

REFERENCES

- Adamson, R.H., *et al.* Carcinogenicity of 2-amino-3-methylimidazo [4, 5-f] quinoline in nonhuman primates: induction of tumors in three macaques. Jpn J Cancer Res 81(1990): 10-14.
- Adzu, B. *et al.* The efficacy of *Sphaeranthus senegalensis* Vaill extract against diarrhea in rats. J Ethnopharmacol 95(2-3)(2004): 173-176.
- Adzu, B. *et al.* Anti-inflammatory and anti-nociceptive effects of *Sphaeranthus senegalensis*. J Ethnopharmacol. 84(2-3)(2003): 169-173.
- Ames, B.N., Lee, F.D., and Durston, W.E. An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc of Natl Acad Sci 70(1973a): 782-786.
- Ames, B.N. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacterial for detection. Proc Natl Acad Sci 70(1973b): 2281-2285.
- Ames, G.F., Biek, D.P. and Spudich, E.N. Duplications of histidine transport genes in *Salmonella typhimurium* and their use for the selection of deletion mutants. J Bacteriol. 136(3)(1978): 1094-1108.
- Amos, S., *et al.* Neuropharmacological effect of the aqueous extract of *Sphaeranthus senegalensis* in mice. J Ethnopharmacol. 78(1) (2001): 33-37.
- Ball, L.M., *et al.* Formation of mutagenic urinary metabolites from 1-nitropyrene in germ free and conventional rats role of the gut flora. Carcinogenesis 12(1991): 1-5.
- Barale, R., *et al.* Vegetable inhibit, in vivo, the mutagenicity of nitrite nitrosable compounds. Mut Res 120(1983): 145-150.
- Bhide, S.V., *et al.* Chemoprevention of mammary tumor virus-induced and chemical carcinogen-induced rodent mammary tumor by natural plant products. Breast Can Res Treat 30(1994): 233-242.
- Binkerd, E.F., and Kolari, O.E. The history and use of nitrate and nitrite in the curing of meat. Food Cosmet Toxicol 13(6)(1975): 655-661.

- Birosova, L., Mikulasova, M., and Vaverkova, S. Antimutagenic effect of phenolic acids. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 149(2)(2005): 489-491.
- Boone C.W., Kelloff G.J., and Malone W.E. Identification of candidate cancer chemopreventive agents and their evaluation in animal models and human clinical trials: a review. Cancer Res 50(1990): 2-9.
- Brusick, D. Prin and methods of toxicol. New York: Raven Press, 1982.
- Buening, M.K., et al. Activation and inhibition of benzo[α]pyrene and aflatoxin B1 metabolism in human liver microsomes by naturally occurring flavonoids. Cancer Res 41(1981): 67-72.
- Busby, W.F., Jr., Penman, B.W., and Crespi, C.L. Human cell mutagenicity of mono- and dinitropyrenes in metabolically competent MCL-5 cells. Mutat Res 322(4)(1994): 233-242.
- Calomme, M. et al. Inhibition of bacterial mutagenesis by Citrus Flavonoids. Planta Med 62(1996): 222-226.
- Cammack, R. et al. Nitrite and nitrosyl compounds in food preservation. Biochimica et Biophysica Acta 1411(1999): 475-488.
- Cantor, K.P. Drinking water and cancer. Cancer Causes Control 8(1997): 292-308.
- Choi, N.W., et al. Consumption of precursors of N-nitroso compounds and human gastric cancer. IARC Sci Publ 84 (1987): 492-6.
- Chansakaow, S., Ruangrunsi, N., and Ishikawa, T.. Isolation of pyropheophorbide a from the leaves of *Atalantia monophylla* (ROXB.) CORR. (Rutaceae) as a possible antiviral active principle against herpes simplex virus type 2. Chem Pharm Bull. 44(7)(1996):1415-1417.
- Chaurasia, S.C., and Jain P.C. Antibacterial activity of essential oils of four medicinal plants. Ind J Hosp Pharm 15(1978): 166-168.
- Chiampanichayakul, S. et al. Inhibitory effects of bitter melon (*Momordica charantia* Linn.) on bacterial mutagenesis and aberrant crypt focus formation in the rat colon. J Med Invest. 48(1-2)(2001): 88-96.
- Commoner, B. et al. Formation of mutagens in beef and beef extract during cooking. Science 41(1978): 3685-3689.

- Cornforth, D.P. Nitric Oxide: Principles and actions. San Diego: Academic Press, 1996.
- Correa, P. et al. Diet and gastric cancer: nutrition survey in high risk area. J Natl Cancer Ins. 70(1983): 673.
- Das, T. et al. Modification of clastogenicity of 3 known clastogens by garlic extract in ice in vivo. Environ Molecular Mutagen 21(1993): 383-388.
- De Flora, S., and Ramel C. Mechanisms of inhibitors of mutagenesis and carcinogenesis. Classification and overview. Mutat Res 202(1988): 285-306.
- De Flora, S. Mechanisms of inhibitors of mutagenesis and carcinogenesis. Mutat Res 402(1998): 151-158.
- De Meester, C. Bacterial mutagenicity of heterocyclic amines found in heat-processed food. Mutat Res. 221(3)(1989): 235-262.
- De Mejia, E.G., Quintanar-Hernandez, A.Q., and Loarca-Pina, G. Antimutagenic activity of carotenoids in green peppers against some nitroarenes. Mutat Res 416(1998): 11-19.
- De Serres, F.J., and Shelby, M.D. Recommendations on data production and analysis using the Salmonella/microsome mutagenicity assay. Mutat Res 64(1979): 159-165.
- Delaquis, P.J., et al. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Inter J Food Microb 74(2002): 101-109.
- Della Greca, M., et al. Cytotoxic 9, 10-Dihydrophenanthrenes from *Juncus effuses* L. Tetrahedron. 49(1993): 3425.
- Della Greca, M., et al. Oleanane Glycosides from *Hydrocotyle ranunculoides*. Phytochemistry. 36(1994): 1479.
- Djuric, Z. In vivo and in vitro formation of glutathione conjugates from the K-region epoxides of 1-nitropyrene. Carcinogenesis 8 (1987): 1781-1787.
- Doll, R., and Peto, R. The cause of cancer: quantitative estimates avoidable risks of cancer in the United States today. J Natl Cancer Ins 66(1981): 1191-1308.
- Duke AJ. Handbook of Medical Herbs. London: CRC Press, 2001.

- Dunkel, V.C., *et al.* Reproducibility of microbial mutagenicity assays 1. Tests with *Salmonella typhimurium* and *Escherichia coli* using a standard protocol. Environ Mutagen suppl2(1984): 1-251.
- Edenharder, R., *et al.* Antimutagenic effects of flavonoids, chalcones and structurally related compounds on the activity of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and other heterocyclic amine mutagens from cooked food. Mutat Res 287(2)(1993): 261-274.
- Edenharder, R., Leopold, C., and Kries, M. Modifying action of solvent extracts from fruit and vegetable residues on 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-f]quinoxaline (MeIQX) induced mutagenesis in *Salmonella typhimurium* TA 98. Mutat Res 341(4)(1995): 303-318.
- Edenharder, R., Rauscher, R. and Platt, K.L. The inhibition by flavonoids of 2-amino-3-methyl-imidazo[4,5-f]quinoline metabolic activation to a mutagen: a structure activity relationship study. Mutat Res 379(1997): 21-32.
- Edenharder, R., and Tang, X. Inhibition of the Mutagenicity of 2-nitrofluorene, 3-nitrofluoranthene and 1-nitropyrene by flavonoids, coumarins, quinines and other phenolic compounds. Food Chem Toxicol 35(1997): 357-372.
- El-Bayoumy, K. *et al.* Comparative tumor initiating activity on mouse skin of 6-nitrobenzo a pyrene, 6-nitrochrysene, 3-nitroperylene, 1-nitropyrene and their parent hydrocarbons. Cancer Letter 16(1982): 333-337.
- El-Bayoumy, K. *et al.* The role of intestinal microflora in the metabolic reduction of 1-nitropyrene to 1-aminopyrene in conventional and germ free rats and in humans. Cancer Letter 19(1983): 311-316.
- Espinosa-Aguirre, J.J. *et al.* Mutagenic active of urban air sample and its modulation chili extracts. Mutat Res 303(1993): 55-61.
- Felton, J.S., *et al.* Mutagens from the cooking of food, I. Improved extraction and characterization of mutagenic fractions from cooked ground beef. Mutat Res 88(1981): 33-44.
- Felton, J.S. *et al.* Health risks of heterocyclic amines. Mutat Res 376(1-2) (1997): 37-41.

- Ferguson, L.R. Role of plant polyphenols in genomic stability. Mutat Res. 475(2001): 89-111.
- Ferguson, L.R. Meat consumption, cancer risk and population groups within New Zealand. Mutat Res 506-7(2002a): 215-224.
- Ferguson, L.R. Natural and human-made mutagens and carcinogens in the human diet. Toxicology 181-182(2002b): 79-82.
- Fine, D.H. *et al.* Endogenous synthesis of volatile nitrosamines: Model calculations and risk assessment. USA: IARC Science publisher, 1976.
- Fleming, T. PDR or Herbal medicine. New Jersey: Medical Economics Company, 2000.
- Forman, D. Are nitrates a significant risk factor in human cancer? Cancer Survey 8(1989): 443-458.
- Gabriele, V., Hiller, K. and Franke, P. On the Structure of Flavonoids from *Hydrocotyle vulgaris* L. Phamazie. 36(1981): 377-379.
- Gelderblom, W.C. *et al.* Cancer initiation by fumonisin B(1) in rat liver-role of cell proliferation. Cancer Lett 169(2001): 127-137.
- Gong, Z.F. *et al.* Survey of chemical constituents and bioactivity of Polygonum L.plants. Chinese Traditional and Herbal Drugs 33(2002): 82-84.
- Graham, S. *et al.* Diet in the epidemiology of gastric cancer. Nutr Cancer 13 (1990): 19-34.
- Gross, G.A. Simple methods for quantifying mutagenic heterocyclic aromatic amines in food products. Carcinogenesis 11(1990): 1597-1603.
- Guenther, E. The Essential Oils. volume 4. New Jersey: D. Van Nostrand company, 1950.
- Gutierrez, R.M.P. Identification of triterpenoids in chloroform extract of *Agarista mexicana* by MS and NMR. Nat Prod Res 20(2)(2006):181-185.
- Handa, T. *et al.* Detection and average content levels of carcinogenic and mutagenic compounds from the particulates on diesel and gasoline engine mufflers. Environment Intitution 9 (1983): 335-341.
- Hankimhun, P. Mutagenic potential of raw and pickled fruits and vegetables treated with nitrite. Master's Thesis, Nutrition, Faculty of Science, Mahidol University, 1997.

- Hargraves, W.A., and Pariza M.W. Purification and mass spectral characterization of bacterial mutagens from commercial beef extract. Cancer Res 43(1983): 1467-1472.
- Hartman, E., and Shankel, D.M. Antimutagens and anticarcinogens: a survey of putative interception molecules. Environ Molecular Mutagen 15(1990): 145-182.
- Hartman, P.E. *et al.* Target sequences for mutagenesis in *Salmonella* histidine-requiring mutants. Environ Molecular Mutagen 8(1986): 631-641.
- Hartman, P.E., Hartman, Z., and Stahl, R.C. Clasification and mapping of spontaneous and induced mutations in the histidine operon of *Salmonella*. Advance Genetic 16(1971): 1-34.
- Hayatsu, H., Arimoto, S., and Neigishi, T. Dietary inhibitors of mutagenesis and carcinogenesis. Mutat Res 202(1988): 429-446.
- Hayatsu, H., and Hayatsu, T. Mutagenicity arising from boiled rice on treatment with nitrous acid. Jap J Cancer Res 80(1989): 1201-1203.
- Heisler, E.C. *et al.* Nitrate and nitrite content of market potatoes. J Agric Food Chem 21(1973): 970-973.
- Herman, A. *et al.* DNA adducts of heterocyclic amine food mutagens: implications for mutagenesis and carcinogenesis. Carcinogenesis 20(3) (1999): 353-368.
- Hoeven, V.D. *et al.* Intercultivar difference with respect to mutagenicity of fava beans (*Vicia faba* L.) after incubation with nitrite. Mutat Res 130(1984): 391
- Howard, P.C., Reed, D.A., and Koop, D.R. Oxidative metabolism of 1-nitropyrene by rabbit liver microsomes and purified microsomal cytochrome P450 isozymes. Cancer Res 48 (1988): 4261-4265.
- Howard, P.C.; Beland, F.A., and Cerniglia, C.E. Redution of the caecinogen 1-Nitropyrene to 1-aminopyrene by rat intestinal bacteria. Carcinogenesis 4(1983): 985-990.
- Huang, M.T., Ferraro, T., and Ho, C.T. Cancer Chemoprevention by Phytochemicals in Fruits and Vegetable. In: C.T. Ho, T. Osawa, N.T. Huang, and R.T. Rosen (ed.). Food Phytochemicals I: Fruits and Vegetables. 1-16. Washibgton DC: The American Chemical Society, 1994.

- Idaomar, M., *et al.* Genotoxicity and antigenotoxicity of some essential oils evaluated by wing spot test of *Drosophila melanogaster*. Mutat Res 513(2002):61-68.
- International agency for research on cancer (IARC). 1-Nitropyrene. IARC Monogr. Eval. Carcinog. Risk Chem. Hum 46 (1989): 321-358.
- Intiyot, Y., *et al.* Antimutagenicity of *Murdannia loriformis* in the *Salmonella* mutation assay and its inhibitory effects on azoxymethane-induced DNA methylation and aberrant crypt focus formation in male F344 rats. J Med Invest. 49(1-2)(2002): 25-34.
- Isono, K., Yourno, J. Chemical carcinogens as frameshift mutagens; *Salmonella* DNA sequence sensitive to mutagenesis by polycyclic carcinogens. Proc Natl Acad Sci 71(1974): 1612-1617.
- Jain, A., and Basal, E. Inhibition of *Propionibacterium acnes*-induced mediators of inflammation by Indian herbs. Phytomedicine 10(1)(2003): 34-38.
- Kada, T., Inoue T., and Namiki, M. Environment desmutagen and antimutagens In: Klewowski E Jr, (ed.). Environment mutagenesis, carcinogenesis and plant biology. 133-151. New York: Praeger, 1982.
- Kamchan, A., *et al.* In vitro calcium bioavailability of vegetables, legumes, and seeds. J Food Comp Anal 17(2004): 311-320.
- Kangsadalampai, K., Butryee, C., and Manoonphol, K. Direct mutagenicity of the polycyclic aromatic hydrocarbon-containing fraction of smoked and charcoal-broiled foods treated with nitrite in acid solution. Food Chem Toxicol 35(1996): 213-218.
- Kangsadalampai, K. and Suharitamrong, S. Food additive-drug interaction induced mutagens and possible prevention. Thai J Pharmacol Sci 20(2)(1996): 107-117.
- Kangsadalampai, K., and Butryee, C. Mutagenicity of some food color interacted with nitrite in acid solution. Thai J Cancer 21(1995): 64-73.
- Kangsadalampai, K., and Peerawong, K. Mutagenic potential of various chicken extracts sold in Thailand after treatment with excess nitrite in the acid condition. MD. Thesis in Nutrition, Bangkok: Faculty of Graduate Studies, Mahidol University, 1997.
- Kapil, V., Sinha, A.K., and Sinha, G.K., Bull Med Ethnobot Res. 4(3-4) (1983):124.

- Karakaya, S., and Kavas, A. Antimutagenic activities of some foods J Science Food and Agri. 79(2)(1999): 237 – 242.
- Kataoka, K., Kinouchi, T., and Ohnishi, Y. Species differences in metabolic activation and inactivation of 1-nitropyrene in the liver. Cancer Res 51 (1991): 3939-3942.
- Katipagduethum, H., Effect of nitrite on mutagenicity of health foods using the Ames test. MD. Thesis in Nutrition, Bangkok: Faculty of Graduate Studies, Mahidol University, 1996
- Kato, T., *et al.* Transformation of arylamines into the direct-acting mutagens by reaction with nitrite. Mutat Res 249 (1991): 243-254.
- Kaur, S., Grover, I.S., and Kumar, S. Antimutagenic potential of extracts isolated from *Terminalia arjuna*. J Environ Pathol Toxicol Oncol. 20(1)(2001):9-14.
- Kaur, K., *et al.* Modulatory effect of phenolic fractions of *Terminalia arjuna* on the mutagenicity in Ames assay. J Environ Pathol Toxicol Oncol. 21(1)(2002): 45-56.
- Khight, T.M., *et al.* Estimation of dietary intake of nitrate and nitrite in Great Britain. Food Chem Toxicol 25(1987): 277-286.
- Kikugawa, K., and Nagao, M. Nitrostable precursors of mutagens in food. In H. Hayatsu (ed.), Mutagen in food : Detection and Preemption. 68-81. Boston: CRC Press, 1990.
- Kinouchi, T., Tsutsui, H., and Ohnishi, Y. Detection of 1-nitropyrene in yakitori grilled chicken. Mutat Res 171 (1986): 105-113.
- Knize, M.G., *et al.* Effect of temperature, patty thickness and fat content on the production of mutagens in fried ground beef. Food Chem Toxicol 23(1985): 1035-1040.
- Knize, M.G., Shen, N.H., and Felton, J.S. A comparison of mutagen production in fried ground chicken and beef: effect of supplemental creatine. Mutagenesis 3(1988): 503-508.
- Kothari, S.K., Bhattacharya, A.K. and Ramesh, S. Essential oil yield and quality of methyl eugenol rich *Ocimum tenuiflorum* L.f. (syn. *O.sanctum* L.) grown in south India as influenced by method of harvest. J Chromatogr A 1054(2004):67-72.
- Kozawa, M., Chenz. Pharnz. Bull. 26(1976), 119

- Krishnan, S., Nair, A., and Ramachandran, G., Ind. J. Chem 38B (1999): 1009.
- Kumar, R.A., *et al.* Anticancer and immunostimulatory compounds from *Andrographis paniculata*. J Ethnopharmacol 92(2-3)(2004): 291-295.
- Kuo, S.M., Lee, K.C. and Lin, L.K. Genotoxicities of nitropyrene and their modulation by apigenin, tannic acid, ellagic acid and indole-3-carbinol in *Salmonella* and CHO systems. Mutat Res 270(1992): 87-95.
- Kusamran, W.R., Tepsuwan, A., and Kupradinum, P. Antimutagenic and anticarcinogenic potentials of some Thai vegetables. Mutat Res. 402(1998): 247-258.
- Kwon, H. C., Zee, O. P. and Lee, K. R. Two New Monogalactosylacylglycerols from *Hydrocotyle ramiflora*. Planta Med. 64(1998): 477.
- Lahiri, M., Maru, G.B., and Bhide, S.V. Effect of plant phrnols, beta-carotene and α -tocopherol on benzo[α]pyrene-induced DNA damage in the mouse forestomach mucosa (target organ) and bone marrow polychromatic erythrocytes (non-target). Mutat Res 303(1993): 97-100.
- Lai, C., Dabney, B.J., and Shaw, C.R. Inhibition of in vitro metabolic activation of carcinogens by wheat sprout extrats. Nutr Cancer 1(3)(1978): 27-30.
- Lai, J.K., Cheng, J.T., and Lin-Shiau S.Y. Enhancement of the mutagenicity of IQ and MeIQ by nitrite in the Salmonella system. Mutat Res 278(4)(1992): 277-287.
- Lasker, J.M., Huang, M.T., and Conney, A.H. In vitro and in vivo activation of oxidative drug metabolism by flavonoids. J. Pharmacol Exp Ther 229(1984): 162-170.
- Layton, D.W. *et al.*, Cancer risk of heterocyclic amines in cooked foods: an analysis and implications for research, Carcinogenesis 16(1995): 39-52.
- Lazutka, J.R. *et al.*, Genotoxicity of dill (*Anethum graveolens* L.), peppermint (*Menthapiperita* L.) and pine (*Pinus sylvestris* L.) essential oils in human lymphocytes and *Drosophila melanogaster*., Food Chem Toxicol 39(5)(2001): 485-492.
- Lee, H., *et al.* Interaction of pine cone extract fraction VI with mutagens. Mutat Res 297(1)(1993): 53-60.

- Levin, D.E., Yamasaki, E., and Ames B.N. A new *Salmonella* tester strain TA97 for the detection of frameshift mutagens: A run of cytosines as a mutational hotspot. Mutat Res 94(1982a): 315-330.
- Levin, D.E., *et al.* A new *Salmonella* tester strain (TA102) with AXT base-pairs at the site of mutation detects oxidative mutagens. Proc Natl Acad Sci USA 79(1982b): 7445-7449.
- Lin, D., *et al.* Identification of *N*-[deoxyguanosine-8-yl]-2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine as the major adduct formed by the food-borne carcinogen. 2-amino-1-methyl-6- phenylimidazo[4,5-*b*]pyridine. with DNA. Chem Res Toxicol 5(1992): 691-697.
- Llanes, P.B.G., and Tannenbaum, S.R. Formation of an activated N-nitroso compound in nitrite treated fava beans (*Vicia faba* L. Carcinogenesis. 3(1982): 1379-1984.
- Lynnette, R.F. Natural and man-made mutagens and carcinogens in the human diet. Mutat Res 113(1999): 173-215.
- Macphee D.G. Effect of a R-factor and caffeine on ultraviolet metabolite in *Salmonella typhimurium*. Mutat Res 18(1973b): 367-70.
- Macphee, D.G. *Salmonella typhimurium* hisG46 (R-Utrecht): Possible use in screening mutagens and carcinogens. Appl Environ Microbiol 25(1973a):1004-1005.
- Maron, D.M., and Ames, B.N. Revised methods for the *Salmonella* mutagenicity test. Mutat Res 113(1983): 173-215.
- Marquard, H., Rufino, F., and Weisburger, J. H. Mutagen activity of nitrite-treated food; Human stomach cancer may be related dietary factors. Science 196(1977): 1000-1001.
- Matsushima, T., *et al.* Factors modulating mutagenicity in microbial tests. In: K.H. Norpoth, R.C. Garner (ed.). Short-Term Test System for Detecting Carcinogens. 273-285. Berlin: Springer, 1980.
- McCann, J., and Ames, B.N. The *Salmonella*/microsome mutagenicity test: Predictive value for animal carcinogenicity. In: H.H. Hiatt, J.D. Weston, J.A. Winsten (ed.). Origin of Human Cancer. 1431-1450 New York: Cold Spring Harbor Laboratory, 1977.

- McCann, J., *et al.* Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Natl Acad Sci USA 72(1975): 5135-5139.
- Medjroubi, K., Benayache, F., and Bermejo, J. Sesquiterpene lactones from *Centaurea musimomum*. Antiplasmodial and cytotoxic activities. Fitoterapia. 76(7-8) (2005):744-746.
- Mesmelstein, R. *et al.* The extraordinary mutagenicity of nitropyrenes in bacteria. Mutat Res. 89(1981): 187-196.
- Miller, E.C., and Miller, J.A. Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules. Cancer. 47(1981): 2327-2345.
- Minnumi, M., *et al.* Natural antioxidants as inhibitors of oxygen species induced mutagenicity. Mutat Res 269(1992): 193-200.
- Mirvish, S.S. The etiology of gastric cancer. Intra-gastric nitrosamide formation and other theories. J Natl Cancer Inst. 71(3)(1983): 629-647.
- Miyazawa, M., Sakano, S., Nakamura, and Kosaka, H. Antimutagenic activity of isoflavones from soybean seeds (*Glycine max* Merrill). J Agric Food Chem 47(1999): 1346-1349.
- Miyazawa, M., and Kohno, G. Suppression of chemical mutagen-induced SOS response by allylbenzenes from *Asiasarum heterotropoides* in the *Salmonella typhimurium* TA1535/PSK1002 umu test. Nat Prod Res. 19(1)(2005): 29-36.
- Moller, L., *et al.* The role of the intestinal microflora in the formation of mutagenic metabolites from the carcinogenic air pollutant 2-nitrofluorene. Carcinogenesis. 9(1988): 823-830.
- Mortelmas, K.E., and Stocker, B.A. Segregation of the mutator property of plasmid R46 from its ultraviolet-protecting property. Mol Gen Genet 167(1979): 317-337.
- Mukundan, M.A., *et al.* Effect of turmeric and curcumin on Bp-DNA adducts. Carcinogenesis 14(1993): 493-496.
- Munro, I.C. *et al.* Safety assessment of ingested heterocyclic amines: initial report. Regul Toxicol Pharmacol 17(1993): S1-S109.
- Munzner, R., and Wever, J. Mutagenic activity in stomach and small intestine of rats after oral administration of nitrosated beef extract. Cancer Lett 25(1984): 225.

- Munzner, R. Modifying action of vegetable juice on the mutagenicity of beef extract and nitrosated beef extract. Food Chem Toxicol 24(8)(1986): 847-849.
- Murakami, A., *et al.* Screening for in vitro anti-tumor promoting activities of edible plants from Thailand. Cancer Lett. 16:95(1-2)(1995):139-146.
- Murata, M., Kobayashi, M., and Kawanishi, S. Nonenzymatic Reduction of Nitro Derivative of a Heterocyclic Amine IQ by NADH and Cu(II) Leads to Oxidative DNA Damage. Biochemistry. 38(1999): 7624-7629.
- Mutsushima, T. *et al.* Factors modulating mutagenicity in microbial tests. In: Norpoth KH, Garner RC, editors. Short-Term test systems for detecting Carcinogens pp 273-85. Berling: Springer. 1980.
- Nagao, M. *et al.*, Dietary carcinogens and mammary carcinogenesis: induction of rat mammary carcinomas by administration of heterocyclic amines in cooked food, Cancer 74(Suppl3) (1994): 1063-1069.
- Nagao, M. Mutagenicity. In: Nagao, M., and Sugimura, T. editors. Food borne carcinogens: Heterocyclic amines. 163-95. Chichester: John Wiley and Sons Ltd; 2000.
- Nakahara, K., and Trakoontivakorn, G. Antioxidant and antimutagenic properties of some edible Thai plants. JIRCAS Newsletter 17(1998).
- Nakahara, K., *et al.* Inventory of indigenous plants and minor crops in Thailand based on bioactivities. 9th JIRCAS International Symposium (2002): 135–139.
- Nakamura, H. and Yamamoto, T. Mutagen and anti-mutagen in ginger, *Zingiber officinale*. Mutat Res 103(2)(1982): 119-126.
- Nakaoki, T. and Morita, N. Medicinal Resources. XVI. Flavonoids of the Leaves of *Castana pubinervis*, *Hydrocotyl wilfordi*, *Sanguisorba hakusanensis*, *Euptelea polyandra*, *Daucus carota* etc. Yakuoaku Zasshi. 80(1960): 1473.
- Namiki, M., *et al.* Chemical aspects of mutagen formation by sorbic acid-sodium nitrite reaction. J Agric Food Chem 29(2)(1981):407-411.
- National Cancer Institute of Thailand. Cancer Incidence and leading sites. Cancer in Thailand Vol.3 Bangkok, 2000.
- Niyazawa M., Kameoka H. Yukagaku. 1974. 23; 746-749.

- Norrish, A.E., *et al.* Heterocyclic amine content of cooked meat and risk of prostate cancer. J Natl Cancer Inst 91(1999): 2038-2044.
- Obaseiki-Ebor, E.E., *et al.* Antimutagenic activity of extracts of leaves of four common edible vegetable plants in Nigeria (west Africa). Mutat Res 302(2)(1993):109-117.
- Ohgaki, H., Takayama, S., and Sugimura, T. Carcinogenicities of heterocyclic amines in cooked food. Mutat Res 259(1991): 399-410.
- Ohshima, H. *et al.* Formation of direct acting genotoxic substances in nitrosate smoked fish and meat products: identification of simple phenolic precursors and phenyldiazonium ions as reactive products. Food Chem Toxicol. 27(1989):193.
- Okai, Y., Higashiokai, K., and Nakamura, S. Identification of heterogeneous antimutagenic activities in the extract of edible brown seaweeds, *Laminaria japonica* (Makonbu) and *Undaria pinnatifida* (Wakame) by the umu gene expression system in *Salmonella typhimurium* (TA 1535/pSK1002). Mutat Res 303(1993): 63-70.
- Palli, D. Epidemiology of gastric cancer. Ann Ist Super Sanita 32(1)(1996):85-99.
- Pasquini, R. *et al.* In vitro protective effects of *Terminalia arjuna* bark extracts against the 4-nitroquinoline-N-oxide genotoxicity. J Environ Pathol Toxicol Oncol. 21(1)(2002): 33-44.
- Peerawong, K., and Kangsadalampai K. Mutagenic potential of various chicken extracts after nitrosation. Mahidol J 5(1998): 5-8.
- Phillips, W.E.. Change in the nitrate and nitrite content of fresh and processed spinach during storage. J Agric Food Chem 16(1968): 88-91.
- Powrie, W., Wu, C., and Stich, H. Browning reaction systems as sources of mutagens and modulators. In Carcinogens and Mutagens in the Environment. 19(1982):121-133.
- Prachayasittikul, S. *et al.* Constituents and Antimicrobial Activity of *Spilanthes acmella* (Linn.) Murr. Biotec Report (2004).
- Prasad, S., Naik, P. and Vijayalaxmi, K.K. Efficiency of *Coleus aromaticus* extract in modifying cyclophosphamide and mitomycin-C induced clastogenicity in mouse bone marrow cells. Indian J Exp Biol. 40(9)(2002): 1020-1025.

- Prival, M.J., King, V.D., and Sheldon, A.T. Jr. The mutagenicity of dialkyl nitrosamines in the Salmonella plate assay. Environ Mutagen 1(1979): 95-104.
- Rahmat, A., *et al.* Determination of total antioxidant activity in three types of local vegetables shoots and the cytotoxic effect of their ethanolic extracts against different cancer cell lines. Asia Pac J Clin Nutr 12 (3)(2003): 308-311.
- Ramsewak, R.S., Erickson, A.J. and Nair, M.G. Bioactive *N*-isobutylamides from the flower buds of *Spilanthes acmella*. Phytochemistry 51(6)(1999): 729-732.
- Rasoamiaranjanahary, L. *et al.* Antifungal diterpenes from *Hypoestes serpens* (Acanthaceae). Phytochemistry 62(3)(2003):333-337.
- Robbana-Barnet, S. *et al.* Heterocyclic amines: Occurance and prevention in cooked food. Environ Health Prospect 104(1996):180-188.
- Rojanapo, W. and Tepsuwan, A. Antimutagenic and mutagenic potentials old Chinese radish. Environ Health Prospect 101(1993): S246-S252.
- Rosenkranz, H.S., and Mermelstein, R. Mutagenicity and geno-toxicity of nitroarenes: all nitro-containing chemicals were not create equal. Mutat Res 114(1983):217-267.
- Rosser, P.F., *et al.* Role of O-acetyltransferase in activation of oxidized metabolites of the genotoxic environmental pollutant 1-nitropyrene. Mutat Res. 369(1996):209-220.
- Sasagawa, C., Muramatsu, M., and Matsushima, T. Formation of mutagens from aminoimidazoazaarenes by nitrite treatment. Mutat Res 203(1988): 386
- Schut, H.A., and Snyderwine, E.G. DNA adducts of heterocyclic amine food mutagens: implications for mutagenesis and carcinogenesis. Carcinogenesis 20(3)(1999): 353-368.
- Schweinsberg, F., and Burkle, V. Nitrite: a co-carcinogen. J. Cancer Res clin Oncol 109(1985): 200-202.
- Seel, D.J., *et al.* N-nitroso compounds in two nitrosated food products in South West Korea. Food Chem Toxicol 32(1994): 1117-1123.
- Shapira, D.A., Goldschmidt, E.E., and Altman, A. Chlorophyll catabolism in senescing plant tissues: In vivo breakdown intermediates suggest different degradative pathways for citrus fruit and parsley leaves. PNAS 7(1987): 1901-1905.

- Shephard, S.E., Wakabayashi, K., and Nagao, M. Mutagenic activity of peptides and the artificial sweetener aspartame after nitrosation. Food Chem Toxicol 31(5)(1993): 323-329
- Shibamoto, T., Nishimura, D., and Mihara, S. Mutagenicity of products obtained from maltol-ascorbic acid browning model system. J Agric Food Chem 29(1981):643-346.
- Shigematsu, N., Kouno, I. and Kawano, N. Quercetin 3-O-(6''-caffeoyl)galactoside) from *Hydrocotyle sibthorpioides*. Phytochemistry. 21(1982): 2156.
- Shimada, T. *et al.* Human liver microsomal cytochrome P450 enzymes involved in the bioactivation of procarcinogens detected by umu gene response in *Salmonella typhimurium* TA1535 rpSK1002. Cancer Res 49(1989):3218-3228
- Skog, K. Cooking procedures and food mutagens: A Literature review. Food Chem Toxicol 31(9)1993: 655-675.
- Snyderwine, E.G., *et al.* Reaction of N-hydroxylamine and N-acetoxy derivatives of 2-amino-3-methylimidazo[4,5-f]quinoline with DNA. Synthesis and identification of N-(deoxyguanosin-8-yl)-IQ. Carcinogenesis 9(1988):1064-1065.
- Spiegelhader, B., Eisenbrand, G., and Preussmann, R. Influence of dietary nitrate on nitrite content of human saliva: Possible relevance to in vivo formation of N-nitroso compounds. Food Chem Toxicol 14(1976): 545-548.
- Srivastava, S.K. *et al.* A flavanone glycoside from *Diplazium esculentum* Phytochemistry 20(4)(1981): 862.
- Starkenmann, C., *et al.* Comparison of Volatile Constituents of *Persicaria odorata*(Lour.) Sojak (*Polygonum odoratum* Lour.) and *Persicaria hydropiper* L. Spach (*Polygonum hydropiper* L.).J Agric Food Chem 54(8)(2006):3067-3071.
- Stavric, B. Antimutagens and anticarcinogens in foods. Food Chem Toxic 32(1)(1994): 79-90.
- Sugimura, T., and Sato, S. Mutagens-carcinogens in foods. Cancer Res (suppl) 43(1983): 2415s-24): 2415s-2421s.
- Sugimura, T., Overview of carcinogenic heterocyclic amines, Mutat Res 376(1997): 211-219.
- Sugimura, T. Multistep carcinogenesis: a 1992 perspective. Science 258(1992):603-607.

- Sugimura, T. Nutrition and dietary carcinogens. Carcinogenesis 21(3)(2000):387-395.
- Suksamrarn, A., *et al.* Antimycobacterial and Antioxidant Flavones from *Limnophila geoffrayi*. Arch Pharm Res 26(10)(2003): 816-820.
- Takahashi, M. *et al.* Quantification of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline(MelQx) in beef extracts by liquid chromatography with electrochemical detection(LCEC). Carcinogenesis 6(1985):1195.
- Tanaka, T., *et al.* Chemoprevention of 4-nitroquinoline 1-oxide-induced oral carcinogenesis by dietary protocatechuic acid during initiation and postinitiation phases. Cancer Res 54(1994): 2359-2365.
- Tang, X., and Edenharder, R. Inhibition of the mutagenicity of 2-nitrofluorene, 3-nitrofluoranthene and 1-nitropyrene by vitamins, porphyrins, and related compounds, and vegetables and fruit juices and solvent extracts. Food Chem Toxicol. 35(1997): 373-378.
- Taungbodhitham, A. K. Thiamin content and activity of antithiamin factor in vegetables of southern Thailand. Food Chem 52(3)(1995): 285-288.
- Taylor, R.T., Fultz, E., and Knize, M.G. Mutagen formation in a model beef supernatant fraction, IV. Properties of the system. Environ Health Perspect. 67(1985):59-74.
- Terada, M., *et al.* Mutagenic activities of heterocyclic amines in Chinese hamster lung cells in culture. Environ Health Perspect 67(1986):117-119.
- Thompson, L.H., *et al.* Genotoxicity of compounds from cooked beef in repair-deficient CHO cells versus *Salmonella* mutagenicity. Mutagenesis 2(1987): 483-487.
- Tokiwa, H., Nakagawa, R., and Horikawa, K. Mutagenic/carcinogenic agents in indoor pollutant; the dinitropyrenes generated by kerosene heaters and fuel gas and liquefied petroleum gas burners. Mutat Res 39(1985):157.
- Tomita, I., *et al.* Mutagenic/carcinogenic potential of DEHP and MEHP. Environ Health Perspect 45(1982):119-125.
- Tsuda, M., *et al.* Use of nitrite and hypochlorite treatments in determination of the contributions of IQ-type heterocyclic amines to the mutagenicities in crude pyrolyzed materials. Mutat Res 147(1985): 335.




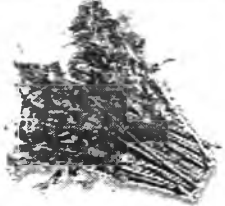

- Vachalkova, A., *et al.* Potential carcinogenic and inhibitory activity of compounds isolated from *Lilium candidum* L. Neoplasma 47(5)(2000): 313-318.
- Van de Braak, S.A.A.J., Leijten, G.C.J.J. Essential Oils and Oleoresins: A Survey in the Netherlands and other Major Markets in the European Union. CBI, Center for the Promotion of Imports from Developing Countries, Rotterdam, p.116, 1999.
- Vinitketkumnuen U., and Suaeyun R. Antimutagenicity of coconut tip extract. Chiang Mai Medical Bulletin 34(2)(1995): 63-66.
- Wakabayashi, K. Appearance of direct-acting mutagenicity of various foodstuffs produced in Japan and Southeast Asia on nitrite treatment. Mutat Res 158(1984): 119-124.
- Wakabayashi, K. Food-derived mutagens and carcinogens. Cancer Lett 52(Suppl)(1992): 2092S-2098S.
- Wargovich, M.J. New dietary anticarcinogens and prevention of gastrointestinal cancer. Dis Color Rectum 31(1988): 72-75.
- Wattenberg L.W. Inhibition of carcinogenesis by minor dietary constituents. Cancer Res 52(1992): 2085S-2091S.
- Wattenberg L.W. Inhibition of neoplasia by minor dietary constituents. Cancer Res 43(5)(1983): 2448S-2453S.
- Wattenberg LW. Anticarcinogens in the diet. In: Enwonwu CO, editor. Diet, Nutrition and Cancer. Meharry Medical college. 1991.
- Wattenberg, L.W. Chemoprevention of cancer. Cancer Res 45(1985): 1-8.
- Wattenberg, L.W. Inhibition of carcinogenesis by minor dietary constituents. Cancer Res 52(Suppl.)1992: 2085s-2091s.
- Wattenberg, L.W. Inhibition of carcinogenesis by naturally-occurring and synthetic compounds. In: Y. Kuraka, D.M. Shankel, M.D. Waters (ed.). Antimutagenesis and Anticarcinogenesis Mechanisms II. 155-166. New York:Plenum Press, 1990.
- Wei, C.I., Kitamura, K., and Shibamoto, T. Mutagenicity of Maillard browning products obtained from a starch-glycine model system. Food Chem Toxicol 19(1981):749-751.

- Weinstein I.B., Cancer prevention: recent progress and future opportunities. Cancer Res 51(18 Suppl.)(1991): 5080S-5085S.
- Weisburger, J.H., and Raineri, R. Dietary factors and the etiology of gastric cancer. Cancer Res 35(1975):3469-3474.
- Weisburger, J.H., Dolan, L., and Pittman, B. Inhibition of PhIP mutagenicity by caffeine, lycopene, daidzin, and genistein. Mutat Res 416(1998): 128-135.
- Wong, B.Y., Lau, B.H., and Teel, R.W. Chinese medicinal herbs modulate mutagenesis, DNA binding and metabolism of benzo[a]pyrene 7,8-dihydrodiol and benzo[a]pyrene 7,8-dihydrodiol-9,10-epoxide. Cancer Lett 29;62(2)(1992):123-131
- World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR), Food, Nutrition and the prevention of cancer: A Global Perspective. American Institute for Cancer Research, Washington, DC, 1997.
- Xu, G., Song, P. and Reed, P.I. The relationship between gastric mucosal changes and nitrite intake via drinking water in a high-risk population for gastric cancer in Moping Country, China. Eur J Cancer Prev 1(16)(1992): 437-443.
- Yahagi, T. Mutagenicity of carcinogenic azo dyes and their derivatives. Cancer Lett 1(1975): 91-96.
- Yahagi, T., Nagao, M., and Matsushima, T. Mutagenicities of N-nitrosamines on *Salmonella*. Mutat Res 48(1977): 121-129.
- Yano, M. Presence of nitrosable mutagen precursors in cooked meat and fish. Mutat Res 202(1988): 119-123.
- Yamanaka, H. Mutagenicities of pyrrolizidine alkaloids in the Salmonella/mammalian-microsome test. Mutat Res 68(1979): 211-216.
- Yang, C.M., *et al.* Herbivorous insect cause deficiency of pigment-protein complex in an oval-pointed cecidomyiid gall of *Mochilus thunbergii* leaf. Botan Bull Academia Sinica 44(2003): 316-321.
- Yano, M., *et al.* Presence of nitrosable mutagen precursors in cooked meat and fish. Mutat Res 202(1988):119-123.

- Yazdanparast, R., and Alavi, M. Antihyperlipidaemia and antihypercholesterolaemic effects of *Anethum graveolens* leaves after the removal of furocoumarins. Cytobios 105(2001):185-191.
- Yen, W.J., and Chen, B.H. Isolation of xanthophylls from Taiwanese orange peels and their effects on the oxidation stability of soybean oil. Food Chem 53(1995): 417-425.
- Yoshikawa, K., *et al.* Antimutagenic activity of extracts from Japanese eggplant. Mutat Res 371(1996): 65-71.
- Zaldivar, R. Geographic pathology of oral, esophageal, gastric and intestinal cancer in Chile. Z krebsforsch 75(1970): 1-3.

APPENDICES

APPENDIX A

Picture	Name	Scientific name	Part of Use
	Pak Chee Lao (Dill) ผักชีลาว	<i>Anethum graveolens</i> Linn	Aerial part
	Pak Ka Yang (ผักแขยง)	<i>Limnophila aromatica</i> Merr	Aerial part
	Pak Pai (ผักไผ่)	<i>Polygonum odoratum</i> Lour.	Aerial part
	Pak Gud (ผักกูด)	<i>Diplazium esculentum</i> (Retz.)	Aerial part
	Pak Krad Hua Wan (ผักคราดหัวแหวน)	<i>Spilanthes acmella</i> Murr .	Aerial part

APPENDIX B

1. Preparation of stock solution and Media (Maron and Ames, 1983)

1.1. Voget Bonner media E stock salt solution (VB salt)

Use : Minimal agar

Ingredient	Per liter
Warm distilled water (45 °C)	670 ml
Magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	10 g
Citric acid monohydrate	100 g
Potassium phosphate, dibasic (anhydrous) (K_2HPO_4)	500 g
Sodium ammonium phosphate ($\text{NaNH}_4\text{HPO}_4 \cdot 4\text{H}_2\text{O}$)	175 g

Add salts in the order indicated to warm water and allow each salt to dissolve completely before adding the next. Adjust the volume to 1 liter. Filter the solutions and then autoclave at 121 °C for 15 min.

1.2. Minimal glucose agar plate

Use : Mutagenicity assay

Ingredient	Per liter
Bacto agar	15 g
Distilled water	930 ml
VB salts	20 ml
40% glucose	50 ml

Add agar to distilled water in a glass bottle. Autoclave at 121 °C for 15 min. when the solution has cooled slightly, add sterile VB salts and sterile 40% glucose. Mix and pour 30 ml into each sterile petri plant. Minimal glucose agar plates were kept in incubator at 37 °C before using. (the VB salts and 40% glucose should be autoclaved separately)

1.3. Oxoid nutrient broth No.2

Use : Growing culture

Dissolve 2.5 g of nutrient broth No.2 in 100 ml distilled water. Transfer 12 ml nutrient broth for each 50 ml Erlenmeyer flask (covered with sterile gauze). Autoclave at 121°C for 15 min.

1.4. Top agar

Use : Mutagenic assay

Ingredient	100 ml
Bacto agar	0.6 g
Sodium chloride (NaCl)	0.5 g
Distilled water	100 ml

Dissolve ingredients in distilled water. Store in a glass bottle. Autoclave for 15 min. At 121 °C and then add 10 ml of 0.5 mM L-histidine/biotin solution and mixed thoroughly by swirling.

1.5. 0.1 M L-histidine HCl stock

Use : Fortification of minimal agar plate

Ingredient	10 ml
L-histidine HCl	0.2096 g
Distilled water	10 ml

Dissolve 0.2096 g of L-histidine HCl (MW 209.63) in 10 ml distilled water. Autoclave at 121 °C for 15 min. Store in a glass bottle 4 °C.

1.6. 1 mM L-histidine HCl stock

Use : Fortification of minimal agar plate.

Ingredient	100 ml
0.1 M L-histidine HCl	1 ml
Distilled water	99 ml

Dilute 1 ml of 0.1 M L-histidine HCl in 99 ml of distilled water. Autoclave at 121 °C for 15 min.

1.7. 1 mM biotin stock

Use : Fortification of minimal agar plate.

Ingredient	100 ml
Biotin	24.43 mg
Distilled water	100 ml

Dissolve biotin (WM 244.3) in distilled H₂O. Warm it until dissolve completely.

Autoclave at 121 °C for 15 min.

1.8. 0.5 mM L-histidine / biotin solution.

Use : Mutagenicity assay (add 10 ml to 100 ml of Top agar)

Ingredient	200 ml
1 mM L-histidine HCl	100 ml
1 mM biotin	100 ml

Mix and autoclave at 121 °C for 15 min.

1.9. 1 M potassium chloride

Use : Na₃PO₄-KCl buffer for mutagenicity assay

Ingredient	100 ml
Potassium chloride	7.456 g
Distilled water	100 ml

Mix and autoclave at 121 °C for 15 min.

1.10. 0.5 M sodium phosphate pH 7.4

Use : Na₃PO₄ – KCl buffer for mutagenicity assay

Ingredient

0.5 M Sodium dihydrogen phosphate (NaH₂ PO₄) (MW 120)

(30 g / 500 ml)

0.5 M Disodium hydrogen phosphate dehydrate (NaH₂ PO₄ .2 H₂O) (MW 177.99)

(44.5 g / 500 ml)

Dissolve 44.5 g disodium hydrogen phosphate dehydrate in 300 ml of distilled water. Add 0.5 M sodium dihydrogen phosphate until to pH 7.4, and then adjust volume to 500 ml. Sterilize by autoclaving for 15 min at 121 °C

1.11. Na_3PO_4 – KCl Buffer

Use : mutagenicity assay

Ingredient	330 ml
0.5 M Sodium Phosphate (Na_3PO_4) pH 7.4	100 ml
1 M Potassium Chloride (KCl)	16.5 ml
Distilled water	213.5 ml
Autoclave for 15 min at 121 °C	

2. Recipes for Some Reagents and Test Chemicals

2.1. 2 M sodium nitrite

Use : Nitrosation

Ingredient	10 ml
Sodium nitrite	1.38 g
Distilled water to	10 ml
Mix and autoclave for 15 min at 121 °C	

2.2. 2 M ammonium sulfamate

Use : reaction mixture

Ingredient	10 ml
Ammonium sulfamate	2.28 g
Distilled water to	10 ml

Dissolve ammonium sulfamate in distilled water and adjust volume. Autoclave for 15 min at 121 °C

2.3. 0.2 N Hydrochloric acid

Use : reaction mixture

Ingredient	100 ml
Conc. Hydrochloric acid	1.66 ml
Sterile distilled water	98.34 ml

Dissolve conc.hydrochloric acid in sterile water. Store in sterile glass tubes or bottle with screw caps.

2.4. 0.3 mg/ml aminopyrene

Use : standard solution for mutagenicity assay

Ingredient	1 ml
Aminopyrene	3 mg
Acetonitrile	1 ml

Dissolve aminopyrene in acetonitrile. Store in sterile vial with screw caps in the freezer. Preparation of this solution must use sterile technique.

Ingredient	1 ml
3 mg/ml aminopyrene	0.1 ml
Acetonitrile	0.9 ml

Dissolve 3 mg/ml aminopyrene in acetonitrile. Store in sterile vial with screw cap in freezer. There preparation must be used sterilr technique.

2.5. 8 mg/ml ampicillin solution

Ingredient	10 ml
Ampicillin sodium	80 mg
Distilled water	10 ml

2.6. 0.1% crystal violet

Ingredient	10 ml
Crystal violet	10 mg
Distilled water to	10 ml

2.5 And 2.6: Store at 4 C in glass bottle with screw cap.

3. Procedure for Reisolation and Growing Culture

Tester strains, TA 98 and TA 100 are grown in Oxoid nutrient broth No.2 and incubated overnight in a 37 °C shaking water bath . The growth period should not exceeded 16 h. these culture are re-isolated by streaking on minimal glucose agar plates which the surface were spread with 0.1 ml of 8 mg/ml ampicillin, 0.3 ml of 0.1 M histidine HCl and 0.1of 1 mM biotin. There plates are incubated at 37 °C for 48 h. After incubation, the 4 single colonies per strain TA 98 and TA 100 are pick and grown in Oxiod nutrient broth No.2 overnight at 37 °C in shaking water bath. Each culture is confirmed genotype of the strains and kept the cultures as the source of bacteria for mutagenicity testing. For each 1.0 ml of culture, add 0.09 ml of spectrophotometric grade DMSO. Combine the culture and DMSO in a sterile tub and distribute 40 µl of the culture aseptically into sterile cryotubes (Nunc). The tubes then transfer to a -80 °C freezer.

4. Confirming Genotype of Tester strains

The broth cultures of TA 98 and TA 100 are used to confirm genotype in the following ways.

4.1 Histidine requirement

The his⁻ character of the strains confirmed by demonstrating the histidine requirement for growth on the minimal glucose agar plates enriched with histidine and biotin.

Procedure :

Plate A : no histidine and biotin

Plate B : 0.1 ml of 1 mM biotin

Plate C : 0.3 ml of 0.1 M His-HCl

Plate D : 0.3 ml of 0.1 M His-HCl + 0.1 ml of 1 mM biotin

Four minimal glucose agar plates are required for each tester strains. Each plate is applied on the surface with 0.1 ml of 1 mM biotin or no application (plate b,c,d,a, respectively) Made a single streak of each strains across these plates. Four strains

could be tested on the same plate Incubated at 37 °C for 24 h. The growing of bacteria on histidine plus biotin plate is the result of histidine requirement.

4.2 R-factor

The R-factor strains (TA 97, TA 98, TA 100 and TA 102) should be tested routinely for the presence of the ampicillin resistance factor because the plasmid is somewhat unstable and can be lost from the bacteria.

Procedure: For each tester strain, add 0.3 ml of fresh overnight culture to a tube containing 0.1 ml of 0.1 M histidine – HCl followed by adding 20 ml of molten top agar containing 0.5 mM, mixed and poured on a minimal glucose agar plate. Rotated the plate to distribute the mixtures and allowed several minutes for agar to become firm. R-factor and *rfa* mutation (see the next section) are performed in the same plate by dividing the plate into 2 areas, one for R-factor and the other for *rfa* mutation. For R-factor, commercial ampicillin disc or filter paper disc containing 8 mg/ml ampicillin is applied on the surface of the agar by using sterile forceps. The disc is pressed lightly to embed in the overlay. The plates are incubated at 37 °C for 24 h. The absence of the clear zones of inhibition around the disc indicates resistance to ampicillin.

4.3 Rfa Mutation

Strains having the deep rough (*rfa*) character should be tested for crystal violet sensitivity.

Procedure : Pipetted 0.1% solution of crystal violet to the sterile filter paper disc (1/4 inch) and transferred the disc to plates, seeded with bacteria (the procedure is similar to R-factor). Incubated at 37 °C for 48 h. The clear zone appeared around the disc indicated the presence of the *rfa* mutation that permitted crystal violet to enter and kill bacteria.

5. Spontaneous Reversion

Spontaneous reversion of the tester strains to histidine independence is measured routinely in mutagenicity experiments and is expressed as the number of

spontaneous revertants per plate. The revertant colonies are clearly visible in a uniform background lawn of auxotrophic bacteria. Each tester strain reverts spontaneously at a frequency that is characteristic of the strain. Nevertheless, there is variability in the number of spontaneous revertants from one experiment to another and from one plate to another, and it is advisable to include at least 2-3 spontaneous mutation control plates for each strain in a mutagenicity assay.

Procedure: 0.2 ml of sterile distilled water (solvent in the experiment) is added to cap tube. Add 0.5 ml of NaPO₄-KCl buffer pH 7.4 in the absence of metabolic activation, 0.1 ml of fresh overnight culture of TA 98 or TA 100, followed plates and left it to become harden. Incubated at 37 °C for 48 h. and the his⁺ revertant colonies were counted.

6. The Response to Standard carcinogen.

Standard carcinogens or positive carcinogen are used routinely in mutagenicity experiment to confirm the reversion property and specificity of each strain. The standard mutagen which used in these experiments is aminopyrene in the absence of metabolic activation. Tester strain which highly response to positive mutagens must be collected.

Procedure: The procedure is as described in spontaneous reversion except aminopyrene (0.06, and 0.12 µl per plate for TA 98 and TA 100, respectively) are used instead of sterile distilled water in absence S-9 mix, respectively. The characteristic of stock culture for TA 98 and TA 100 as the source of bacteria for mutagenicity is

- a) Contained R-factor (pKM 101) and rfa mutation
- b) His⁺ requirement
- c) low spontaneous reversion
- d) highly response to standard carcinogen

After the characteristic of the culture was tested, the mutagenicity test was started.

VITA

Miss Nisita Komsakorn was born on August 11, 1978 in Bangkok, Thailand. She received her Bachelor's degree of Sciences in Pharmacy in 2000 from the Faculty of pharmaceutical sciences, Chulalongkorn University, Thailand. She has five-year experiences in hospital pharmacy. Now she is working as marketing executive for the British Dispensary (L.P.) Co., Ltd.

