

The Diverse Cytotoxicity Evaluation of *Lactobacillus* Discovered from Sheep Milk

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Abbreviations

LAB: *Lactobacillus*; Lp: *Lactobacillus plantarum*; Lr: *Lactobacillus rhamnosus*; La: *Lactobacillus acidophilus*

The pharmacology research, for the evaluation of the toxicity, is become vital in regards to the nutraceuticals products development [1,7-10]. The cytotoxicity studies of the nutraceutical products, especially the *lactobacillus* (LAB) formulation are gaining importance due to its industrial application [11, 12]. The interaction of the probiotics cells with the living host cells may rarely precipitate toxicity [1-3,13, 14,17,18]. The interaction of the LAB in pathophysiological conditions such as cancer is gaining importance as an alternative source of functional food [14-16].

To support the claim MTT test was performed on three different strains of *Lactobacillus* i.e., Lp- *Lactobacillus plantarum*; Lr- *Lactobacillus rhamnosus* and La- *Lactobacillus acidophilus*. The culture of Lp and La were discovered by our team from sheep milk and whole genome sequence was deposited in NCBI database with reference number- PYBS00000000 and PYBS01000000 respectively [5,6].

In this test, the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) was used [4]. This reagent passes through the viable cells and enters the mitochondria, producing the dark blue/purple insoluble compound called as formazan. This is observed only in viable cells, as the yellow tetrazolium salt from MTT reagent is reduced to colored purple dye only in the living cells. These viable colored cells get homogenized and soluble because of

the use of organic solvent like isopropanol resulting in the formation of the formazan. This complex-colored compound is measured by the spectrophotometric method. The dead cells never show these activities thus, this assay gives the count of the living cells in the form of the viability count and determines the number of metabolically active cells.

Figure 1: Effects of the cell-free lyophilized filtrate of LAB on the viability of HCT 115 cell line for various concentration by MTT assay. Values are expressed as viability mean ratio \pm SD*, n = 3.

The viability study conducted by MTT assay on HCT 115 cell lines shows a decrease in percentage cell viability, an approximately around 40% in case of the Lp for its highest dose of 50 μ g/ml and further, showing the strongest anti-proliferative activity as compared to control group. In the case of Lp, expect the dose of 10 μ g/ml the other doses from 20 μ g/ml to 50 μ g/ml shows the anti-proliferative activity. Similarly, the cell viability results obtained

in case of Lr, and La are 74% and 88% on HCT 115 cell line for its highest dose of 50 µg/ml with respect to the control group. La, which is used as the reference control group, not show any anti-proliferative activity.

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