

Acknowledgments

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References

1. Steinfeld, H., Hann, C., and Blackburn, H., Livestock-environment interactions. 1997, Commission of the European Communities, Food and Agriculture Organization of the United Nations, World Bank.
2. Ministry of Agriculture, Forestry and Fisheries, The white paper for food and agriculture. 1998.
3. Ministry of Agriculture, Forestry and Fisheries, The basic law on food, agriculture and rural areas (Provisional Translation)1999.
4. Ministry of Agriculture, Forestry and Fisheries, The statistical chart for self-supporting feed. 1998.
5. Takano, N. and Y. Yamashita, The innovation of technology and strategy of silage utilization for Japanese beef production. 1990, Tokyo: Tsukijishokan.
6. Seale, D. R., Bacterial inoculants as silage additives. J. Appl. Bacteriol., 1986. (Symp. Suppl.): 9-26.
7. Daniel, P., H. Honig, F. Weise and E. Zimmer, Wirkung von propionsäure bei der grünfuttersilierung. Wirtschaftseigene Futter, 1970. **16**:239-256.
8. Jonsson, A. and G. Pahlow, Systematic classification and biochemical characterization of yeasts growing in grass silage inoculated with *Lactobacillus* cultures. Anim Res Develop, 1984. **20**:7-22.
9. O'Kiely, P. and R. Muck, Aerobic deterioration of Lucerne (*Medicago sativa*) and Maize (*Zea mays*) silage-effects of yeasts. J Sci Food Agric, 1992. **59**:139-144.
10. Ohyama, Y., Aerobic deterioration of silage and the way of the prevention. Chikusan no Kenkyu (Animal Husbandry), 1981. **35**:997-1120.
11. Woolford, M. K., A Review : The detrimental effects of air on silage. J Appl Bacteriol, 1990. **68**: 101-116.
12. McDonald, P., N. Henderson and S. Heron, The biochemistry of silage. 2nd ed. 1991, Great Britain: Chalcombe publications.

13. Kung, L. J., A. C. Sheperd, A. M. Smagala, K. M. Endres, C.A. Bessett, N. K. Ranjit, N. K. and J. L., Glancey, The effect of preservatives based on propionic acid on the fermentation and aerobic stability of corn silage and total mixed ration. *J. Dairy Sci*, 1998. **81**:1322-1330.
14. Sasaki, H., Microbiological studies on grass silage fermentation. *Memories of the Faculty of Agriculture Hokkaido University*, 1972. **8**(3):189-251.
15. Tanaka, O. and S. Ohmomo, A repeatable model system for silage fermentation in culture tubes. *Biosci Biotech Biochem*, 1994. **58**(8):1407-1411.
16. Tanaka, O. and S. Ohmomo, A new device for a model system of silage fermentation by a plastic bag. *Grassland Science*, 1995. **41**(1):55-59.
17. Organization, J. D. C., *Handbook for increasing the milk demand '98*. 1998,
18. Kisaalita, W. S., Pinder, L. K. and Lo, K. V., Acidogenic fermentation of lactose. *Biotechnol Bioeng*, 1987. **30**:88-95.
19. Vienne, P. and von Stocker, U., Metabolic, physiological and kinetic aspects of the alcoholic fermentation of whey permeate by *Kluyveromyces fragilis* NRRL 665 and *Kluyveromyces lactis* NCYC 571. *Enzyme Microb Technol*, 1985. **7**:287-294.
20. Duvnjak, Z., Houle, C., Mok, and Lok, K. L., Production of ethanol and biomass from various carbohydrates by *Kluyveromyces fragilis*. *Biotechnol Lett*, 1987. **9**:343-346.
21. Mahmoud, M. M. and Kosikowski, F. V., Alcohol and single cell protein production by *Kluyveromyces* in concentrated whey permeates with reduced ash. *J Dairy Sci*, 1982. **65**:2082-2087.
22. El-Samragy, Y. A., Chen, J. H. and Zall, R. R., Amino acid and mineral profile of yeast biomass produced from fermentation of cheddar whey permeate. *Process Biochem.*, 1988. **2**:28-30.
23. Gomez, A. and Castillo, F. J., Production of biomass and β -D-galactosidase by *Candida pseudotropicalis* grown in continuous culture on whey. *Biotechnol Bioeng*, 1983. **25**:1341-1357.
24. Belem, M. A. F. and Lee, B. H., Production of bioingredients from *Kluyveromyces marxianus* grown on whey: an alternative. *Critical Review in Food Science and Nutrition*, 1998. **38**:565-598.

25. Walker, G. M., Yeast physiology and biotechnology. 1998, West Sussex: John Wiley & Sons Lt.
26. Mir, Z. and Mir, P. S., Effect of the addition of live yeast (*Saccharomyces cerevisiae*) on growth and carcass quality of steers fed high-forage or high-grain diets and feed digestibility and in situ degradability. J Anim Sci, 1994. **72**:537-545.
27. Robinsin, P. H., Chalupa, W., Sniffen, C. J., Julian, W. E., Sato, H., Watanabe, K., Fujieda, T. and Suzuki, H., Ruminally protected lysine and methionine for lactating dairy cows fed a ration designed to meet requirements for microbial and postruminal protein. J Dairy Sci, 1998. **81**:1364-1373.
28. Bevan, E. A. and M. Makower. The physiological basis of the killer character in yeast. in XIth Int Congr Genet. 1963.
29. Polonelli, L., S. Conti, M. Gerloni, W. Magliani and C. Chezzi, Interfaces of the yeast killer phenomenon. Critical Reviews in Microbiology, 1991. **18**(1):47-87.
30. Gunge, N., A. Tamaru, F. Ozawa and K. Sakaguchi, Isolation and characterization of linear deoxyribonucleic acid plasmids from *Kluyveromyces lactis* and the plasmid-associated killer character. J Bacteriol, 1981. **145**:382-390.
31. Sugisaki, Y., Gunge, N., Sakaguchi, K., Yamasaki, M. and Tamura, G., *Kluyveromyces lactis* killer toxin inhibits adenylate cyclase of sensitive yeast cells. Nature, 1983. **304**:464-466.
32. Kimura, T., Kitamoto, N., Kito, Y., Iimura, Y., Shirai, T., Komiya, T., Furuichi, Y., Sakka, K. and Ohmiya, K., A novel yeast gene, *RHK1*, is involved in the synthesis of the cell wall receptor for HM-1 killer toxin that inhibits β -1, 3-glucan synthesis. Mol Gen Genet, 1997. **254**:139-147.
33. Schmitt, M. J., Klavehn, P., Wang, J., Schüig, I and Tipper, D. J., Cell cycle studies on the mode of action of yeast K28 killer toxin. Microbiology, 1996. **142**:2655-2662.
34. Ouchi, K. and H. Akiyama, Breeding of useful killer sake yeasts by repeated back-crossing. J Ferment Technol, 1976. **54**:615-623.
35. Hara, S., Y. Iimura and K. Otsuka, Breeding of useful killer wine yeasts. Am J Enol Vitic, 1980. **31**(1):28-33.
36. Hara, S., Iimura, Y., Oyama, H., Kozeki, T., Kitano, K., and Otsuka, K., The breeding of cryophilic killer wine yeasts. Agric Biol Chem, 1981. **45**:1327-1334.

37. Heard, G. M. and G. H. Fleet, Occurrence and growth of killer yeasts during wine fermentation. *Appl Environ Microbiol*, 1987. **53**(9):2171-2174.
38. Sulo, P., S. Michalcáková, and V. Reiser, Construction and properties of K1 type killer wine yeasts. *Biotechnology Letters*, 1992. **14**(1):55-60.
39. Akiyama, Y., Growth of microorganisms during moromi making, In *The Brewing*, K. Otsuka, Editor. 1989, Yokendo: Tokyo. pp 33-34.
40. Okada, S., Lactic acid bacteria which lives with yeast, In *The challenge from yeasts - Applied yeast*, G. Tamura Nojiro, K., Akiyama, Y., and Kolzumi, T., Editor. 1997, Gihoudou: Tokyo. pp 66-75.
41. Utter, M. F. and H. M. Kolenbrander, Formation of oxalacetate by CO₂ fixation on phosphoenolpyruvate., In *The Enzymes*, P.D. Boyer, Editor. 1972, Academic Press: New York. pp 117-168.
42. Perea, J. and C. Gancedo, Isolation and characterization of a mutant of *Saccharomyces cerevisiae* defective in phosphoenolpyruvate carboxykinase. *Arch Microbiol*, 1982. **132**:141-143.
43. Valdés-Hévia, M. D., Guerra, R. and C., Gancedo, Isolation and characterization of the gene encoding phosphoenolpyruvate carboxykinase from *Saccharomyces cerevisiae*. *FEBS Lett*, 1989. **258**:313-316.
44. Stucka, R., M. D. Valdés-Hévia, C. Gancedo, C. Schwarzlose and H. Feldmann, Nucleotide sequence of the phosphoenolpyruvate carboxykinase gene from *Saccharomyces cerevisiae*. *Nucleic Acids Res*, 1988. **16**:10926.
45. Krautwurst, H., Encinas, M. V., Marcus, F., Latshaw, S. P., Kemp, R. G., Frey, P. A., and Cardemil, E., *Saccharomyces cerevisiae* phosphoenolpyruvate carboxykinase: Revised amino acid sequence, site-directed mutagenesis, and microenvironment characteristics of cysteines 365 and 458. *Biochemistry*, 1995. **34**:6382-6388.
46. Proft, M., D. Grzesitza and K. Entian, Identification and characterization of regulatory elements in the phosphoenolpyruvate carboxykinase gene *PCK1* of *Saccharomyces cerevisiae*. *Mol Gen Genet*, 1995. **246**:367-373.
47. Kuzhandaivelu, N., K. W. Jones, A. K. Martin and R. C. Dicson, The signal for glucose repression of the lactose-galactose regulon is amplified through subtle modulation of transcription of the *Kluyveromyces lactis* *KI-GAL4* activator gene. *Mol Cel Biol*, 1992. **12**:1924-1931.

48. Zaror, I., F. Marcus, D. L. Moyer, J. Tung and J. R. Shuster, Fructose-1,6-bisphosphatase of the yeast *Kluyveromyces lactis*. *Eur J Biochem*, 1993. **212**:193-199.
49. Wésolowski-Louvel, M. and H. Fukuhara, A map of the *Kluyveromyces lactis* genome. *Yeast*, 1995. **11**:211-218.
50. Ozier-Kalogeropoulos, O., Malpertuy, A., Boyer, J., Tekala, F. and B., Dujon, Random exploration of the *Kluyveromyces lactis* genome and comparison with that of *Saccharomyces cerevisiae*. *Nucleic Acids Research*, 1998. **26**:5511-5524.
51. Wésolowski-Louvel, M., K. D. Breunig and H. Fukuhara, *Kluyveromyces lactis*, in Nonconventional yeasts in biotechnology, K. Wolf, Editor. 1996, Springer-Verlag: Berlin. pp140-201.
52. Van den Berg, J. A., Van der Laken, K. J., Van Ooyen, A. J. J., Renniers, T., Rietveld, K., Schaap, A., Brake, A. J., Bishop, R. J., Schulz, K., Moyer, D., Richman, M. and J. R. Scuster, *Kluyveromyces* as a host for heterologous gene expression: Expression and secretion of prochymosin. *Bio/Technology*, 1990. **8**:135-139.
53. Sasaki, Y. and H. Sasaki, Studies on microflora in beet-top silage. (II) Yeasts In beet-top silage. *Jpn J Zootech Sci*, 1966. **37**:458-464.
54. Rose, M. D., P., Novick, J. H. Thomas, D., Botstein and G. R., Fink, A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. *Gene*, 1987. **60**:237.
55. Ogata, T., Y. Okumura, M. Tadenuma and G. Tamura, Improving transformation method for Industrial yeasts: Construction of *ADH1-APT2* gene and using electroporation. *J Gen Appl Microbiol*, 1993. **39**:285-294.
56. Chen X-J., M. Saliola, C. Falcone, M. M. Bianchi and H. Fukuhara, Sequence organization of the circular plasmid pKD1 from the yeast *Kluyveromyces drosophilicarum*. *Nucleic Acids Res*, 1986. **14**:4471-4481.
57. Walter, T. and H.-J. Holtke, Colony and plaque hybridization with the digoxigenin system. *Colloquium*, 1992. **2**:8-9.
58. Tanaka, O., Kimura, H., Takahashi, E., Ogata, S., S. Ohmomo, Screening of lactic acid bacteria for silage inoculants by using a model system of silage fermentation. *Biosci Biotech Biochem*,

59. Cai, Y., Benno, Y., Ogawa, M., Ohmomo, S., Kumai, S. and T. Nakase, Influence of *Lactobacillus* spp. from an inoculant of *Weissella* and *Leuconostoc* spp. from forage crops on silage fermentation. *Appl Environ Microbiol*, 1998. 64:2982-2987.
60. Sambrook, J., E. F. Fritsch and T. Maniatis, Molecular Cloning: A Laboratory Manual. 2nd ed. 1989, New York: Cold Spring Harbor Laboratory Press.
61. Southern, E. M., Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol*, 1975. 98:503-517.
62. Sanger, F., S. Nicklen and A. R. Coulson, DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA*, 1977. 74:5463-5467.
63. Ito, H., Y. Fukada, K. Murata and A. Kimura, Transformation of intact yeast cells treated with alkali cations. *J Bacteriol*, 1983. 153:163-168.
64. Kaiser, C., S. Michaelis and A. Mitchell, Method in Yeast Genetics. 1994, NY: Cold Spring Harbor Laboratory Press. pp137-138.
65. Stam, J. C., J. Kwakman, M. Meijer and A. R. Stuitje, Efficient isolation of the linear DNA killer plasmid of *Kluyveromyces lactis*: Evidence for location and expression in the cytoplasm and characterization of their terminally bound proteins. *Nucleic Acids Res*, 1986. 14(17):6871-6884.
66. Sánchez, M., F. J. Iglesias, C. Santamaría and A. Domínguez, Transformation of *Kluyveromyces lactis* by electroporation. *Appl Environ Microbiol*, 1993. 59:2087-2092.
67. Wolfe, R. G. and J. B. Neelands, Some molecular and kinetic properties of heart malic dehydrogenase. *J Biol Chem*, 1956. 221:61-69.
68. Foy, J. J. and J. K. Bhattacharjee, Gluconeogenesis in *Saccharomyces cerevisiae*: Determination of fructose-1,6-bisphosphatase activity in cells grown in the presence of glycolytic carbon sources. *J Bacteriol*, 1977. 129:978-982.
69. Somers, J. M. and E. A. Bevan, The inheritance of the killer character in yeast. *Genetic Res Camb*, 1969. 13:71.
70. Ishiyama, M., miyazono, Y., Sasamoto, K., Ohkura, Y. and K. Ueno, A highly water-soluble disulfonated tetrazolium salt as a chromogenic indicator for NADH as well as cell viability. *Talanta*, 1997. 44:1299-1305.

71. Kobayashi, T., Tabuchi, T., A method employing a tribasic sodium phosphate buffered reagent for estimating semimicro quantities of reducing sugars. *Nippon Nogeikagaku Kaishi*, 1953. **28**:171-173.
72. Institute, S., SAS/STAT User's Guide1988, SAS Institute Inc: Cary N. C.
73. Beggs, J. D., Transformation of yeast by a replicating hybrid plasmid. *Nature*, 1978. **275**:104-109.
74. Karube, I., E. Tamiya and H. Matsuoka, Transformation of *Saccharomyces cerevisiae* spheroplasts by high electric pulse. *FEBS Lett*, 1985. **182**(1):90-94.
75. Hashimoto, H., H. Morikawa, Y. Yamada and A. Kimura, A novel method for transformation of intact yeast cells by electroporation of plasmid DNA. *Appl Microbiol Biotechnol*, 1985. **21**:336-339.
76. Shimon, J. R. and K. McEntee, A rapid procedure for transformation of intact *Saccharomyces cerevisiae* by electroporation . *Biochem Biophys Res Com*, 1989. **164**(3):1157-1164.
77. Delorme, E., Transformation of *Saccharomyces cerevisiae* by electroporation. *Appl Environ Microbiol*, 1989. **55**(9):2242-2246.
78. Meilhoc, E., J.-M. Masson and J. Telisse, High efficiency transformation of intact yeast cells by electric field pulses. *BioTechnology*, 1990. **8**(3):223-227.
79. Ouchi, K. and T. Yamamoto, Killer phenomenon in yeast: Biosynthesis, mode of action, and practical use of killer toxin. *Biseibutsu*, 1986. **2**:27-41.
80. Demain, A. L., H. J. Phaff and C. P. Kurtzman, The industrial and agricultural significance of yeasts. In *The Yeasts, a taxonomic study*, C.P.and J.W.F. Kurtzman, Editor 1998, Elsevier Science B. V.: Amsterdam. pp 13-19.
81. Sugisaki, Y., N. Gunge, M. Yamasaki and G. Tamura, Characterization of a novel killer toxin encoded by a double-strand linear DNA plasmid of *Kluyveromyces lactis*. *Eur J Biochem*, 1984. **141**:241-245.
82. Visser, W., W. A. Scheffers, W. H. Batenburg-van Der Vugte and J. P. Van Dijken, Oxygen requirements of yeasts. *Appl Environ Microbiol*, 1990. **56**:3785-3792.

83. Gunge, N. and K. Sakaguchi., Intergeneric transfer of deoxyribonucleic acid killer plasmids, pGK1 and pGK2, from *Kluyveromyces lactis* into *Saccharomyces cerevisiae* by cell fusion. J Bacteriol, 1981. **147**:155-160.
84. Alvear, M., M. V. Encinas, R. G. Kemp, S. P. Latshaw and E. Cardemil, ATP-dependent *Saccharomyces cerevisiae* phosphoenolpyruvate carboxykinase: isolation and sequence of a peptide containing a highly reactive cysteine. Biochem Biophys Acta, 1992. **1119**:35-38.
85. Linss, J., S. Goldenberg, J. A. Urbina and L. M. Amzel, Cloning and characterization of the gene encoding ATP-dependent phospho-enol-pyruvate carboxykinase in *Trypanosoma cruzi*: Comparison of primary and predicted secondary structure with host GTP-dependent enzyme. Gene, 1993. **136**:69-77.
86. Mercado, J. J. and J. M. Gancedo, Regulatory regions in the yeast *FBP1* and *PCK1* genes. FEBS Lett, 1992. **311**:110-114.
87. Lodi, T., D. O'Connor and P. Goffini, Carbon catabolite repression in *Kluyveromyces lactis*: Isolation and characterization of the *KIDLD* gene encoding the mitochondrial enzyme D-lactate ferricytochrome c oxidoreductase. Mol Gen Genet, 1994. **244**:622-629.
88. Niwa, O., K. Sakaguchi, and N., Gunge, Curing of the killer deoxyribonucleic acid plasmids of *Kluyveromyces lactis*. J. Bacteriol, 1981. **148**(3):988-990.
89. Delgado, M. A. and C. Gancedo, Mapping of the *PCK1* gene encoding phosphoenolpyruvate carboxykinase on chromosome XI of *Saccharomyces cerevisiae*. FEMS Microbiol. Lett, 1992. **92**:125-128.
90. Kurtzman, C. P., Classification of the ascomycetous taxa *Pichia* E. C. Hansen emend., In The yeasts, a taxonomic study, C.P. and J.W.F. Kurtzman, Editor. 1998, Elsevier Science B. V.: Amsterdam.273-352.
91. OECD, Safety considerations for biotechnology- Scale up of microorganisms as biofertilizers. 1995.
92. Wolfe, K. H. and Neilands, J. B., Molecular evidence for an ancient duplication of the entire yeast genome. Nature, 1997. **387**:708-713.
93. Lachance, M. A., *Kluyveromyces* van der Walt emend. van der Walt, In The yeasts, a taxonomic study, C.P. and J. W.F. Kurtzman, Editor. 1998, Elsevier Science B. V.: Amsterdam. pp 227-247.

94. Bonekamp, F. J. and J. Oosterom, On the safety of *Kluyveromyces lactis* - A review. *Appl Microbiol Biotechnol*, 1994. **41**:1-3.
95. Redenbaugh, K., Hiatt, W., Martineau, B., Kramer, M., Sheehy, R., Sanders, R., Houck, C. and Emlay, D., Safety assesment of genetically engineered fruits and vegetables - A case study of the FLAVER SAVERTM TOMATO. 1992, Boca Ranton: CRC Press.
96. Unit, W. H. O. F. S., Health aspects of marker genes in genetically modified plants - Report of a WHO Workshop1993.
97. Sor, F., M. Wésolowski and H. Fukuhara, Inverted terminal repetitions of the two linear DNA associated with the killer character of the yeast *Kluyveromyces lactis*. *Nucleic Acids Res*, 1983. **11**:5037-5044.
98. Kikuchi, Y., K. Hirai, N. Gunge and F. Hishinuma, Hairpin plasmid-a novel killer DNA of perfect hairpin structure. *EMBO J*, 1985. **4**:1881-1886.
99. Gunge, N. and C. Yamane, Incompatibility of linear DNA killer plasmids pGKL1 and pGKL2 from *Kluyveromyces lactis* with mitochondrial DNA from *Saccharomyces cerevisiae*. *J Bacteriol*, 1984. **159**:533-539.
100. Sugisaki, Y., N. Gunge, K. Sakaguchi, M. Yamasaki and G. Tamura, Transfer of DNA killer plasmids from *Kluyveromyces lactis* to *Kluyveromyces fragilis* and *Candida pseudotropicalis*. *J Bacteriol*, 1985. **164**(3):1373-1375.
101. Stumm, C., Hermers, J. M. H., Middelbeek, E. J., Croes, A. F. and de Vries, G. J. M. L., Killer sensitive relationship in yeast from natural habitats. *Antonie van Leeuwenhoek*, 1975. **43**:125-128.
102. Buttler, A. R., White, J. H. and Stark, J. R., Analysis of the responce of *Saccharomyces cerevisiae* cells to *Kluyveromyces lactis* toxin. *J General Microbiol*, 1991. **137**:1749-1757.
103. Stark, M. J. R., A. Boyd, A. J. Mileham and M. A. Roman, The plasmid-encoded killer system of *Kluyveromyces lactis*; A review. *Yeast*, 1990. **6**:1-29.
104. Buttler, A. R., O'donnell, R. W., Martin, V. J., Gooday, G. W. and Stark, M. J. R., *Kluyveromyces lactis* toxin has an essential chitinase activity. *Eur J Biochem*, 1991. **199**:483-488.

105. Kawamoto, S., Arai, N., Kobayashi, M., kawahara, K., Iwahashi, H., Tanabe, C., Hatori, H., Ohno, T. and Nakamura, T., Isolation and characterization of mutants *Saccharomyces cerevisiae* resistant to killer toxin of *Kluyveromyces lactis*. *J Ferment Bioeng*, 1990. **70**:222-227.
106. Butler, A., Porter, M. and Stark. M. J. R., Intracellular expression of *Kluyveromyces lactis* toxin γ subunit mimics treatment with exogeneous toxin and distinguishes two classes of toxin-resistant mutant. *Yeast*, 1991. **7**:617-625.
107. Kawamoto, S., Sasaki, T., Itahashi, S., Hatsuyama, Y. and Ohno, T., A mutant allele *skt5* affecting protoplast regeneration and killer toxin resistance has double mutations in its wild-type structural gene in *Saccharomyces cerevisiae*. *Biosci Biotech Biochem*, 1993. **57**:1391-1393.
108. Burtler, A. R., White, J. H., Folawiyo, Y., Edlin, A., Gardiner, D. and Stark. M. J. R., Two *Saccharomyces cerevisiae* genes which control sensitivity to G₁ arrest induced by *Kluyveromyces lactis* toxin. *Mol Cell Biol*, 1994. **14**:6306-6316.
109. Kishida, M., Tokunaga, M., Katayose, Y., Yajima, H., Kawamura, A. W. and Hishinuma, F., Isolation and genetic characterization of pGKL killer-insenstitive mutants (*ki*) from *Saccharomyces cerevisiae*. *Biosci Biotech Biochem*, 1996. **60**:798-801.
110. Yajima, H., Tokunaga, M., awamura, A. W. and Hishinuma,, Characterization of *IKI1* and *IKI3* genes conferring pGKL killer sensitivity on *Saccharomyces cerevisiae*. *Biosci Biotech Biochem*, 1997. **61**:704-709.

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Original paper

1. Kitamoto, H. K., S. Ohmomo and T. Nakahara, Selection of killer yeasts (*Kluyveromyces lactis*) to prevent aerobic deterioration in silage making. *J Dairy Sci*, 1993. **76** (3):803-811.
2. Kitamoto, H. K. and T. Nakahara, Isolation of L-methionine-enriched mutant of *Kluyveromyces lactis* grown on whey permeate. *Process Biochem*, 1994. **29** (2):127-131.
3. Kitamoto, H. K., S. Ohmomo and Y. Iimura, Isolation and nucleotide sequence of the gene encoding phosphoenolpyruvate carboxykinase from *Kluyveromyces lactis*. *Yeast*, 1998. **14** (10):963-967.
4. Kitamoto, H. K., S. Ohmomo, K. Amaha, T. Nishikawa and Y. Iimura, Construction of *Kluyveromyces lactis* killer strains defective in growth on lactic acid as a silage additive. *Biotechnology Letters*, 1998. **20** (8):725-728.
5. Kitamoto, H. K., A. Hasebe, S. Ohmomo, E. G. Suto, M. Muraki, and Y. Iimura, Prevention of aerobic spoilage of maize silage by a genetically modified killer yeast, *Kluyveromyces lactis*, defective in ability to grow on lactic acid. *Applied and Environmental Microbiology*, 1999. **65** (10):4697-4700.