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ABSTRACT

The aim of this study is to isolate potential lactic acid bacteria (LAB) for developing the rapid malolactic fermentation (MLF) process for wine production. The isolated LAB were identified for their genus using morphological and physiological techniques. Moreover, the species of these isolates were identified using molecular biology techniques. The selected LAB were then tested as a pure starter culture for malolactic fermentation during wine processing and wine quality were evaluated by sensory evaluation.

Seventy-four LAB were isolated from the fermentation broth of mixture between grape juice and MRS media using cyclohexamide as inhibitor of yeast growth. The isolated LAB were identified as Lactobacillus spp. (62 isolates), Leuconostoc spp. (3 isolates) and Pediococcus spp. (2 isolates) by physiological and morphology techniques. Furthermore, the fermentation mixture of grape juice and tomato juice medium (medium specific for the growth of LAB) were employed for to isolation. The 98 isolated LAB were selected from the above fermentation broth. Those LAB were identified as Lactobacillus spp. (60 isolates), Leuconostoc spp. (28 isolates), Pediococcus spp. (6 isolates), Streptococcus spp. (2 isolates), Lactococcus spp. (1 isolate) and Enterococcus spp. (1 isolate) by physiological and morphology techniques. Lactobacillus spp. was found to be a predominant genus comparing to the other isolates.

The molecular identification of O. oeni from isolated LAB, together with a PCR-RFLP and specific species PCR were performed. No RFLP patterns from PCR fragment of selected LAB were similar to the RFLP pattern of O. oeni (reference strain). When Lactobacillus spp. were determined by species-specific PCR, the results shown that 25 isolates from 32 isolates of homofermentative Lactobacillus spp. were detected as Lb. plantarum. The 25 isolates of Lb. plantarum containing malolactic enzyme gene (mle) were investigated by molecular biological technique using degenerate primers designed from partial mle gene sequences. The results revealed that all isolates contain mle gene. The 9 isolates of Lb. plantarum which exhibit rapid growth in tomato juice-glucose-fructose-malate broth (TGFM) containing 0.2 % L-malic acid and pH 5.5 were selected to tested the expression of malolactic enzyme. The results shown that all isolates have ability to degrade L- malic acid to L-lactic acid. Lb. plantarum isolated LP 2.25, CB 2.31 and BO 2.84 which dominate highly malolactic activity.
were selected for testing the capability to reduce L-malic acid in synthetic medium containing 8 % ethanol at pH 3.7 (as in wine condition). The results revealed that CB 2.31 and BO 2.84 were able to grow and to degrade L-malic acid in this condition.

Isolates CB 2.31 and BO 2.84 were tested as starter to improve malolactic fermentation in wine containing 8, 10 and 11.7 % (v/v) alcohol. The results indicated that *Lb. plantarum* BO 2.84 was the most effective LAB isolate to degrade L-malic acid (0.96 g/l) in wine containing 8 % (v/v) alcohol, while *Lb. plantarum* CB 2.31 was the most effective LAB isolate to degrade L-malic acid (1.02 g/l) in wine containing 10 % (v/v) alcohol. Moreover, *Lb. plantarum* CB 2.31 is the most effective LAB isolate to degrade L-malic acid (0.63 g/l) in wine containing 11.7 % (v/v) alcohol. Finally, it was be found that the *Lb. plantarum* CB 2.31 and BO 2.84 had a higher capability to degrade L-malic acid to L-lactic acid as compare to *O. oeni Viniflora® LS oenos* (DSM 7008) (a commercial strain).

The sensory evaluation of wine samples from wine containing 8, 10 and 11.7 % (v/v) alcohol with different strain of LAB starter were determined by German Agricultural Society (DLG method) and ranking test. By using DLG methods to evaluate five sensory characteristic of wine i.e. brightness, colour, odour, taste and overall liking, the results revealed that, there were no significant differences (p≤ 0.05) in each group of wines containing 8 %, 10 % and 11.7 % (v/v) alcohol. In term of ranking test, the results revealed that wine containing 8 % (v/v) alcohol without added LAB exhibited the most acceptances from testers. Wine containing 10 % (v/v) alcohol with *Lb. plantarum* CB 2.31 was highly accepted comparing to the others. Finally, wine containing 11.7% (v/v) alcohol with four different conditions showed no significant differences.