATION AND PROP -ERTIES OF THE CELL WALL-ASSOCIATED BETA-1,3- GLUCANASE. J. BIOL. CHEM, 249:1717-1728. 37.FISKE, M. J., TOBEY-FINCHER, K. L. AND FUCHS, R. L. (1990) CLONING OF TWO GENES FROM B -ACILLUS CIRCULANS WL-12 WHICH ENCODE 1,3-BETA-GLUCANASE ACTIVITY. J. GEN. MICROBIOL, 136(12): 2377-2383. 38.HIEN, N. H. AND FLEET, G. H. (1983) SEPARATION AND CHARCTERIZATION OF SIX -(1 3)-GL -UCANASE FROM SACCHAROMYCES CEREVISIAE. J. BACTERIAL, 156:1204-1213. 39.KARINEN, P. AND LEHTOMAELI, I. (1990) PROCEDURE FOR PRODUCING FINE FIBRE, AND F -INE FIBRE.PCT-INTERNATIONAL-PATENT-APPLICATION; WO 90/10392 A1, FI 89-1110 (19890308) [ALKO, SF-00101 HELSINKI, FINLAND]. 40.KOBAYASHI, R., MIWA, T., YAMAMOTO, S. AND NAGASAKI, S. (1980) PURIFICATION AND CHARACT -ERIZATION OF A YEAST CELL-LYTIC ENZYME OF A SPECIES OF RHIZOCTONIA. J. FERMENT. TECHN -OL, 58(3):319-326. 41.KOBAYASHI, R., MIWA, T., YAMAMOTO, S. AND NAGASAKI, S. (1981) PROPERTIES AND MODE OF AC -TION OF BETA-1,3-GLUCANASE FROM RHIZOCTONIA SP.

[FUNGI]. J. FERMENT. TECHNOL, 59(1):21-26. 42. KUSAMA, S., KUSAKABE, I. AND MURAKAMI, K. (1986) PURIFICATION AND SOME PROPERTIES OF DE-TA-1,3-GLUCANASE FROM STREPTOMYCES SP. AGRIC. BIO. CHEM, 50(5): 1101-1106.

43. LAURENT, T. C. AND KILLANDER, J. (1964) A THEORY OF GEL FILTRATION AND ITS EXPERIMENTAL VERIFICATION. J. CHROMATOGR, 14:317-330. 44. LEE, C. J., HORSLEY, R. D., MANTHEY, F. A. AND SCHWARZ, P. B. (1997) COMPARISONS OF -GLUCAN CONTENT OF BARLEY AND OAT. CEREAL CHEM, 74(5): 571-575. 45. I. U. B. (1984) ENZYME NOMENCLATURE; RECOMMENDATIONS OF THE NOMENCLATURE COMMITTEE OF THE INTERNATIONAL UNION OF BIOCHEMISTRY. ACADEMIC PRESS, ORLANDO. 46. MARTIN, D. F., PRIEST, F. G., TODD, C. AND GOODFELLOW, M. (1980) DISTRIBUTION OF BETA -GLUCANASES WITHIN THE GENUS BACILLUS. APPL. ENVIRON. MICROBIOL, 40(6): 1136-1138. 47. MCCLEARY, B. V. (2000) IMPORTANCE OF ENZYME PURITY AND ACTIVITY IN THE MEASUREMENT OF TOTAL DIETARY FIBER AND DIETARY FIBER COMPONENTS. JOURNAL-OF-AOAC-INTERNATIONAL, 83 (4): 997-1005. 48. MERATAD, O. AND MCNAB, J. M. (1975) THE EFFECT OF HEAT TREATMENT AND ENZYME SUPPLEMENTATION ON THE NUTRITIVE VALUE OF BARLEY FOR BROILER CHICKS. BR. POULT, 16(1): 68-72. 49. MOSS, B. R., BEECHLER, C. W., NEWMAN, C. W. AND EI-NEGOUMY, A. M. (1977) ENZYME SUPPLEMENTATION OF BROILERS RATIONS. POULT. SCI, 56:1741-1745. 50. NOTARIO, V. (1982) BETA-GLUCANASES FROM CANDIDA ALBICANS : PURIFICATION, CHARACTERIZATION AND THE NATURE OF THEIR ATTACHMENT TO CELL WALL COMPONENT [FUNGI]. J. GEN. MICROBIOL, 128:747-759. 51. NOTARIO, V., VILLA, T. G., BENITEZ, T. AND VILLANUEVA, J. R. (1976) BETA-GLUCANASE IN THE YEAST CRYPTOCOCCUS ALBIDUS VAR. AERIUS. PRODUCTION AND SEPARATION OF BETA-GLUCANASES IN ASYNCHRONOUS CULTURE. CAN. J. MICROBIOL, 22(2):261-268. 52. PETERSEN, B. O., KRAH, M., DUUS, J. O. AND THOMSEN, K. K. (2000) A TRANSGLYCOSYLATING 1,3(4)-BETA-GLUCANASE FROM RHODOTHERMUS MARINUS. NMR ANALYSIS OF ENZYME REACTIONS. EUROPEAN JOURNAL OF BIOCHEMISTRY, 267 (2) : 361-369. 53. PETERSSON, D. AND AMAN, P. (1989) ENZYME SUPPLEMENTATION OF A POULTRY DIET CONTAINING RYE AND WHEAT. BR. J. NUTR, 62:139-146. 54. PITSON, S. M., SEVIOUR, R. J. AND MCDUGALL, B. M. (1997) PURIFICATION AND CHARACTERIZATION OF AN EXTRACELLULAR BETA -GLUCOSIDASE FROM THE FILAMENTOUS FUNGUS ACREMONIUM PERISICINUM AND ITS PROBABLE ROLE IN BETA -GLUCAN DEGRADATION. ENZYME AND MICROBIAL TECHNOLOGY, 21:182-190. 55. PITSON, S. M., SEVIOUR, R. J., AND MCDUGALL, B. M. (1993) NONCELLULOLYTIC FUNGAL BETA -GLUCANASES: THEIR PHYSIOLOGY AND REGULATION. ENZYME AND MICROBIAL TECHNOLOGY, 15 (3) :178-192. 56. PITSON, S. M., SEVIOUR, R. J. AND MCDUGALL, B. M. (1996) PROTEOLYTIC INACTIVATION OF AN EXTRACELLULAR (1,3)-BETA-GLUCANASE FROM THE FUNGUS ACREMONIUM PERISICINUM IS ASSOCIATED WITH GROWTH AT NEUTRAL OR ALKALINE MEDIUM PH. FEMS-MICROBIOLOGY-LETTERS, 145 (2) 287-293. 57. REESE, E. T. (1977) RECENT ADVANCES IN PHYTOCHEMISTRY 11 (LOEWUS, F. A. AND RONECKLES, V. C., EDS) PLENUM, NEW YORK, PP. 311-367. 58. REYES, F. AND LAHOZ, R (1977) VARIATION IN LYSIS OF WALLS OF SCLEROTINIA FRUCTIGENA WITH AGE OF CULTURE. J. GEN. MICROBIOL, 98:607-610. 59. RIDOUT, C. J., COLEY-SMITH, J. R. AND LYNCH, J. M. (1986) ENZYME ACTIVITY ELECTROPHORETIC PROFILE OF EXTRACELLULAR PROTEIN INDUCED IN TRICHODERMA SPP. BY CELL WALLS OF RHIZOCTONIA SOLANI. J. GEN. MICROBIOL, 132(8):2345-2352. 60. SANCHEZ, M., NOMBELA, C., VILLANUEVA, J. R. AND SANTOS, T (1982) PURIFICATION AND PARTIAL CHARACTERIZATION OF A DEVELOPMENTALLY REGULATED 1,3-BETA-GLUCANASE FROM PENCILLIUM ITALICUM [FUNGI]. J. GEN. MICROBIOL, 128:2047-2053. 61. SANTOS, T., VILLANUEVA, J. R. AND NOMBELA, C. (1977) PRODUCTION AND CATABOLITE REPRESENTATION OF PENICILLIUM ITALICUM BETA-GLUCANASES. J. BACTERIOL, 129(1):52-58. 62. SANTOS, T., VILLANUEVA, J. R. AND NOMBELA, C. (1978) REGULATION OF BETA-1,3-GLUCANASE SYNTHESIS IN PENICILLIUM ITALICUM. J. BACTERIOL, 133(2):542-548. 63. SHINGO, N. AND TAKAHISA, H. (1993) PURIFICATION AND PROPERTIES OF AN EXTRACELLULAR ENDO-1,4-BETA-GLUCANASE FROM SUSPENSION-CULTURED POPLAR CELLS. PLANT CELL PHYSICAL, 34(7) : 1009-1013. 64. SHINSHI, H. AND KATO, K. (1983) PHYSICAL AND CHEMICAL PROPERTIES OF BETA-1,3-GLUCANASE FROM CULTURED TOBACCO CELLS [NICOTIANA TABACUM]. AGRIC. BIOL. CHEM, 47(7):1455-1460. 65. SMITH, D. (1982) LIQUID NITROGEN STORAGE OF FUNGI. TRANS. BR. MYCOL. SOC, 79: 415-421. 66. SMITH, D. (1984). MAINTENANCE OF FUNGI. IN B.E KIRSOP AND J. J. S. SNELL (EDS.), MAINTENANCE OF MICROORGANISM: A MANUAL OF LABORATORY METHODS. ACADEMIC PRESS, LONDON. PP. 83-108. 67. SONE, Y., R. OKUDA, N. WADA, E. KISHIDA, AND A. MISAKI. (1985). STRUCTURE AND ANTITUMOR ACTIVITIES OF POLYSACCHARIDES ISOLATED FROM FRUITING BODY AND GROWING CULTURE OF MYCELIUM OF GANODERMA ALUCIDIUM. AGRIC. BIOL. CHEM, 49:2641-2653. 68. SOUTH, J. B. (1997) ESTIMATION OF ENDO BETA-GLUCANASE ACTIVITY IN MALT USING A VISCOMETRIC METHOD. JOURNAL-OF-THE-INSTITUTE-OF-BREWING, 103 (1):3-6. 69. STASINOPOULOS, S. J., AND R. J. SEVIOUR. (1989). EXOPOLYSACCHARIDE FORMATION BY ISOLATES OF CEPHALOSPORIUM AND ACREMONIUM. CHEM. PHARM. BULL, 39(3):798-800. 70. TAKASHI AKIYAMA, HANAE KAKU, NAOTO SHIBUYA. (1998). PURIFICATION, CHARACTERIZATION AND NH₂-TERMINAL SEQUENCING OF AN ENDO-(1,3,4)-GLUCANASE FROM RICE (ORYZA SATIVA) BRANN. PLANT SCIENCE, 134: 3-10. 71. TOTSUKA, A. AND USUI, T. (1986) SEPARATION AND CHARACTERIZATION OF THE ENDO-BETA-(1 TO

3)-D-GLUCANASE FROM RHIZOCTONIA SOLANI. AGRIC. BIOL.CHEM, 50(3):543-550. 73.TURNER, P. D. (1965). INFECTION OF OIL PALMS BY GANODERMA. PHYTOPATHOLOGY, 55:937-943. 74.VILLA, T. G., NOTARIO, V., BENITEZ, T. AND VILLANUEVA, J. R. (1976) PURIFICATION OF AN EXO-1,3-BETA-GLUCANASE FROM CANDIDA UTILIS. CAN. J. BIOCHEM, 54(1):927-934. 75.WALSH, G. A., POWER, R. F. AND HEADON, D. R. (1993) ENZYMES IN THE ANIMAL FEED INDUSTRY .TRENDS IN BIOTECHNOL, 11:424-428. 76.WALSH, G. A., MURPHY, R. A., KILLEEN, G. F., HEADON, D. R. AND POWER, R. F. (1995) TECHNICAL NOTE: DETECTION AND QUANTIFICATION OF SUPPLEMENTATION FUNGAL β -GLUCANASE ACTIVITY IN ANIMAL FEED. J. ANIM. SCI, 73:1074-1076. 77.WANG G., ZHANG J., MIZUNO T., ZHUANG C., ITO H., MAYUZUMI H.,OKAMOTO H. AND LI J.(1993) .ANTITUMOR ACTIVE POLYSACCHARIDES FROM THE CHINESE MUSHROOM SONG SHAN LINGZHI, THE FRUITING BODY OF GANODERMA TSUGAE. BIOSCI. BIOTECH. BIOCHEM, 57(6):894-900. 78.WOOD, P. J.(1994) EVALUATION OF OAT BRAN AS A SOLUBLE FIBER SOURCE. CHARACTERIZATION OF OAT β -GLUCAN AND ITS EFFECTS ON GLYCERNIC RESPONSE. CARBOHYDRATE POLYMERS, 25:331-336. 79.WOOD, P. J. (1981) THE USE OF DYE-POLYSACCHARIDE INTERACTIONS IN BETA-D-GLUCANASE ASSAY .CARBOHYDR. RES, 94:C19. 80.YAMAMOTO, R. AND NEVINS, D. J. (1983) DEGRADATION OF A GLUCAN CONTAINING BETA-(1 TO 3) AND BETA-(1 TO 6) LINKAGES BY EXO-(1 TO 3)-BETA-D-GLUCANASE [ISOLATED FROM A JAPANESE MARINE ALGA KNOWN AS "ARAME", EISENIA BICYCLIS]. CARBOHYDR. RES, 122(2):217-226.