INTRODUCTION
Phytochemicals, a type of isoflavones, representing a major group of phytoestrogens are the beneficial composites to health.1 Soy isoflavones complements are used to treat several chronic diseases; cancer cells cardiovascular diseases and osteoporosis.2 Biological activities of soy isoflavones and aglycones that are in two groups of isoflavones are originating from their aglycones (genistin, daidzin), but not from their glycoside forms (genistin, daidzin). Isoflavone aglycones have been shown to be more quickly and efficiently absorbed into intestines than isoflavone glucosides. β-glucosidases can be used to convert isoflavone glucosides to aglycones.3 Microorganisms are synthesized in cells with β-glucosidase enzymes. β-glucoside bond breaking glucosides isoflavones ensure aglycones transformation.4 Thus, the utility increases with the concentration of isoflavones in free form.

MATERIALS & METHODS

Bacteria
Pure cultures of 39 Lactobacillus spp. were obtained from the Gazi University Culture Collection.

Assay of β-glucosidase activity
β-glucosidase activity was determined at 24 h of incubation in de Man, Rogosa and Sharpe (MRS). The β-glucosidase activity was determined by measuring the rate of hydrolysis of p-nitrophenyl-β-D-glucopyranoside (pNPG). The amount of p-nitrophenol released was measured using a spectrophotometer (Hitachi) at 420 nm. One unit of the enzyme activity was defined as the amount of β-glucosidase that released 1 nmol of p-nitrophenol from the substrate pNPG per milliliter per min under assay conditions. The specific activity was expressed as units of enzyme per milligram of the protein.5 The protein concentration was determined with Bradford Reagent. The enzymatic activity was determined in the supernatant of the cultures and in the cells free extract.

Hydrolysis of isoflavone glucosides
The highest β-glucosidase specific enzyme activity determined L. rhamnosus MB9A, L. casei SC1 and L. rhamnosus EA1 strains grown in MRS medium were inoculated at 2% (v/v) and incubated for 24 h at 37°C. Culture broth, 0.2 mL, was added to 1.8 mL, 0.5 M potassium phosphate buffer, pH 7.5 containing 100 µg genistin or daidzin. The mixture was held at 45°C for 30 min and then boiled for 10 min. The composition of isoflavones was analyzed by HPLC6.

RESULTS
In the present study, human-being, nutritional and animal originated 39 Lactobacillus species were used. β-glucosidases enzyme activities of the cultures were identified by using p-nitrophenyl-β-D-glucopyranosidase (pNPG) as a substrate. In these strains, β-glucosidase specific enzyme activity were determined varies from 0.250-4.500 U/mg. For β-glucosidase enzyme belonging to L. rhamnosus MB9A (4,500 U/mg), L. rhamnosus EA1 (2,670 U/mg), and L. casei SC1 (3,000 U/mg) strains was showed high β-glucosidase specific enzyme activity (Table 1).

Table 1. β-Glucosidase enzyme, specific activity and protein content in Lactobacillus spp.

Strains belonging to the genus Lactobacillus, p-nitrophenyl-β-D-glucopyranosidase (pNPG) as a substrate using the β-glucosidase enzyme activities and their product (p-nitrophenol) formed, p-nitrophenyl-β-D-glucopyranosidase (pNPG) containing the mixture turned into a yellow color formation is observed in the product (Picture 1).

REFERENCES