IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 4, March 2022
Impact Factor: 6.252

Cytotoxic Assay on Bacteriocin B25 produced by Marine *Lactobacillus pentosus* B25

Dr. Bharti Wadekar
Assistant Professor, Microbiology ID,
ZSCT's Thakur Shyamnarayan Degree College, Kandivali East, Mumbai
bhartiwadekar@tsdcmumbai.in

Abstract: *The in vitro antimicrobial efficiency of the bacteriocin B25 is well documented, however its clinical application needs investigation, as its toxicity may well be totally different in in vitro (haemolytic and bactericide activity in blood and toxicity towards traditional human cell lines). The cytotoxic result of bacteriocin on traditional human blood cells (lymphocytes) was evaluated by MTT assay. Inhibition of cell proliferation was quantitated. a gentle cytotoxic result of bacteriocin was found on lymphocytes. The bacteriocin concentration fifty µg/mL to two hundred µg/mL used and the death of lymphocytes was ascertained in bacteriocin treated lymphocytes, twenty µg/mL to a hundred and sixty µg/mL from concentration, when a hundred and sixty µL there have been not found any changes in lymphocytes. It had shown the gentle toxicity. The IC50 worth was obtained as a hundred and sixty µL +/-20 µL. The Student's two-tailed t-test was accustomed verify applied mathematics significance of the variations between untreated cells and cells treated with the varied concentrations of bacteriocin.*

Keywords: Toxicity, Bacteriocin, MTT Assay, Lymphocytes

I. INTRODUCTION

In recent decades, bacteriocins have received substantial attention as antimicrobial compounds. Though bacteriocins are preponderantly exploited as food preservatives, they're currently receiving raised attention as potential clinical antimicrobials and as doable immune-modulating agents. Infections caused by antibiotic-resistant bacterium are declared as a world threat to public health. Bacteriocins represent a possible answer to the present worldwide threat because of their broad or slender spectrum activity against antibiotic-resistant bacterium. Notably, despite their role in food safety as natural alternatives to chemical preservatives. In gift study, we have a tendency to studied on toxicity of bacteriocin ageing human lymphocytes.

1.1 Cytotoxicity Assay

While finding out the toxicity of nisin and colicins against civilized simian virus 40-transfected human colon (SV40-HC) and Vero monkey urinary organ (Vero) cells (Murunds et al., 2003).He had shown SV40-HC cells incontestable larger sensitivity than Vero cells. The result of bacteriocin made by *Lactococcus sp*. HY 449 against skin-inflammatory bacterium like *Staph epidermidis* ATCC 12228, *Staph aureus* ATCC 65389, *Strep pyogenes* ATCC 21059 and *Propionibacterium acnes* ATCC 6919 (Oh *et al*., 2005) and ascertained the LC50 of bacteriocin on human formative cell was 50 mg/mL at that the inhibition of cell proliferation wasn't ascertained. More Kumar *et al.,* (2014) evaluated each native and rec-pediocin from *Pediococcus acidilactici* MTCC 5101 for his or her toxicity against HepG2 (a hepatocellular carcinoma cell line), Norse deity (a cervical adenocarcinoma), MCF7 (a mamma adenocarcinoma) and Sp2/0-Ag14 (a spleen lymphoblast) cell lines and according a considerably higher toxicity of rec-pediocin and injury of body polymer in bacteriocin tested cell lines. Sevda *et al.,* (2014), had according workplace need to possess sure antineoplastic properties and investigated the antiproliferative effects of the acellular filtrate and therefore the acellular preserved filtrate of three workplace (Lactic Acid Bacteria: *Pediococcus pentosaceus*, *Eubacterium plantarum*, and *Weissella confusa*) on human body part carcinoma cell line Caco-2. The toxicity assay of bacteriocins made by *E. durans* GEn09 and *L. lactis subsp. lactis* GLc03 that was done by Todorov et al., in 2014 and obtained lowest toxicity values were 172 µg/ml and 295 µg/ml.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 4, March 2022
Impact Factor: 6.252

II. Material and Method

2.1 Toxicity Assay

In vitro pharmacology and connected sciences have historically evaluated the consequences of assorted agents on cell growth and proliferation by perceptive the changes in cell numbers and in cell morphology. Current customary approaches embody the assays conducted to live distinct cell growth and metabolism connected endpoints, like the activity of intracellular enzymes, integrity of cellular membranes, deoxyribonucleic acid synthesis, and ATP standing (Schroterova *et al.,* 2009).

2.1.1 Cell Culture Medium:

DMEM (Glutamax, Life Technologies , USA) was supplemented with low toxin craniate bovine blood serum (FBS, Life Technologies, USA), one hundred U/ml antibiotic drug (Gibco), one hundred µg/ml antibiotic (Gibco), two metric linear unit L- amino acid (Gibco), twenty five metric linear unit HEPES buffer (Life Technologies, USA) and one metric linear unit Na pyruvate, lymphocytes separation medium (Himedia , India)

2.1.2 Preparation of Lymphocytes:

4 ml of human blood sample in heparinised vials was collected. The human erythrocytes were lysed by RBC lysis buffer. The PBMCS cells were washed (centrifuge at one hundred x g for ten min) with ten metric capacity unit of sterile DMEM double. The cells were re-suspended in one metric capacity unit of sterile Dulbecco's changed eagle medium. Cells were counted by haemocytometer exploitation W.B.C. diluting fluid. (cell concentration at $1x10^6$ cells/ml with Dulbecco's changed eagle medium (DMEG) supplemented with I Chronicles of Penicillin-streptomycin answer and 100% FBS. The approximate yield of cells from 4 ml of blood varies between 10^7-10^8. The 100 µl of cell suspension seeded during a 096 well culture plate.

2.2 Determination of Cell Viability by MTT Assay

The cell viability result of Bacteriocin on traditional blood cells was evaluated by Vybrant® MTT Cell Proliferation Assay Kit (Life technologies INC.USA). The cells were plated at ~ 1×10^3cells in every well of ninety six well plate in 100 µl of various medium and zero, 20, 40, 80, 100 µl samples were superimposed to every well. Every samples were continual three times for every cell line. Cell viability determined 24h when incubation in carbonic acid gas apparatus at 37^0C. MTT (5 mg/ml in PBS) was superimposed to every well and incubated for four h. The procedure was followed in line with manufacturer's directions. The absorbance was recorded at 490 nm employing a ninety six well Multiscan Ascent (Thermo INC.USA). The restrictive result of Bacteriocin on cell growth was assessed as p.c cell viability, wherever cells while not treatment were thought of 100% viable.

III. Result and Discussion

3.1 Determination of IC50

Initially, lymphocytes were treated with the Bacteriocin and antiproliferative result was evaluated by MTT assay. As shown in (Figure one) sample 1 doesn't induce any death in traditional human blood cells.

The cytotoxic result of bacteriocin on traditional human blood cells (lymphocytes) was evaluated by MTT assay. Inhibition of cell proliferation was quantitated. A light cytotoxic result of bacteriocin was found on lymphocytes. The bacteriocin concentration fifty µg/mL to two hundred µg/mL used and the death of lymphocytes was ascertained in bacteriocin treated lymphocytes, 20 µg/mL to 160 µg/mL from concentration, when 160 µL there have been not found any changes in lymphocytes. It had shown the gentle toxicity. The IC50 worth was obtained as 160 µL +/-20 µL. The Student's two-tailed t-test was accustomed confirm applied math significance of the variations between untreated cells and cells treated with the varied concentrations of bacteriocin.

In our study, we have a tendency to evaluated the antiproliferative effects of the bacteriocin B25 of *L. pentosus* B25 on blood cells lymphocytes. The bacteriocin B25 was found to gentle inhibition the expansion of blood cells lymphocytes during a dose-dependent manner as detected by the MTT assay. Among the foremost typically used assays square measure those who square measure conducted to live the metabolic activity of viable cells exploitation quantitative chemical analysis changes supported tetrazolium salt reduction. The MTT assay utilizes 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 4, March 2022
Impact Factor: 6.252

bromide (MTT) reduction to blue colored formazan by metabolically active cells. The intensity of the color of the dissolved formazan is proportional to the amount of viable cells. The MTT assay was tested for its validity with numerous cell lines (Mossmann, 1983). A dose-dependent response has been according for the anticarcinogenic and/or antimutagenic skills of some carboxylic acid microorganism (Salminen *et al.*, 1998). Paolillo *et al*., (2009) studied the cytotoxic result of live cells of L. plantarum on Caco-2 cells and according L. Plantarum exhibited no important result on the viability of Caco-2cells twelve h post treatment compared to cells alone, when twenty four h and forty eight h of exposure, the amount of viable cells was 92 ± 3d and 91 ± 3-d severally, each over the controls (73.6 ± 2.9% and 68.25 ± 2.8%, respectively).

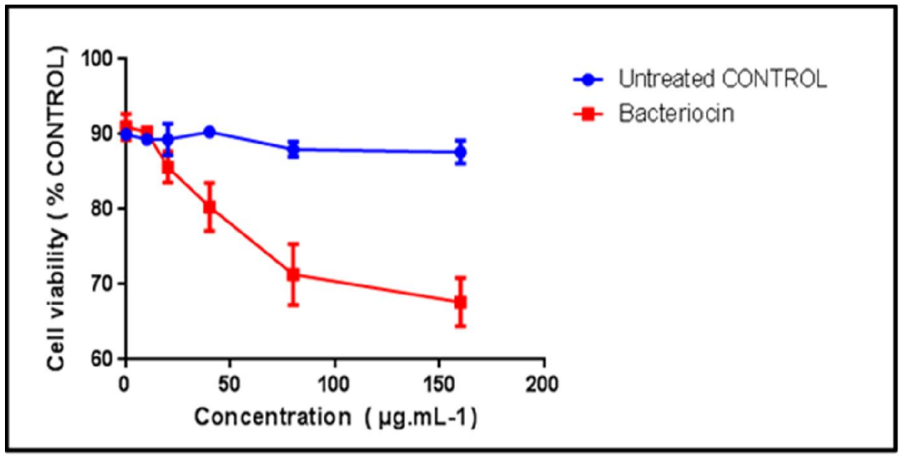

Figure 1: Result of bacteriocin on growth in Human blood (lymphocytes) cells.

IV. Conclusion

Bacteriocins have properties because of that they'll be used fora broad vary of food and medical applications. Thus, the protection of bacteriocins needs a lot of attention. Antimicrobial activity of bacteriocins has been wide studied; but, there's an absence of accessible in vitro and in vivo knowledge concerning safety and toxicity of bacteriocins. so as to concern the protection of bacteriocins, investigation of their immunogenicity and each in vitro and in vivo toxicity is critical. In fact, for this purpose, totally different assays square measure required to be performed to assess their toxicity in organism cells, ability to induce caspase-mediated cell death, growth restrictive result, lysis activity, acute and subchronic toxicity, etc. in our finding, bacteriocin B25 had shown the gentle toxicity against human somatic cell lymphocytes.

References

[1]. Farkas-Himsley H., Cheung R. (1976). Bacterial proteinaceous products (bacteriocins) as cytotoxic agents of neoplasia. *Cancer Res.* 36, 3561–3567.

[2]. Hetz C., Bono M. R., Barros L. F., Lagos R. (2002). Microcin E492, a channel-forming bacteriocin from *Klebsiella pneumoniae*, induces apoptosis in some human cell lines. *Proc. Natl. Acad. Sci. U.S.A.* 99, 2696–2701. 10.1073/pnas.052709699

[3]. Kumar B., Balgir P. P., Kaur B., Mittu B., Chauhan A. (2012). *In vitro* cytotoxicity of native and rec-pediocin cp2 against cancer cell lines: a comparative study. *Open Access Sci. Rep.* 1, 316–321. 10.4172/2153-2435.1000183

[4]. Kumar, B., Balgir, P., Kaur, B., Mittu, B. and Chauhan, A. (2014). In vitro cytotoxicity of native and rec-pediocin CP2 against cancer cell lines: a comparative study. Probiotics in dairy foods: a review. *Nutr Food Sci.* 44, 71–88. http://dx.doi.org/10.4172/scientificreports.316 Khan SU.

[5]. Murinda, S.E., Rashid, K.A., Roberts, R.F. (2003). In vitro assessment of the cytotoxicity of nisin, pediocin, and selected colicins on simian virus 40-transfected human colon and Vero monkey kidney cells with trypan blue staining viability assays. *Journal Food Protec*. 66(5), 847-53.

[6]. Salminen, S. (1999). Probiotics: Scientific support for use. *Food Technology*. 53(11), 66.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 4, March 2022
Impact Factor: 6.252

[7]. Schroterova, L., Kralova, V., Voracova, A., Haskova, P., Rudolf, E., Cervinka, M. (2009). Antiproliferative effects of selenium compounds in colon cancer cells: comparison of different cytotoxicity assays. *Toxicol In Vitro*. 23, 1406–1411.

[8]. Sevda, E.R., Koparal, A., Kivanc, M. (2015). Cytotoxic effects of various lactic acid bacteria on Caco-2 cells. *Turkish Journal of Biology*. 39, 23-30. DOI: 10.3906/biy-1402-62.

[9]. Todorov, S. (2014). P3-17 Cytotoxicity of Bacteriocins Produced by Four *Enterococcus faecium* Strains Isolated from Cheese. International association for food protection. *Technol. Biotechnol*. 44(3), 435–439.