

# Production of Lactic Acid Bacteria Transformant Containing Extracellular Superoxide Dismutase by Gene Engineering Techni

王妙齡、陳小玲；陳全木

E-mail: 9403791@mail.dyu.edu.tw

## ABSTRACT

Reactive oxygen species (ROS) such as superoxide anion are associated with the pathogenesis of a variety of diseases. Extracellular superoxide dismutase (EC-SOD, SOD3) is the only known extracellular enzyme designed to scavenge the superoxide anion. SOD3 is capable of preventing and curing diseases resulting from the accumulation of superoxide anion, such as cardiovascular disease, lung disease, neurological disorders, and inflammation. Our study was focus on the generation of the recombinant Lactic acid bacteria (LAB), *Lactobacillus casei*, containing the human extracellular superoxide dismutase (hSOD3) gene fragment and the feasibility of large-scale production. In the first instance, the hSOD3 gene was amplified by polymerase chain reaction (PCR), and was cloned into *L. casei* expression vector pLP3537. The recombinant plasmid hSOD3/pLP3537 via electroporation was introduced into *L. casei* and used erythromycin to select the LAB transformants. The recombinant hSOD3 was purified from *L. casei* transformants using fast protein liquid chromatography (FPLC) with heparin affinity column. The purified enzyme showed two bands with apparent molecular masses of 38 and 50 kDa on SDS-PAGE and Western blot. The subunit molecular sizes of recombinant hSOD3 were 38 and 50 kDa representing monomer and dimer, respectively. Antioxidative activity of recombinant hSOD3 was determined by SOD assay kit, and the result showed that expressed hSOD3 in recombinant *L. casei* actually possessed enzymatic activity.

Keywords : Extracellular superoxide dismutase ; *Lactobacillus casei* ; Antioxidative activity

## Table of Contents

|                                  |      |                                       |     |                                      |      |
|----------------------------------|------|---------------------------------------|-----|--------------------------------------|------|
| 目錄 封面內頁 簽名頁 授權書.....             | iii  | 中文摘要.....                             | iv  | 英文摘要.....                            | v    |
| 誌謝.....                          | vi   | 目錄.....                               | vii | 圖目錄.....                             | viii |
| 表目錄.....                         | viii | 第一章 緒言.....                           | ix  | 第二章 文獻檢討.....                        | x    |
| 2.1 超氧歧化?之分類.....                | 4    | 2.1.1 銅鋅型超氧歧化?.....                   | 4   | 2.1.2 錳型超氧歧化?.....                   | 5    |
| 2.1.3 鐵型超氧歧化?.....               | 6    | 2.2 超氧歧化?之反應機制.....                   | 9   | 2.3 胞外超氧歧化?之特性.....                  | 12   |
| 2.3.1 胞外超氧化物歧化?之分子與生物特性.....     | 12   | 2.3.2 肝素結合區域.....                     | 15  | 2.3.3 肝素結合區域之蛋白質水解作用.....            | 18   |
| 2.3.4 胞外超氧歧化?之基因多型性.....         | 20   | 2.4 胞外超氧歧化?與疾病之關係.....                | 21  | 2.4.1 心血管疾病.....                     | 21   |
| 2.4.1.1 胞外超氧歧化?與一氧化氮.....        | 21   | 2.4.1.2 動脈粥狀硬化症.....                  | 24  | 2.4.1.3 高血壓.....                     | 28   |
| 2.4.1.4 糖尿病.....                 | 31   | 2.4.2 肺部疾病.....                       | 34  | 2.4.2.1 胞外超氧歧化?於肺部之表現情形.....         | 34   |
| 2.4.2.2 急性肺損傷.....               | 35   | 2.4.2.3 肺部纖維化.....                    | 37  | 2.4.2.4 肺部發炎.....                    | 39   |
| 2.4.3 類風濕性關節炎.....               | 42   | 2.5 超氧化物歧化?之代謝.....                   | 44  | 2.6 利用微生物系統表現外源性超氧歧化?.....           | 45   |
| 2.6.1 原核系統.....                  | 45   | 2.6.2 真核系統.....                       | 47  | 第三章 材料與方法.....                       | 49   |
| 3.1 人類胞外超氧化物歧化?於乳酸菌表現型載體之建構..... | 49   | 3.1.1 人類胞外超氧化物歧化?之聚合?連鎖反應擴增.....      | 49  | 3.1.2 選殖片段與載體之接合反應.....              | 50   |
| 3.1.3 大腸桿菌之轉型作用.....             | 51   | 3.1.4 小量質體之DNA萃取.....                 | 53  | 3.1.5 核酸限制酵素之截切作用.....               | 55   |
| 3.1.6 DNA片段之回收.....              | 55   | 3.1.7 乳酸菌表現型載體之構築.....                | 56  | 3.2 重組乳酸菌菌株之轉型與篩選.....               | 60   |
| 3.2.1 乳酸菌勝任細胞之製備.....            | 60   | 3.2.2 電穿孔轉型作用.....                    | 60  | 3.2.3 重組乳酸菌菌株之篩選.....                | 61   |
| 3.3 乳酸菌轉型株之胞內蛋白質分析.....          | 62   | 3.3.1 乳酸菌轉型株之胞內蛋白萃取.....              | 62  | 3.3.2 硫酸十二酯鈉-聚丙烯醯胺凝膠電泳分析.....        | 63   |
| 3.3.3 西方吸漬法分析.....               | 65   | 3.3.4 重組人類胞外超氧化物歧化?之初步純化.....         | 68  | 3.3.5 胞外超氧化物歧化?活性測定方法.....           | 70   |
| 第四章 結果與討論.....                   | 74   | 4.1 人類胞外超氧化物歧化?基因cDNA序列之選殖.....       | 74  | 4.2 人類胞外超氧化物歧化?基因於乳酸菌表現型載體之 建構.....  | 74   |
| 4.3 人類胞外超氧化物歧化?重組載體之核酸定序分析.....  | 78   | 4.4 人類胞外超氧化物歧化?於乳酸菌轉型株之蛋白質表 現分 析..... | 81  | 4.5 人類胞外超氧化物歧化?於乳酸菌轉型株之蛋白質表 現分析..... | 84   |
| 4.6 重組人類胞外超氧化物歧化?之初步純化.....      | 87   | 4.7 重組人類胞外超氧化物歧化?之抗氧化活性分析.....        | 89  | 第五章 結 論.....                         | 95   |
| 參考文獻.....                        | 96   | 附錄.....                               | 122 | 作者 小傳.....                           | 122  |

## REFERENCES

- 尤新。2001。機能性醱酵製品，pp.52-64。藝軒圖書出版社，台北市。謝達仁。1996。基因治療。內科學誌 7:224-230。Adachi, T., H. Ohta, K. Hirano, K. Hayashi, and S. L. Marklund. 1991. Nonenzymic glycation of human extracellular superoxide dismutase. *Biochem. J.* 279:263-267. Adachi, T., T. Kodera, H. Ohta, K. Hayashi, and K. Hirano. 1992a. The heparin binding site of human extracellular superoxide dismutase. *Arch. Biochem. Biophys.* 297:155-161. Adachi, T., H. Ohta, H. Yamada, A. Futenma, K. Kato, and K. Hirano. 1992b. Quantitative analysis of extracellular superoxide dismutase in serum and urine by ELISA with monoclonal antibody. *Clin. Chim. Acta.* 212:89-102. Adachi, T., H. Ohta, K. Hayashi, K. Hirano, and S. L. Marklund. 1992c. The site of nonenzymic glycation of human extracellular superoxide dismutase in vitro. *Free Radic. Biol. Med.* 13:205-210. Adachi, T., M. Nakamura, H. Yamada, A. Futenma, K. Kato, and K. Hirano. 1994. Quantitative and qualitative changes of extracellular superoxide dismutase in patients with various diseases. *Clin. Chim. Acta.* 229:123-131. Adachi, T., H. Yamada, Y. Yamada, N. Morihara, N. Yamazaki, T. Murakami, A. Futenma, K. Kato, and K. Hirano. 1996a. Substitution of glycine for arginine-213 in extracellular superoxide dismutase impairs affinity for heparin and endothelial cell surface. *Biochem. J.* 313:235-239. Adachi, T., N. Morihara, N. Yamazaki, H. Yamada, A. Futenma, K. Kato, and K. Hirano. 1996b. An arginine-213 to glycine mutation in human extracellular superoxide dismutase reduces susceptibility to trypsin-like proteinases. *J. Biochem. (Tokyo)* 120:184-188. Adamson, I. Y. and D. H. Bowden. 1974. The pathogenesis of bleomy cininduced pulmonary fibrosis in mice. *Am. J. Pathol.* 77:185-197. Beck, B. L., L. B. Tabatabai, and J. E. Mayfield. 1990. A protein isolated from *Brucella abortus* is a Cu-Zn superoxide dismutase. *Biochemistry.* 29:372-376. Beckman, J. S., T. W. Beckman, J. Chen, P. A. Marshall, and B. A. Freeman. 1990. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. U.S.A.* 87:1620-1624. Beckman, J. S., M. Carson, C. D. Smith, W. H. Koppenol. 1993. ALS, SOD and peroxynitrite. *Nature.* 364:584. Benov, L. T. and I. Fridovich. 1994. *Escherichia coli* expresses a copper- and zinc-containing superoxide dismutase. *J. Biol. Chem.* 269:25310-25314. Beyer, W. F. Jr. and I. Fridovich. 1991. In vivo competition between iron and manganese for occupancy of the active site region of the manganese-superoxide dismutase of *Escherichia coli*. *J. Biol. Chem.* 266:303-308. Bowler, R. P. and J. D. Crapo. 2002a. Oxidative stress in airways: is there a role for extracellular superoxide dismutase? *Am. J. Respir. Crit. Care Med.* 166:S38-S43. Bowler, R. P., M. Nicks, D. A. Olsen, I. B. Thogersen, Z. Valnickova, P. Hojrup, A. Franzusoff, J. J. Enghild, and J. D. Crapo. 2002b. Furin proteolytically processes the heparin-binding region of extracellular superoxide dismutase. *J. Biol. Chem.* 277:16505-16511. Bravard, A., L. Sabatier, F. Hoffschir, M. Ricoul, C. Luccioni, and B. Dutrillaux. 1992. SOD2: a new type of tumor-suppressor gene? *Int. J. Cancer.* 51:476-480. Burkhardt, H., F. Hartmann, and M. L. Schwingel. 1986. Activation of latent collagenase from polymorphonuclear leukocytes by oxygen radicals. *Enzyme.* 36:221-231. Canvin, J., P. R. Langford, K. E. Wilks, and J. S. Kroll. 1996. Identification of sodC encoding periplasmic [Cu,Zn]-superoxide dismutase in *Salmonella*. *FEMS. Microbiol. Lett.* 136:215-220. Carliz, A. and D. Touati. 1986. Isolation of superoxide dismutase mutants in *Escherichia coli*: is superoxide dismutase necessary for aerobic life? *EMBO. J.* 5:623-630. Carlsson, L. M., J. Jonsson, T. Edlund, and S. L. Marklund. 1995. Mice lacking extracellular superoxide dismutase are more sensitive to hyperoxia. *Proc. Natl. Acad. Sci. U.S.A.* 92:6264-6268. Chang, S. K. and H. M. Hassan. 1997. Characterization of superoxide dismutase in *Streptococcus thermophilus*. *Appl. Environ. Microbiol.* 63:3732-3735. Darley-Usmar, V., H. Wiseman, and B. Halliwell. 1995. Nitric oxide and oxygen radicals: a question of balance. *FEBS. Lett.* 369:131-135. DiSilvestro, R. A., F. L. Yang, and E. A. David. 1992. Species-specific heterogeneity for molecular weight estimates of serum extracellular superoxide dismutase activities. *Comp. Biochem. Physiol. B.* 101:531-534. Donnelly, J. K. K. M. Mclellan, J. L. Walker, and D. S. Robison. 1989. Superoxide dismutase in foods: a review. *Food Chem.* 33:243-270. Dubman, N. P., D. E. Wilcken, J. Wang, J. F. Lynch, D. Macey, and P. Lundberg. 1993. Disordered methionine/homocysteine metabolism in premature vascular disease. Its occurrence, cofactor therapy, and enzymology. *Arterioscler. Thromb.* 13:1253-1260. Edlund, A., T. Edlund, K. Hjalmarsson, S. L. Marklund, J. Sandstrom, M. Stromqvist, L. Tibell. 1992. A nonglycosylated extracellular superoxide dismutase variant. *Biochem. J.* 288:451-456. Enghild, J. J., I. B. Thogersen, T. D. Oury, Z. Valnickova, P. Hojrup, J. D. Crapo. 1999. The heparin-binding domain of extracellular superoxide dismutase is proteolytically processed intracellularly during biosynthesis. *J. Biol. Chem.* 274:14818-14822. Esterbauer, H., J. Gebicki, H. Puhl, and G. Jurgens. 1992. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radic. Biol. Med.* 13:341-390. Fasske, E. and K. Morgenroth. 1983. Experimental bleomycin lung in mice. A contribution to the pathogenesis of pulmonary fibrosis. *Lung.* 161:133-146. Fattman, C. L., J. J. Enghild, J. D. Crapo, L. M. Schaefer, Z. Valnickova, and T. D. Oury. 2000. Purification and characterization of extracellular superoxide dismutase in mouse lung. *Biochem. Biophys. Res. Commun.* 275:542-548. Fattman, C. L., C. T. Chu, S. M. Kulich, J. J. Enghild, and T. D. Oury. 2001. Altered expression of extracellular superoxide dismutase in mouse lung after bleomycin treatment. *Free Radic. Biol. Med.* 31:1198-1207. Fattman, C. L., L. M. Schaefer, and T. D. Oury. 2003. Extracellular superoxide dismutase in biology and medicine. *Free Radic. Biol. Med.* 35:236-256. Fiedler, S. and R. Wirth. 1988. Transformation of bacteria with plasmid DNA by electroporation. *Analytical. Biochem.* 170:38-44. Finkelstein, J. D. 1998. The metabolism of homocysteine: pathways and regulation. *Eur. J. Pediatr.* 157:S40-S44. Folz, R. J., L. Peno-Green, and J. D. Crapo. 1994. Identification of a homozygous missense mutation (Arg to Gly) in the critical binding region of the human EC-SOD gene (SOD3) and its association with dramatically increased serum enzyme levels. *Hum. Mol. Genet.* 3:2251-2254. Folz, R. J., J. Guan, M. F. Seldin, T. D. Oury, J. J. Enghild, and J. D. Crapo. 1997. Mouse extracellular superoxide dismutase: primary structure, tissue-specific gene expression, chromosomal localization, and lung in situ hybridization. *Am. J. Respir. Cell Mol. Biol.* 17:393-403. Folz, R. J., A. M. Abushamaa, and H. B. Suliman. 1999. Extracellular superoxide dismutase in the airways of transgenic mice reduces inflammation and attenuates lung toxicity following hyperoxia. *J. Clin.*

Invest. 103:1055-1066. Forman, H. J. and I. Fridovich. 1973. On the stability of bovine superoxide dismutase. The effects of metals. *J. Biol. Chem.* 248:2645-2649. Fridovich I. 1986. Superoxide dismutases. *Adv. Enzymol. Relat. Areas. Mol. Biol.* 58:61-97. Fukai, T., Z. S. Galis, X. P. Meng, S. Parthasarathy, and D. G. Harrison. 1998. Vascular expression of extracellular superoxide dismutase in atherosclerosis. *J. Clin. Invest.* 101:2101-2111. Fukai, T., M. R. Siegfried, M. Ushio-Fukai, K. K. Griendling, and D. G. Harrison. 1999. Modulation of extracellular superoxide dismutase expression by angiotensin II and hypertension. *Circ. Res.* 85:23-28. Fukai, T., M. R. Siegfried, M. Ushio-Fukai, Y. Cheng, G. Kojda, and D. G. 2000. Harrison. Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. *J. Clin. Invest.* 105:1631-1639. Fukai, T., R. J. Folz, U. Landmesser, and D. G. Harrison. 2002. Extracellular superoxide dismutase and cardiovascular disease. *Cardiovasc. Res.* 55:239-249. Fukui, S., T. Ookawara, H. Nawashiro, K. Suzuki, and K. Shima. 2002. Postischemic transcriptional and translational responses of EC-SOD in mouse brain and serum. *Free Radic. Biol. Med.* 32:289-298. Gao, B., S. C. Flores, S. K. Bose, and J. M. McCord. 1996. A novel *Escherichia coli* vector for oxygen-inducible high level expression of foreign genes. *Gene.* 176:269-270. Gasson, M. J. 1983. Genetic transfer systems in lactic acid bacteria. *Antonie van Leeuwenhoek.* 49:275-282. Gregory, E. M. and I. Fridovich. 1974. Oxygen metabolism in *Lactobacillus plantarum*. *J. Bacteriol.* 117:166-169. Geller, B. L. and D. R. Winge. 1983. A method for distinguishing Cu,Zn- and Mn-containing superoxide dismutases. *Anal. Biochem.* 128:86-92. Gerlach, D., W. Reichardt, and S. Vettermann. 1998. Extracellular superoxide dismutase from streptococcus pyogenes type 12 strain is manganese-dependent. *FEMS. Microbiol. Lett.* 160:217-224. Ghio, A. J., H. B. Suliman, J. D. Carter, A. M. Abushama, and R. J. Folz. 2002. Overexpression of extracellular superoxide dismutase decreases lung injury after exposure to oil fly ash. *Am. J. Physiol. Lung Cell Mol. Physiol.* 283:L211-L218. Griendling, K. K. and M. Ushio-Fukai. 2000. Reactive oxygen species as mediators of angiotensin II signaling. *Regul. Pept.* 91:21-27. Haddad, I. Y., H. Ischiropoulos, B. A. Holm, J. S. Beckman, J. R. Baker, and S. Matalon. 1993. Mechanisms of peroxynitrite- induced injury to pulmonary surfactants. *Am. J. Physiol.* 265:L555-L564. Halliwell, B. 1989. Free radicals, reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis. *Br. J. Exp. Pathol.* 70:737-757. He, H. J., Q. S. Yuan, G. Z. Yang, and X. F. Wu. 2002. High- level expression of human extracellular superoxide dismutase in *Escherichia coli* and insect cells. *Protein. Expr. Purif.* 24:13-17. Hjalmarsson, K., S. L. Marklund, A. Engstrom, and T. Edlund. 1987. Isolation and sequence of complementary DNA encoding human extracellular superoxide dismutase. *Proc. Natl. Acad. Sci. U.S.A.* 84:6340-6344. Hopkin, K. A., M. A. Papazian, and H. M. Steinman. 1992. Functional differences between manganese and iron superoxide dismutases in *Escherichia coli* K-12. *J. Biol. Chem.* 267:24253-24258. Hornig, B., U. Landmesser, C. Kohler, D. Ahlersmann, S. Spiekermann, A. Christoph, H. Tatge, and H. Drexler. 2001. Comparative effect of ace inhibition and angiotensin II type 1 receptor antagonism on bioavailability of nitric oxide in patients with coronary artery disease: role of superoxide dismutase. *Circulation.* 103:799-805. Inaoka, T., Y. Matsumura, and T. Tsuchido. 1998. Molecular cloning and nucleotide sequence of the superoxide dismutase gene and characterization of its product from *Bacillus subtilis*. *J. Bacteriol.* 180:3697-3703. Iyama, S., T. Okamoto, T. Sato, N. Yamauchi, Y. Sato, K. Sasaki, M. Takahashi, M. Tanaka, T. Adachi, K. Kogawa, J. Kato, S. Sakamaki, and Y. Niitsu. 2001. Treatment of murine collageninduced arthritis by ex vivo extracellular superoxide dismutase gene transfer. *Arthritis Rheum.* 44:2160-2167. John, St. G. and H. M. Steinman. 1996. Periplasmic copper-zinc superoxide dismutase of *Legionella pneumophila*: role in stationary-phase survival. *J. Bacteriol.* 178:1578-1584. Karlsson, K. and S. L. Marklund. 1987. Heparin-induced release of extracellular superoxide dismutase to human blood plasma. *Biochem. J.* 242:55-59. Karlsson, K. and S. L. Marklund. 1988a. Extracellular superoxide dismutase in the vascular system of mammals. *Biochem. J.* 255: 223-228. Karlsson, K. and S. L. Marklund. 1988b. Heparin-, dextran sulfate-, and protamine-induced release of extracellular superoxide dismutase to plasma in pigs. *Biochim. Biophys. Acta.* 967:110-114. Karlsson, K. and S. L. Marklund. 1988c. Plasma clearance of human extracellular superoxide dismutase C in rabbits. *J. Clin. Invest.* 82:762-766. Karlsson, K. and S. L. Marklund. 1989. Binding of human extracellular superoxide dismutase C to cultured cell lines and to blood cells. *Lab. Invest.* 60:659-666. Karlsson, K., A. Edlund, J. Sandstrom, and S. L. Marklund. 1993a. Proteolytic modification of the heparin-binding affinity of extracellular superoxide dismutase. *Biochem. J.* 290:623-626. Karlsson, K., J. Sandstrom, A. Edlund, T. Edlund, and S. L. Marklund. 1993b. Pharmacokinetics of extracellular superoxide dismutase in the vascular system. *Free Radic. Biol. Med.* 14: 185-190. Karlsson, K., J. Sandstrom, A. Edlund, and S. L. Marklund. 1994. Turnover of extracellular superoxide dismutase in tissues. *Lab. Invest.* 70:705-710. Kawamura, N., T. Ookawara, K. Suzuki, K. Konishi, M. Mino, and N. Taniguchi. 1992. Increased glycosylated Cu,Zn superoxide dismutase levels in erythrocytes of patients with insulin- dependent diabetes mellitus. *J. Clin. Endocrinol. Metab.* 74:1352-1354. Kim, W. J., B. Ray, and M. C. Johnson. 1992. Plasmid transfers by conjugation and electroporation in *pediococcus acidilactici*. *J. Appl. Bacterio.* 72:201-207. Kojda, G. and D. Harrison. 1999. Interactions between NO and reactive oxygen species: pathophysiological importance in atherosclerosis, hypertension, diabetes and heart failure. *Cardiovasc. Res.* 43:562-571. Kroll, J. S., P. R. Langford, J. R. Saah, and B. M. Loynds. 1993. Molecular and genetic characterization of superoxide dismutase in *haemophilus influenzae* type b. *Mol. Microbiol.* 10:839-848. Kroll, J. S., P. R. Langford, K. E. Wilks, and A.D. Keil. 1995. Bacterial [Cu,Zn]-superoxide dismutase: phylogenetically distinct from the eukaryotic enzyme, and not so rare after all! *Microbiology.* 141:2271-2279. Landmesser, U., R. Merten, S. Spiekermann, K. Buttner, H. Drexler, and B. Hornig. 2000. Vascular extracellular superoxide dismutase activity in patients with coronary artery disease: relation to endothelium-dependent vasodilation. *Circulation.* 101:2264-2270. Laukkanen, M. O.; P. Lehtolainen, P. Turunen, S. Aittomaki; P. Oikari, S. L. Marklund, and S. Yla-Herttuala. 2000. Rabbit extracellular superoxide dismutase: expression and effect on LDL oxidation. *Gene.* 254:173-179. Laukkanen, M. O., P. Leppanen, P. Turunen, T. Tuomisto, J. Naarala, S. and Yla-Herttuala. 2001. EC-SOD gene therapy reduces paracetamol-induced liver damage in mice. *J. Gene Med.* 3:321-325. Laursen, J. B., S. Rajagopalan, Z. Galis, M. Tarpey, B. A. Freeman, D. G. and Harrison. 1997. Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. *Circulation.*

95:588-593. Lebovitz, R. M., H. Zhang, H. Vogel, J. Jr. Cartwright, L. Dionne, N. Lu, S. Huang, and M. M. Matzuk. 1996. Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proc. Natl. Acad. Sci. U. S. A.* 93:9782-9787.

Ledwozyw, A. 1991. Protective effect of liposome-entrapped superoxide dismutase and catalase on bleomycin-induced lung injury in rats. II. Phospholipids of the lung surfactant. *Acta. Physiol. Hung.* 78:157-162.

Lee, I. M. 1999. Antioxidant vitamins in the prevention of cancer. *Proc. Assoc. Am. Physicians.* 111:10-15.

Li, Q., R. Bolli, Y. Qiu, X. L. Tang, Y. Guo, and B. A. French. 2001. Gene therapy with extracellular superoxide dismutase protects conscious rabbits against myocardial infarction. *Circulation.* 103:1893—1898.

Lim, J.H., Y. G. Yu, I. G. Choi, J. R. Ryu, B. Y. Ahn, S. H. Kim, and Y. S. Han. 1997. Cloning and expression of superoxide dismutase from *Aquifex pyrophilus*, a hyperthermophilic bacterium. *FEBS. Lett.* 406:142-146.

Loenders, B., E. Van Mechelen, S. Nicolai, N. Buysens, N. Van Osselaer, P. G. Jorens, J. Willems, A. G. Herman, and H. Slegers. 1998. Localization of extracellular superoxide dismutase in rat lung: neutrophils and macrophages as carriers of the enzyme. *Free Radic. Biol. Med.* 24:1097-1106.

Lookene A, Stenlund P, Tibell LA. 2000. Characterization of heparin binding of human extracellular superoxide dismutase. *Biochemistry.* 39:230-236.

Lynch, R. E. and B. C. Cole. 1980. *Mycoplasma pneumoniae*: a prokaryote which consumes O<sub>2</sub> and generates O<sub>2</sub><sup>-</sup>, but which lacks SOD. *Biochem. Biophys. Res. Commun.* 96:98-105.

Malinowski, D. P. and I. Fridovich. 1979. Subunit association and side-chain reactivities of bovine erythrocyte superoxide dismutase in denaturing solvents. *Biochemistry.* 18:5055-5060.

Marklund, S. L. 1982. Human copper-containing superoxide dismutase of high molecular weight. *Proc. Natl. Acad. Sci. U.S.A.* 79:7634-7638.

Marklund, S. L., E. Holme, and L. Hellner. 1982. Superoxide dismutase in extracellular fluids. *Clin. Chim. Acta.* 126:41- 51.

Marklund, S. L. 1984a. Extracellular superoxide dismutase and other superoxide dismutase isoenzymes in tissues from nine mammalian species. *Biochem. J.* 222:649-655.

Marklund, S. L. 1984b. Extracellular superoxide dismutase in human tissues and human cell lines. *J. Clin. Invest.* 74:1398- 1403.

Marklund, S. L. 1984c. Properties of extracellular superoxide dismutase from human lung. *Biochem. J.* 220:269-272.

Marklund, S. L., A. Bjelle, and L. G. Elmqvist. 1986. Superoxide dismutase isoenzymes of the synovial fluid in rheumatoid arthritis and in reactive arthritides. *Ann. Rheum. Dis.* 45:847-851.

Marklund S. L. 1992. Regulation by cytokines of extracellular superoxide dismutase and other superoxide dismutase isoenzymes in fibroblasts. *J. Biol. Chem.* 267:6696-6701.

Marklund, S. L., P. Nilsson, K. Israelsson, I. Schampi, M. Peltonen, and K. Asplund. 1997. Two variants of extracellular superoxide dismutase: relationship to cardiovascular risk factors in an unselected middle-aged population. *J. Intern. Med.* 242:5-14.

McCord, J. M., B. Gao, J. Leff, and S. C. Flores. 1994. Neutrophil-generated free radicals: possible mechanisms of injury in adult respiratory distress syndrome. *Environ. Health Perspect.* 102(Suppl. 10):57-60.

McCully, K. S. 1996. Homocysteine and vascular disease. *Nat. Med.* 2:386-389.

Miyoshi, A., T. Rochat, J. J. Gratadoux, Y. Le Loir, S. C. Oliveira, P. Langella, and V. Azevedo. 2003. Oxidative stress in *Lactococcus lactis*. *Genet. Mol. Res.* 2003. 2:348- 259.

Monboisse, J. C., P. Braquet, A. Randoux, and J. P. Borel. 1983. Non-enzymatic degradation of acid-soluble calf skin collagen by superoxide ion: protective effect of flavonoids. *Biochem. Pharmacol.* 32:53-58.

Monboisse, J. C., G. Bellon, J. Dufer, A. Randoux, and J. P. Borel. 1987. Collagen activates superoxide anion production by human polymorphonuclear neutrophils. *Biochem. J.* 246:599- 603.

Monboisse, J. C., G. Bellon, A. Randoux, J. Dufer, and J. P. Borel. 1990. Activation of human neutrophils by type 1 collagen. Requirement of two different sequences. *Biochem. J.* 270:459-462.

Moncada, S. 1997. Adventures in pharmacology, aspirin, prostacyclin and nitric oxide. *Methods. Find. Exp. Clin. Pharmacol.* 19 Suppl A:3-5.

Mruk, D., C. H. Cheng, Y. H. Cheng, M. Y. Mo, J. Grima, B. Silvestrini, W. M. Lee, and C. Y. Cheng. 1998. Rat testicular extracellular superoxide dismutase: its purification, cellular distribution, and regulation. *Biol. Reprod.* 59:298-308.

Mruk, D., B. Silvestrini, M. Y. Mo, and C. Y. Cheng. 2002. Antioxidant superoxide dismutase - a review: its function, regulation in the testis, and role in male fertility. *Contraception.* 65:305-311.

Mugge, A., R. P. Brandes, R. H. Boger, A. Dwenger, S. Bode-Boger, S. Kienke, J. C. Frolich, and P. R. Lichtlen. 1994. Vascular release of superoxide radicals is enhanced in hypercholesterolemic rabbits. *J. Cardiovasc. Pharmacol.* 24:994-998.

Mullarkey, C. J., D. Edelstein, and M. Brownlee. 1990. Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. *Biochem. Biophys. Res. Commun.* 173:932-939.

Natvig, D. O., K. Imlay, D. Touati, and R. A. Hallewell. 1987. Human copper-zinc superoxide dismutase complements superoxide dismutase-deficient *Escherichia coli* mutants. *J. Biol. Chem.* 262:14697-14701.

Nonaka, H., T. Tsujino, Y. Watari, N. Emoto, and M. Yokoyama. 2001. Taurine prevents the decrease in expression and secretion of extracellular superoxide dismutase induced by homocysteine: amelioration of homocysteine-induced endoplasmic reticulum stress by taurine. *Circulation.* 104:1165-1170.

Norrod, P. and S. Morse. 1979. Absence of superoxide dismutase in some strains of *Neisseria gonorrhoeae*. *Biochem. Biophys. Res. Commun.* 90:1287-1294.

Nygaard, O., J. E. Nordrehaug, H. Refsum, P. M. Ueland, M. Farstad, and S. E. Vollset. 1997. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N. Engl. J. Med.* 337:230—236.

Oda, A., C. Bannai, T. Yamaoka, T. Katori, T. Matsushima, and K. Yamashita. 1994. Inactivation of Cu,Zn superoxide dismutase by in vitro glycosylation and in erythrocytes of diabetic patients. *Horm. Metab. Res.* 26:1-4.

Ohara, Y., T. E. Peterson, and D. G. Harrison. 1993. Hypercholesterolemia increases endothelial superoxide anion production. *J. Clin. Invest.* 91:2546-2551.

Ohta, H., T. Adachi, and K. Hirano. 1993. The nature of heterogeneous components of extracellular superoxide dismutase purified from human umbilical cords. *Free Radic. Biol. Med.* 15:151-158.

Ookawara, T., T. Kizaki, S. Ohishi, M. Yamamoto, O. Matsubara, and H. Ohno. 1997. Purification and subunit structure of extracellular superoxide dismutase from mouse lung tissue. *Arch. Biochem. Biophys.* 340:299-304.

Oury, T. D., L. Y. Chang, S. L. Marklund, B. J. Day, and J. D. Crapo. 1994. Immunocytochemical localization of extracellular superoxide dismutase in human lung. *Lab. Invest.* 70:889-898.

Oury, T. D., B. J. Day, and J. D. Crapo. 1996a. Extracellular superoxide dismutase: a regulator of nitric oxide bioavailability. *Lab. Invest.* 75:617-636.

Oury, T. D., J. D. Crapo, Z. Valnickova, and J. J. Enghild. 1996b. Human extracellular superoxide dismutase is a tetramer composed of two disulphide-linked dimers: a simplified, high-yield purification of extracellular superoxide dismutase. *Biochem. J.* 317:51-57.

Oury,

T. D., B. J. Day, and J. D. Crapo. 1996c. Extracellular superoxide dismutase in vessels and airways of humans and baboons. *Free Radic. Biol. Med.* 20:957-965.

Oury, T. D., K. Thakker, M. Menache, L. Y. Chang, J. D. Crapo, B. J. Day. 2001. Attenuation of bleomycin-induced pulmonary fibrosis by a catalytic antioxidant metalloporphyrin. *Am. J. Respir. Cell Mol. Biol.* 25:164-169.

Pardo, A., M. Selman, K. Ridge, R. Barrios, and J. I. Sznajder. 1996. Increased expression of gelatinases and collagenase in rat lungs exposed to 100% oxygen. *Am. J. Respir. Crit. Care Med.* 154:1067-1075.

Petersen, S. V., T. D. Oury, Z. Valnickova, I. B. Thogersen, P. Hojrup, J. D. Crapo, and J. J. Enghild. 2003. The dual nature of human extracellular superoxide dismutase: one sequence and two structures. *Proc. Natl. Acad. Sci. U. S. A.* 100:13875-13880.

Powell, I. B., M. G. Achen, A. J. Hillier, B. E. Davidson. 1988. A simple and rapid method for genetic transformation of lactic streptococci by electroporation. *Appl. Environ. Microbiol.* 54:655-660.

Radi, R., J. S. Beckman, K. M. Bush, and B. A. Freeman. 1991. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch. Biochem. Biophys.* 288:481-487.

Rajagopalan, S., S. Kurz, T. Munzel, B. A. Freeman, K. K. Griendling, and D. G. Harrison. 1996. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation: contribution to alterations of vasomotor tone. *J. Clin. Invest.* 97:1916-1923.

Roy, D. G., T. R. Klaenhammer, and H. M. Hassan. 1993. Cloning and expression of the manganese superoxide dismutase gene of *Escherichia coli* in *Lactococcus lactis* and *Lactobacillus gasseri*. *Mol. Gen. Genet.* 239:33-40.

Russell, W. M. and T. R. Klaenhammer. 2001. Efficient system for directed integration into the *Lactobacillus acidophilus* and *Lactobacillus gasseri* chromosomes via homologous recombination. *Appl. Environ. Microbiol.* 63:4361-4364.

Salvemini, D., E. Mazzon, L. Dugo, D. P. Riley, I. Serraino, A. P. Caputi, and S. Cuzzocrea. 2001a. Pharmacological manipulation of the inflammatory cascade by the superoxide dismutase mimetic, M40403. *Br. J. Pharmacol.* 132:815-827.

Salvemini, D., E. Mazzon, L. Dugo, I. Serraino, A. De Sarro, A. P. Caputi, and S. Cuzzocrea. 2001b. Amelioration of joint disease in a rat model of collagen-induced arthritis by M40403, a superoxide dismutase mimetic. *Arthritis. Rheum.* 44:2909-2921.

Sanchez-Quesada, J. L., R. Homs-Serradesanferm, J. Serrat-Serrat, J. R. Serra-Grima, F. Gonzalez-Sastre, and J. Ordonez-Llanos. 1995. Increase of LDL susceptibility to oxidation occurring after intense, long duration aerobic exercise. *Atherosclerosis.* 118:297-305.

Sanders J. W., K. J. Leenhouts, A. J. Haandrikman, G. Venema, and J. Kok. 1995. Stress response in *Lactococcus lactis*: cloning, expression analysis, and mutation of the lactococcal superoxide dismutase gene. *J. Bacteriol.* 177:5254-5260.

Sandstrom, J., L. Carlsson, S. L. Marklund, and T. Edlund. 1992. The heparin-binding domain of extracellular superoxide dismutase C and formation of variants with reduced heparin affinity. *J. Biol. Chem.* 267:18205-18209.

Sandstrom, J., K. Karlsson, T. Edlund, and S. L. Marklund. 1993. Heparin-affinity patterns and composition of extracellular superoxide dismutase in human plasma and tissues. *Biochem. J.* 294(Part 3):853-857.

Sandstrom, J., P. Nilsson, K. Karlsson, and S. L. Marklund. 1994. 10-fold increase in human plasma extracellular superoxide dismutase content caused by a mutation in heparin-binding domain. *J. Biol. Chem.* 269:19163-19166.

Saul, R. L. and B. N. Ames. 1986. Background levels of DNA damage in the population. *Basic. Life. Sci.* 38:529-535.

Sentman, M. L., L. M. Jonsson, and S. L. Marklund. 1999. Enhanced alloxan-induced  $\beta$ -cell damage and delayed recovery from hyperglycemia in mice lacking extracellular superoxide dismutase. *Free Radic. Biol. Med.* 27:790-796.

Sessa, W. C., K. Pritchard, N. Seyedi, J. Wang, and T. H. Hintze. 1994. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. *Circ. Res.* 74:349-353.

Sherman, L., N. Dafni, J. Lieman-Hurwitz, and Y. Groner. 1983. Nucleotide sequence and expression of human chromosome 21-encoded superoxide dismutase mRNA. *Proc. Natl. Acad. Sci. U. S. A.* 80:5465-5469.

Sorsa, T., K. Suomalainen, and V. J. Uitto. 1990. The role of gingival crevicular fluid and salivary interstitial collagenases in human periodontal diseases. *Arch. Oral. Biol.* 35:193S-196S.

Steinberg, D. 1995. Role of oxidized LDL and antioxidants in atherosclerosis. *Adv. Exp. Med. Biol.* 369:39-48.

Stenlund, P., M. J. Lindberg, and L. A. Tibell. 2002. Structural requirements for high-affinity heparin binding: alanine scanning analysis of charged residues in the C-terminal domain of human extracellular superoxide dismutase. *Biochemistry.* 41:3168-3175.

Stralin, P. and S. L. Marklund. 2001. Vasoactive factors and growth factors alter vascular smooth muscle cell EC-SOD expression. *Am. J. Physiol. Heart Circ. Physiol.* 281:H1621- H1629.

Stromqvist, M., J. Holgersson, and B. Samuelsson. 1991. Glycosylation of extracellular superoxide dismutase studied by high-performance liquid chromatography and mass spectrometry. *J. Chromatogr.* 548:293-301.

Su, W. Y., R. Folz, J. S. Chen, J. D. Crapo, and L. Y. Chang. 1997. Extracellular superoxide dismutase mRNA expressions in the human lung by in situ hybridization. *Am. J. Respir. Cell Mol. Biol.* 16:162-170.

Suliman, H. B., L. K. Ryan, L. Bishop, and R. J. Folz. 2001. Prevention of influenza-induced lung injury in mice overexpressing extracellular superoxide dismutase. *Am. J. Physiol. Lung Cell Mol. Physiol.* 280:L69-L78.

Takatsu, H., H. Tasaki, H. N. Kim, S. Ueda, M. Tsutsui, K. Yamashita, T. Toyokawa, Y. Morimoto, Y. Nakashima, and T. Adachi. 2001. Overexpression of EC-SOD suppresses endothelial cell-mediated LDL oxidation. *Biochem. Biophys. Res. Commun.* 285:84-91.

Taniguchi, N. 1990. Superoxide dismutases: significances in aging, diabetes, ischemia and cancer. *Rinsho. Byori.* 38:876- 881.

Tate, R. M. and J. E. Repine. 1983. Neutrophils and the adult respiratory distress syndrome. *Am. Rev. Respir. Dis.* 128:552- 559.

Tibell, L., K. Hjalmarsson, T. Edlund, G. Skogman, A. Engstrom, and S. L. Marklund. 1987. Expression of human extracellular superoxide dismutase in Chinese hamster ovary cells and characterization of the product. *Proc. Natl. Acad. Sci. U. S. A.* 84:6634-6638.

Tibell, L., R. Aasa, and S. L. Marklund. 1993. Spectral and physical properties of human extracellular superoxide dismutase: a comparison with Cu,Zn superoxide dismutase. *Arch. Biochem. Biophys.* 304:429-433.

Tibell, L. A., E. Skarfstad, and B. H. Jonsson. 1996. Determination of the structural role of the N-terminal domain of human extracellular superoxide dismutase by use of protein fusions. *Biochim. Biophys. Acta.* 1292:47-52.

Tibell, L. A., I. Sethson, and A. V. Buevich. 1997. Characterization of the heparin-binding domain of human extracellular superoxide dismutase. *Biochim. Biophys. Acta.* 1340:21-32.

Torii, K., K. Iida, Y. Miyazaki, S. Saga, Y. Kondoh, H. Taniguchi, F. Taki, K. Takagi, M. Matsuyama, and R. Suzuki. 1997. Higher concentrations of matrix metalloproteinases in bronchoalveolar lavage fluid of patients with adult respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* 155:43-46.

van Poppel, G. and H. van den Berg. 1997. Vitamins and cancer. *Cancer. Lett.* 114:195-202. Wang, X. L., N. Duarte, H. Cai, T. Adachi, A. S. Sim, G. Cranney, and D. E. Wilcken. 1999. Relationship between total plasma homocysteine, polymorphisms of homocysteine metabolism related enzymes, risk factors, and coronary artery disease in the Australian hospital-based population. *Atherosclerosis.* 146:133-140. White, C. R., T. A. Brock, L. Y. Chang, J. Crapo, P. Briscoe, D. Ku, W. A. Bradley, S. H. Gianturco, J. Gore, B. A. Freeman, and M. M. Tarpey. 1994. Superoxide and peroxynitrite in atherosclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 91:1044-1048. Wilcken, D. E. and B. Wilcken. 1976. The pathogenesis of coronary artery disease. A possible role for methionine metabolism. *J. Clin. Invest.* 57:1079-1082. Wilcken, D. E., X. L. Wang, T. Adachi, H. Hara, N. Duarte, K. Green, and B. Wilcken. 2000. Relationship between homocysteine and superoxide dismutase in homocystinuria: possible relevance to cardiovascular risk. *Arterioscler. Thromb. Vasc. Biol.* 20:1199-1202. Willems, J., A. Zwijsen, H. Slegers, S. Nicolai, J. Bettadapura, J. Raymackers, and T. Scarcez. 1993. Purification and sequence of rat extracellular superoxide dismutase B secreted by C6 glioma. *J. Biol. Chem.* 268:24614- 24621