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Research article

MOLECULAR CHARACTERIZATION OF MAP30 GENE FROM *MOMORDICA CHARANTIA L.*

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ABSTRACT: Natural products especially from plants have been used for the treatment of various diseases. Momordica charantia or bitter melon, grows in the tropical area is popularly consumed as vegetables and has high medicinal values .It is one of the most promising alternative medicines used as anti-HIV, anti-ulcer, anti-inflammatory, antileukemic, anti-microbial, anti-diabetic, and anti-tumor. Proteins like momordin, alpha- and beta-momorcharin and cucurbitacin B of *Momordica charantia* were also tested for possible anticancerous effects .Chemical analogs of these proteins have been developed, patented, and named MAP30.The MAP30gene was amplified from *Momordica charantia* leaves by Polymerase Chain Reaction .The resultant product was amplified **approximately 861 bp.**

Key words: Gene, *Momordica charantia*, Charecterization

INTRODUCTION

A relatively common food item, *Momordica charantia* (Bitter melon) was traditionally used for incredible array of conditions by people in tropical regions. Further, it is an excellent source of health benefiting flavonoids. It also contains a good amount of vitamin-A. Together, these compounds help act as protective scavengers against oxygen-derived free radicals and reactive oxygen species that play a role in various diseases, tumours and aging processes. The leaves and fruits have both been used occasionally to make teas and beer or to season soups in the Western world. Bitter melon as an unripe fruit is commonly eaten as a vegetable and has been used as a folk remedy for tumors, asthma, skin diseases, and blood sugar levels. In 1980s, the seeds were investigated in China as a potential contraceptive. It contains an array of novel and biologically active phytochemicals, including (triterpenes), proteins and steroids. Triterpenes have been clinically demonstrated to possess the ability to inhibit the *guanylate cyclase* enzyme (Taylor L., 2002). This enzyme is thought to be linked to the mutagenic signaling of leukemia, and solid tumors (Taylor L., 2002). Phytochemicals that are (Barbieri *et al.*,, 1993)named, momordin, cucurbitacin *B* and alpha- and beta - momorcharin, of these proteins was developed (MAP- 30) and reported to be able to inhibit prostate tumor growth (Wang Y. X., Neamati N.1999). MAP30 is a single-stranded protein containing 263 amino acids 30 kDa!, reported to possess anti-HIV and anti-tumor activity (Lee-Huang *et al.*, 1995a.).

MATERIALS AND METHODS

DNA extraction from *Momordica charantia* leaves

The genomic DNA was extracted from the fresh leaves of *Momordica charantia* by Cetyltrimethyl Ammonium Bromide (CTAB) method. Fresh plant material (0.5g) was ground in a pre-sterilized pestle and mortar with liquid nitrogen until a fine powder was obtained and transferred to sterile eppendorf tube. To this added 2ml of pre-heated (65ºC) extraction buffer (100 mMTris (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% PVP, 0.1% Mercaptoethanal) and incubated for 1 hour in water bath at 65ºC. Then tubes were centrifuged at 10,000 rpm for 10 min at room temperature. The supernatant was collected into new eppendorf tubes and to this added equal volumes of chloroform and Isoamyl alcohol (24:1) and centrifuged the tubes at 10,000 rpm for 10 min. After centrifugation take the upper aqueous phase with the help of micropipette and transfer into a clean new eppendorf tube.

To this add equal volume of ice cold isopropanol then incubated at -20ºC for 1hour. After incubation, the tubes were taken out and centrifuged at 13,000 rpm for 20 min at 4ºC. The supernatant was discarded and the pellet was washed with 70% alcohol and again centrifuged at 13,000 rpm at 4ºC for 10 min, discarded the supernatant, air dried the pellets and dissolved in 100µl of sterile distilled water. The DNA samples were quantified by nano drop spectrophotometer and stored at -20ºC for further use.

Amplification of MAP30 gene by PCR

Polymerase Chain Reaction (PCR) PCR was performed in 25 µl of reaction mixture by using 1X PCR reaction buffer, 2.5 mM of MgCl2, 10mM of dNTPs, 10 pmols of each primer (*primer-F:* 5' ATGGTGGTATGCTTACTACTTTCTT-3' and *Primer- R:* 5'- CTTGGGGAATCTGTTGTGAATTGA- 3'), 2.5 U/ µl of Taq DNA polymerase (Fermentas, USA) and 100 ng of DNA template. The amplification was performed in a PCR machine (Biorad, USA). The conditions for amplification of MAP30 gene are; initial denaturation of 94°C for 5min, denaturation of 94°C for 40 sec, annealing of 55°C for 40 sec, extension of 72°C for 1min followed by 35cycles and final extension of 72°C for 7min. Analysis of PCR products by Agarose Gel Electrophoresis of DNA was performed as described by Sambrook *et al.*,2001 The amplified products was recorded through 1% agarose gel and visualised in a UV gel documentation system (Alpha Innotech, USA) after staining with ethidium bromide (10 mg/ml).

RESULTS AND DISCUSSION

The genomic DNA was isolated from the fresh leaves of *Momordica charantia* by Cetyltrimethyl Ammonium Bromide (CTAB) method. The amount of DNA and purity of DNA (260/280 ratio) was measured in Nanodrop spectrophotometer. The genomic DNA was subjected to PCR by using specific primers of MAP30 gene. The amplified PCR product was amplified approximately at 861 bp (fig.1). The band size was similar to the expected band for MAP30. *Momordica charantia* has been suggested to potentiate function of natural killer cells in cancer patients (Pongnikorn, S.2003, Cunnick, J. E., 1990). More recently, MAP30, a 30-kDa protein isolated from seeds of *Momordica charantia*, has shown promising effects for treating tumors and HIV infection (S. Lee-Huang, H. C. Chen *et al.*, 1995). Recent interest in MAP30 has been stimulated by reports (S Lee-Huang *et al.*,1995) of potent anti-tumor activity against human cancer cell lines and inhibition of HIV-1 infection in lymphocytes and monocytes, and viral replication in HIV-infected cells. MAP30 toxicity is specific to tumor-transformed or viral-infected cells. It shows no adverse effects on normal cells, making it a candidate for clinical applications.

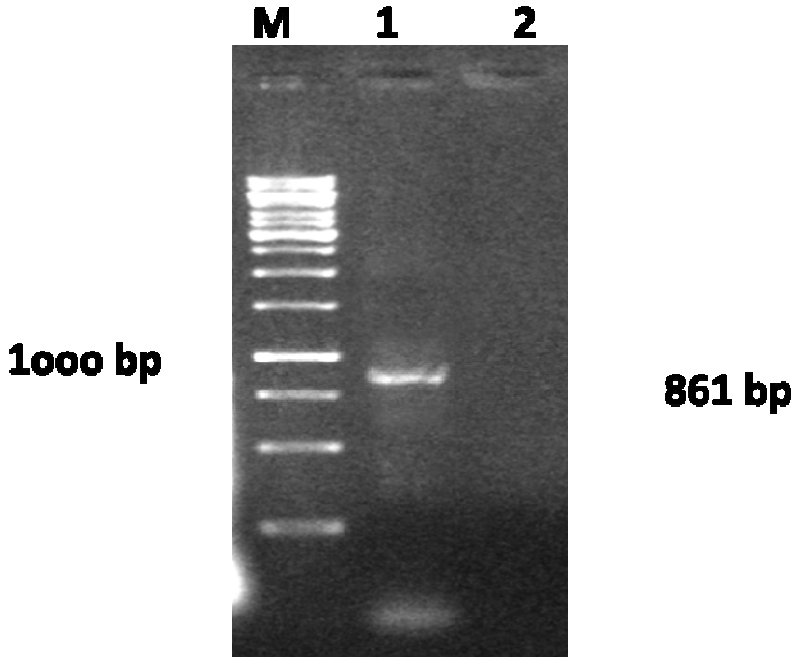

Fig.1: Identification of MAP30 gene in 1% agarose gel electrophoresis.

Lane M: 1 Kb DNA Ladder, 1: MAP30 PCR amplicon

CONCLUSION

The objective of this study was to develop and optimize a PCR method that permits sensitive and accurate detection method for MAP30 gene in *Momordica charantia*.

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