Genotoxicity Analysis of Kefir Powder

李兆桐、孫芳君

E-mail: 387162@mail.dyu.edu.tw

ABSTRACT

Kefir is a fermented milk product by kefir grain. Kefir has been certidied with antibacteria, antifungal, antivirus, anti-allergy, anti-inflammatory, and blood pressure-lowering activities. The kefir powder was subjected to genotoxicity analysis. In this study, Ames test, mouse lymphoma tk assay, chromosome aberrations assay, cytokinesis-block micronucleus assay, and mice peripheral blood micronucleus assay were performed in accordance with OECD guidelines for the testing of Chemicals. These results reveal that the kefir powder was not genotoxic in five independent assays.

Keywords : kefir、genotoxicity、Ames test、mouse lymphoma tk assay, chromosome aberrations assay、cytokinesis-block micronucleus assay、mice peripheral blood micronucleus assay

Table of Contents

REFERENCES

1. 石春海。遺傳學第七章，浙江大學。2005。 2. 經濟部智慧財產局。發明專利說明書，第096135346號。2011。 3. Abraham, A.G., Antoni, G.L., Characterization of kefir grains grown in cow ’ milk, J. airy e ., 66, 327-333, 1999. 4. Ames, B.N., McCann, J., Yamasaki, E., Methods for detecting carcinogensand mutagens with the Salmonella/mammalian-microsome mutagenicity test, Mutat. Res.,31, 347-364, 1975. 5. Angulo, L., Lopez, E., and Lema, C., Microflora present in kefir grainsof the Galician region (North-West of Spain), J. Dairy Res., 60, 263-267, 1993. 6. Applegate, M.L., Hozier, J.C., On the complexity of mutagenic events atthe mouse lymphoma tk locus, Cold Spring Harbor Laboratory Press, New York, 213-224, 1987. 7. Applegate, M.L., Moore, M., Broder, C.B., Burrell, A., Juhn, G, Kaswek, K.L., Lin, P.R., Wadhams, A., Hozier, J.C., Molecular dissection of mutations at the heterozygous thymidine kinase locus in mouse lymphoma cells. Proc. Natl. Acad. Set. USA 87, 51-55, 1990. 8. Arai, K., Murota, I., Hayakawa, K., Kataoka, M., Mitsuoka, T., Effects of administration of pasteurized fermented milk to mice on the life-spanand intestinal flora, J. Food Sci., 33, 219-223, 1980. 9. Blazak, W.R., Stewart, B.R., Galperin, I., Allen, K.L., Rudd, C.J., Mitchell, A.D., Caspary, W.J., Chromosome analysis of trifluorothymidine-resistant L5178Y mouse lymphoma cell colonies. Environ. Mutagen., 8, 229-240, 1986. 10. Brialy, C., Rivalland, P., Coiffard, L., Roeck H.Y., Microbiolgical study of lyophilised dairy kefir, Folia Microbiol., 40, 198-200, 1995. 11. Carere, A., Benigni R., Strategies and government regulations, Carcinogen. Mutagen., 10, 199-208, 1990. 12. Clementi, F., Gobbetti, M., Rossi, J., Carbon dioxide synthesis by immobilized yeast cells in kefir production, Milchwissenschaft., 44, 70-74, 1989. 13. Clements, J., The Mouse Lymphoma Assay, Mutat. Res., 455, 97-110, 2000 14. Clive, D., Batson, A.G., Turner, N.T., The ability of L5178Y/TK+/- mouse lymphoma cells to detect single gene and viable chromosome mutations: valuation and relevance to mutagen and carcinogen screening. The Predictive Value of Short-Term Screening Tests in Carcinogenicity Evaluation. Elsevier/North Holland Biomedical Press, New York, 103-123, 1980. 15. Clive, D., McCuen, R., Spector, J.F.S., Piper, C, Mavournin, K.H., Specific gene mutations in L5178Y cells in culture. A report of the U.S. Environmental Protection Agency Gene-Tox Program, Mutat. Res., 115, 225-251, 1983. 16. Clive, D., Moore, M., Historical overview of the mouse lymphoma tk+/- mutagenicity assay, Cold Spring Harbor Laboratory Press, New York,25-36, 1987. 17. David, K., Chromosome aberration testing in genetic toxicology-past, present and future, Mutat. Res., 404, 173-185, 1998. 18. Dearfield, K.L., Auletta, A.E., Cimino, M.C., Moore M., Consideration in the US environmental protection agency's testing approach for mutagenicity, Mutat. Res., 258, 259-283, 1991. 19. Dertinger, S.D., Bishop, M.E., McNamee, J.P., Hayashi, M., Suzuki, T., Asano, N., Nakajima, M., Saito, J., Moore, M., Torous, D.K., Macgregor, J.T., Flow cytometric analysis of micronuclei in peripheral blood reticulocytes: I. Intra- and interlaboratory comparison with microscopic scoring, Toxicol Sci., 94(1), 83-91, 2006. 20. Duitschaever, C.L., Kemp, N., Smith, A.K., Microscopic studies of the microflora of kefir grains and of kefir made by different methods, Milchwissenschaft., 43, 479-481, 1988. 21. Eastmond, D.A., Tucker, J.D., Identification of aneuploidyinducing agentsusing cytokinesis-blocked human lymphocytes and an antikinetochore antibody, Env. Mol. Mutagen., 13(1), 34-43, 1989. 22. Fahrig, R., Lang R., Madle S., General strategy for the assessment of genotoxicity. Mutat. Res., 252, 161-163, 1991. 23. Farnworth, E.R., Mainville, unpublished data, 2000. 24. Farnworth, E.R., Kefir: from folklore to regulatory approval, Med. Food.,1, 57-68, 1999. 25. Farnworth, E.R., Properties and characteristics of kefir: a fermented milkprobiotic, Abs. No. PM332, 16th International Congress of Nutrition, Montreal, July 27-August 1, 1997. 26. Fernando, L.O., Aitor, R., Natalia, E., Javier, G., Kefir: A symbiotic yeasts-bacteria community with alleged healthy capabilities, Rev. Iberoam Micol., 23, 67-74, 2006. 27. Fenech, M., Morley, A.A., Cytokinesis-block micronucleus method in human lymphocytes: effect of in vivo ageing and low dose X-irradiation, Mutat. Res., 161(2), 193-198, 1986. 28. Fenech, M., Morley, A.A., Measurement of micronuclei in lymphocytes. Mutat. Res., 147(1-2), 29-36, 1985. 29. Fenech, M, The in vitro micronucleus technique, Mutat. Res., 455, 81-95, 2000. 30. Fenech, M., The advantages and

disadvantages of the cytokinesis-block micronucleus method, Mutat. Res., 392(1-2), 11-18, 1997. 31. Galloway, S.M., Bloom, A.D., Resnick, M., Margolin, B.H., Nakamura, F., Archer, P., Zeiger, E., Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories, Environ. Mutagen., 7, 1-51,1985. 32. Garrote, G.L., Abraham, A.G., Antoni, G.L., Inhibitory power of kefir: the role of organic acids, J. Food Prot., 63, 364-369, 2000. 33. Hata, Y., Yamamoto, M., Ohni, M., Nakajima, K., Nakamura, Y., Takano, T., A placebo-controlled study of the effect of sour milk on blood pressure in hypertensive subjects, J. Clin. Nutr., 64, 767-771, 1996. 34. Heddle, J.A., Rapid in vivo test for chromosomal damage, Mutat. Res., 18, 187-190, 1973. 35. Hozier, J., Sawyer, J., Clive, D., Moore, M., Cytogenetic distinction between the TK+ and TK- chromosomes in the L5178Y TK+/- 3.7.2 C cell line, Mutat. Res., 105, 451-456, 1982. 36. Hozier, J., Sawyer, J., Clive, D., Moore, M., 1985. Chromosome 11 aberrations in small-colony L5178Y TK-/- mutants early in their clonal history, Ann. N.Y. Acad. Sci., 107, 423-425, 1985. 37. ICH Topic S2A, Genotoxicity: Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Harmonised Tripartite Guideline, September, 1995. 38. Kado, N.Y., Langley, D., Eisenstadt, E., A simple modification of the Salmonella liquid incubation assay: increased sensitivity for detecting mutagens in human urine, Mutat. Res., 122, 25-32, 1983. 39. Karaki, H., Doi, K., Sugano, S., Uchiwa, H., Sugai, R., Murakami, U., Takemoto, S., Antihypertensive effect of tryptic hydrolysate of milk casein in spontaneously hypertensive rats, Comp. Bioc. B., 96, 367-371, 1990. 40. Kemp, N., Kefir, the champagne of cultured dairy products, J. Cult. Dairy Prod., 29-30, 1984. 41. Kooiman, P., The chemical structure of kefiran, the water-soluble polysaccharide of the kefir grain, Carbohydr. Res., 7, 200-211, 1968. 42. Koroleva, N.S., Robinson, R.K., Products prepared with lactic acid bacteria and yeasts, in therapeutic properties of fermented milks, Elsevier Applied Sci., 159-179, 1991. 43. Kramers, P.G., Knaap, A.G., Heijden, C.A., Taalman, R.D., Mohn G.R., Role of genotoxicity assays in the regulation of chemicals in the Netherlands: Considerations and experience, Mutagenesis., 6, 487-493, 1990. 44. Liechty, M.C., Scalzi, J.M., Sims, K.R., Crosby, Jr., Spencer, D.L., Davis,L.M., Caspary, W.J., Hozier, J.C., Analysis of large and small colony L5178Y tk-/- mouse lymphoma mutants by loss of heterozygosity (LOH)and by whole chromosome 11 painting: detection or recombination, Mutagenesis., 13(5), 461-474, 1998. 45. Linossier, J.P., Dousset, X., Stimulation de la croissance et du metabolisme de Lactobacillus kefir par Candida kefir, Microbiol, Aliments Nutr., 12, 341-351, 1994. 46. Lshidate, M.Jr., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., Matsuoka, A., Primary mutagenicity screening of food additives currently used in Japan. Food Chem. Toxicol., 22, 623-636, 1984. 47. Maeda, H., Zhu, X., Suzuki, S., Suzuki, K., Kitamuar, S., Structural characterization and biological activities of an exopolysaccharide kefrian produced by Lactobacillus kefiranofaciens WT-2B, J. Agric. Food Chem., 52, 5533-5538, 2004. 48. Maeno, M., Yamamoto, N., Takano, T., Identification of an Antihypertensive Peptide from Casein Hydrolysate Produced by a Proteinase fromLactobacillus helveticus CP790, J. Dairy Sci., 79, 1316-1321, 1996. 49. Maeno, M., Nakamura, Y., Mennear, J.H., Bernard, B.K., Studies of theToxicological Potential of Tripeptides (L-Valyl-L-prolyl-L-proline and L-Isoleucyl-L-prolyl-L-proline): III. Single- and/or Repeated-Dose Toxicitiesof Tripeptides-Containing Lactobacillus helveticus- Fermented Milk Powder and Casein Hydrolysate in Rats, Int J. Toxicol., 24(4), 13-23, 2005. 50. Maeno, M., Mizuno, S., Mennear, J.H., Bernard, B.K., Studies of the toxicological potential of tripeptides (L-valyl-L-prolyl-L-proline and L-isoleucyl-L-prolyl-L-proline): VIII. Assessment of cytotoxicity and clastogenicity of tripeptides-containing casein hydrolysate and Lactobacillus helveticus-fermented milk powders in Chinese hamster lung cells, Int J. Toxicol.,24(4), 97-105, 2005. 51. Mann, E.J., Kefir and koumiss, Dairy Ind. Int., 50, 11-12, 1985. 52. Mann, E.J., Kefir and koumiss, Dairy Ind. Int., 54, 9-10, 1989. 53. Margulis, L., Brockman, J., and Matson, K., From kefir to death, New York, 69-78, 1995. 54. Marshall, R., Obe, G., The application of chromosome painting to clastogenicity testing in vitro, Environ. Mol. Mutagen, submitted for publication, 32(3), 212-22, 1998. 55. Marshall, V.M., Cole, W.M., Methods for making kefir and fermented milks based on kefir, J. Dairy Res., 52, 451-456, 1985. 56. Marshall, V.M., Cole, W.M., Brooker, B.E., Observations on the structure of kefir grains and the distribution of the microflora, J. Appl. Bacteriol., 57, 491-497, 1984. 57. Matsushima, T., Genotoxicity of New Japanese Chemicals. Mutation andthe Environment, Part E. Wiley-Liss, Inc. 249-255, 1990. 58. McCann, J., Choi, E., Yamasaki, E., Ames, B.N., Detection of carcinogens in the Salmonella/microsome test. Assay of 300 chemicals, Proc. Natl.Acad. Sci. U.S.A. 72, 5135-5139, 1975. 59. Miiller, L., Kasper, P., Madle, S., The quality of genotoxicity testing ofdrugs, experiences of a regulatory agency with new and old compounds,Mutagenesis., 6, 143-149, 1991. 60. Miller, B., Albertini, S., Locher, F., Thybaud, V., Lorge, E., Comparative evaluation of the in vitro micronucleus test and the in vitro chromosome aberration test: industrial experience, Mutat. Res., 392, 45-59, 1997. 61. Mitchell, A.D., Auletta, A.E., Clive, D., Kirby P.E., Moore M., MyhrB.C., The L5178Y tk+/- mouse lymphoma specific gene and chromosomal mutation assay: a phase III report of the U.S. Environmental Protection Agency Gene-Tox. Program, Mutat. Res., 394, 177-303, 1997. 62. Mortelmans, K., Zeiger, E., The Ames Salmonella/microsome mutagenicity assay, Mutat. Res., 455, 29-60, 2000. 63. Mouse Lymphoma Thymidine Kinase Gene Mutation Assay: InternationalWorkshop on Genotoxicity Tests Workgroup Report—Plymouth, UK 2002,Mutat. Res., 540, 127-140, 2003. 64. Natarajan, A.T., Obe, G., Heddle J.A., Mutagenicity testing with cultured mammalian cells: cytogenetic assays, Mutagenicity: New Horizons in Genetic Toxicology, Academic Press, New York, 171-213, 1982. 65. OECD. TG471, Bacterial reverse mutation test, OECD guideline for the testing of chemicals, 10, 1997. 66. OECD. TG473, In vitro mammalain chromosome aberration test, OECD guideline for the testing of chemicals, 10, 1997. 67. OECD. TG474, Mammalian erythrocyte micronucleus test, OECD guideline for the testing of chemicals, 10, 1997. 68. OECD. TG476, In vitro mammalian cell gene mutation test, OECD guideline for the testing of chemicals, 10, 1997. 69. OECD. TG487, In vitro mammalian cell micronucleus test, OECD guideline for the testing of chemicals, 10, 2010. 70. Oleinichenko, E.V., Mitrokhin, S.D., Nonikov, V.E., Minaev, V.I., Acipole efficacy in prevention of enteric dysbacteriosis due to antibacterial therapy, Anitibiot. Khimioter., 44, 23-25, 1999. 71. Ormisson, A.A., Soo, T.R., Effect of lactic acid milk and kefir on the indicators of

acid-base equilibrium of arterial blood in healthy young children and patients with acute pneumonia and acute bronchitis, Pediatriia,10, 37-38, 1976. 72. Osada, K., Nagira, K., Teruya, K., Tachibana, H., Shirahata, S., Murakami, H., Enhancement of interferon- β production with sphingomyelin fromfermented milk, Biotherapy., 7, 115-123, 1994. 73. Ottogalli, G., Galli, A., Resmini, P., Volonterio, G., Composizione microbiologica, chimica et ultrastruttura dei ganuli di kefir, Ann. Micr., 23, 109-121, 1973. 74. Quir?s, A., Ledesma, B.H., Ramos, M., Amigo, L., Recio, I., Angiotensin-converting enzyme inhibitory activity of peptides derived from caprinekefir, J. Dairy Res., 88, 3480-3487, 2005. 75. Roberts M., Yarunin S., Danone moves into Russian kefir market, New Nutr Business., 6, 22-24, 2000. 76. Rosi, J., Rossi, J., I Microrganismi del kefir: I fermenti lattici, Technol.Lattiero-Casearia Sci., 29, 291-305, 1978. 77. Sato, S., Regulation of food-related carcinogens in Japan. Regul. Tox. Pharmacol., 11, 149-157, 1990. 78. Savage, R.K., An introduction to chromosomal aberrations, MRC Radiation and Genome Stability Unit, Harwell, Didcot, OX11 0RD, UK, 1999. 79. Savage, R.K., Micronuclei : pitfalls and problems, 34 city Road, Tilehurst, Reading, RG31 5HB, UK, 2000. 80. Schmid, W., Micronucleus test, Mutat. Res., 31, 9-15, 1975. 81. Sugimura, T., Sato, S., Nagao, M., Yahagi, T., Matsushima, T., Seino, Y., Takeuchi, M., Kawachi, T., Overlapping of carcinogens and mutagens, Fundamental of Cancer Prevention, University Park Press, Baltimore, 191-215, 1976. 82. Tamime, A.Y., Muir, D.D., Wszolek, M., Kefir and koumiss, Dairy Ind. Int.,64, 32-33, 1999. 83. The Collaborative Study Group for the Micronucleus Test (CSGMT), Evaluation of the rat micronucleus test with bone marrow and peripheral blood: summary of the ninth collaborative study by CSGMT/JEMS MMS,Environ. Mol. Mutagen., 32, 84-1001, 1998. 84. Toba, T., Abe, S., Adachi, S., Modification of KPL medium for polysaccharide production by Lactobacillus sp. isolated from kefir grain, Jpn. J.Zootechnol. Sci., 58, 987 – 990, 1987. 85. Toba, T., Abe, S., Arihara, K., Adachi, S., A medium for the isolation of capsular bacteria from kefir grains, Agric. Biol. Chem., 50, 2673-2674, 1986. 86. Tolbert, P.E., Shy, C.M., Allen, J.W., Micronuclei and other nuclear anomalies in buccal smear: a field test in snuff users, J. Epidemiology, 134,840-850, 1991. 87. Tolbert, P.E., Shy, C.M., Allen, J.W., Micronuclei and other nuclear anomalies in buccal smears: methods development, Mutat. Res., 271(1), 69-77, 1992. 88. Torous, D.K., Dertinger, S.D., Hall, N.E., Tometsko, C.R., Enumeration of micronucleated reticulocytes in rat peripheral blood: a flow cytometric study, Mutat. Res., 465(1-2), 91-99, 2000. 89. Viljoen, B.C., The interaction between yeasts and bacteria in dairy environments, J. Food Microbiol., 69, 37-44, 2001. 90. Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H., Tennant, R.W.,Evaluation of four in vitro genetic toxicity tests for predicting rodent carcinogenicity: confirmation of earlier results with 41 additional chemicals,Environ. Mol. Mutagen., 16, 1-14, 1990. 91. Zeiger E., Milman H.A., Weisburger E.K., The Salmonella mutagenicity assay for identification of presumptive carcinogens, Handbook of Carcinogen Testing, Noyes Publishers, Park Ridge, 83-99, 1985. 92. Zoryana C., Azeddine E., Willi S., In vivo micronucleus test with flow cytometry after acute and chronic exposures of rats to chemicals, Mutat.Res., 626, 26-33, 2007.