

India. It was maintained as broth and agar cultures in Brain Heart Infusion medium at 37°C.

### **Production and purification of native pediocin CP2**

*P. acidilactici* MTCC 5101 was grown overnight in MRS broth; pH 6.5 at 37°C. Native pediocin CP2 was purified by conventional method of adsorption-desorption [11]. Bacteriocin preparation was then filter sterilized using Millipore 0.45 µm filters [12].

### **Pediocin activity assay**

Bacteriocin activity in native and recombinant cell cultures can also be determined using spot-on-lawn assay [13]. It was carried out by spotting 5 µl CFS or dilutions of pure bacteriocin preparations on MRS bottom agar plates overlaid with 3-4 ml TGE soft agar containing 6 log units of *L. monocytogenes*. Plates were incubated at 37°C for 24 h and inhibition zones were scored. Bacteriocin titre was expressed as reciprocal of the highest dilution showing a definite zone of inhibition or cell lysis in the resultant lawn culture.

### **Production and purification of recombinant pediocin CP2**

Recombinant pediocin was expressed using T7 driven pET32(b)-

tissues were fed with the fresh medium. Cell counts were taken using haemocytometer. After the required exposure time, MTT assay was carried out to determine overall cell viability.

### **MTT cell viability assay**

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was carried out according to the protocol given by Shashi et al. [15]. Viability of the cells exposed to bacteriocin, was measured as a % of control. The absorbance was measured at 550 nm using a microplate reader. The results were expressed as mean ± standard deviation. Statistical significance was determined by one-way ANOVA test. P < 0.05 was considered significant.

### **DNA fragmentation assay**

Fragmentation of the genomic DNA was studied in the most

in the treatment of cancers especially of Hodgkin disease and germinal cancers for more than 30 years [22]. Pyocins F and S produced by *Pseudomonas aeruginosa* show structure homology with bacteriophage tails [23]. Antineoplastic activity of pyocin has been established against mouse hepatocarcinoma and lymphoblastic leukemia using HepG2 and Im9 cell lines, whereas human fetal foreskin fibroblast was unaffected [24]. Its uptake is possibly mediated by iron-related receptors in bacterial cells [25] and transferrin receptors in mammalian cells [26].

This mechanism is reinforced by the fact that iron deprivation stops cell division in G1/S and leads to apoptosis in some neoplastic cell lines [27]. However, detailed *in vivo* investigation is required on potential use of pediocins as therapeutic agents or prophylactic compounds.

Carl Vogt [28] described the principle of apoptosis which shows it as a programmed death of cells, which may occur even in multicellular organisms. Various biochemical changes such as cell membrane damage, cell shrinkage, nuclear fragmentation, chromatin condensation and genetic DNA fragmentation take place during apoptosis. DNA fragmentation takes place at the end of apoptosis, which includes activation of calcium and magnesium dependent nucleases that degrade genomic DNA of susceptible cells. Currently used anticancer drugs have been shown to induce apoptosis in susceptible cells [29]. Nuclear DNA of cells that have entered in the phase of apoptosis shows a characteristic ladder pattern of oligonucleosomal fragments, which is regarded as the hallmark of apoptosis [14]. A series of studies have provided convincing evidence suggesting that the antimicrobial peptides or bacteriocins produced by lactic acid bacteria inhibit growth of cancer cells [30]. Inhibition of cell proliferation by colicins [17], microcin [18], pediocin [19] and pyocin [20] has been established in breast carcinoma, breast adenocarcinoma, osteosarcoma, leiomyosarcoma, fibrosarcoma, T cell lymphoma, cervix carcinoma, Burkitt lymphoma, pulmonary carcinoma, colon adenocarcinoma, lymphoblastic leukemia, and hepatocarcinoma.

The results presented here indicate cytotoxic effect of rec-pediocin on various cancerous cell lines tested in the study. The cytotoxic effect on cancerous cells from human origin was also reported earlier [31].

The uniqueness of the bacteriocins lies in their interaction with the cell surface without penetrating the target cells, yet affecting cell division and DNA synthesis [32]. Bacteriocins are highly specific in their membrane interaction which is related to the unique receptors found in different bacterial species or types [33]. Preliminary experiments with rec-pediocin have shown its cytotoxicity against cancerous cell lines and which is attributed through the induction of programmed cell death or apoptosis. In future, this information could be integrated and exploited to fully explore the suitability of rec-pediocin as *in vivo* therapeutics.

## References

1. Kheadr E, Bernoussi, N, Lacroix C, Fliss I (2004) Comparison of the sensitivity of bacteriocins. *International Dairy Journal* 14: 1041-1053.
2. Le Blay G, Lacroix C, Zihler A, Fliss I (2007) *In vitro* inhibition activity of nisin A, nisin Z, pediocin PA-1 and antibiotics against common intestinal bacteria. *Letts Appl Microbiol* 45: 252-257.
3. Dabour N, Zihler A, Kheadr E, Lacroix C, Fliss I (2009) *In vivo* study on the effectiveness of pediocin PA-1 and *Pediococcus acidilactici* UL5 at inhibiting *Listeria monocytogenes*. *Int J Food Microbiol* 133: 225-233.
4. Bernbom N, Jelle B, Brogren CH, VJ Eén FK, H5583 120.689d0tici m Td(. ta 1 Tf10.562 0 Td(. Int 0(Ap11 Micrcteria. ): 225-233.)TJEMC ET/Span 70/Lang (en)/MCID 566 B

