e - ISSN - 2348 - 2168
Print ISSN - 2348 - 215X

Acta Biomedica Scientia

Journal homepage: www.mcmed.us/journal/abs

ANTIMICROBIAL ACTIVITY OF WHEY PROTEIN FROM MILK AGAINST DIFFERENT MICROBIAL PATHOGENS

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Article Info

Received 29/01/2015
Revised 16/02/2015
Accepted 19/03/2015

Keywords :-
Milk, Whey protein,
Antibacterial activity,
Antifungal activity.

ABSTRACT

The work was carried out to isolate, characterize and to find out the antimicrobial activity of the whey protein from cow milk. In this study proteins were isolated and lyophilized and characterized by thin layer chromatography and SDS PAGE. Meanwhile, its antimicrobial activity was tested under in vitro conditions against the antibacterial pathogenic strains such as *E.coli, S.aureus, K.pneumoniae, B.subtilis, P.aeroginsa* and pathogenic antifungal strains such as *Aspergillus niger, A. flavus* and *candida albicans.* In this study, the derived whey protein showed an maximum and minimum antimicrobial activity against all the microorganisms both bacteria and fungi. According to the zone of inhibition (ZOI) the maximum zone was measured against *S.aureus and A. niger*.

INTRODUCTION

Milk is a complex medium containing a variety of proteins, lipids, carbohydrates, vitamins and other molecules of functional or bioactive properties. Generally, milk is rich in proteins that are classically grouped into two main classes, such as major milk proteins (α,β and k-caseins Example – lactalbumin and lactoglobulin) and minor milk proteins such as, lysozyme, lactoferrins, lactoperoxidase and immunoglobulins. Milk proteins play an important role in mammals due to their direct and indirect antimicrobial activity in addition to other important physiological and health promoting functions [1]. Researchers reported that some peptides derived from milk protein have been found to be active against a broad range of pathogenic organisms (Such as, *Escherichia coli*, *Helicobacter* sp., *Listeria* sp., *Salmonella* sp., and *Staphylococcus* sp.,) yeast and filamentous fungi [2,3].

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Whey is a liquid by-product and it is widely accepted to contain many valuable constituents. These include especially proteins that possess important nutritional and biological properties particularly with regard to promotion of health, as well as prevention of diseases and health conditions [4]. They have an immunomodulatory, antibacterial, antiviral and antifungal properties, and it is proteins are primarily associated with whey proteins such as immunoglobulins, lactoferrin, lactoperoxidase and lysozyme [5,6]. Thus the present study has carried out to isolate, characterize and find out the antimicrobial activity of the whey protein present in cow milk.

MATERIALS AND METHODS

Collection of sample

Milk sample were collected from nearby area of Katpadi, Vellore, Tamil Nadu, India. Samples are taken freshly in sterilized air tight containers; that were brought to the laboratory for whey protein extraction and further processing.

Extraction of Whey protein

1000ml of milk was taken in a sterilized conical flask and the pH were adjusted to 4.5 by the addition of

Hydrochloric acid. Then, it was centrifuged at 8000 rpm for 15mts to remove the precipitated whey. Then the supernatant was filtered on a Whatman No.1 Filter paper and the collected whey extract was lyophilized and it was stored in a deep freezer at -30 C for future use [7].

Estimation and Characterization of whey protein

The protein content in the sample was estimated by using Bradford assay and it was characterized by TLC and SDS page analysis.

Antimicrobial Activity

The pathogenic bacteria such as, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Bacillus subtilis* and fungi such as, *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* were used as a test organism in this study. The antibacterial assay was done by using well diffusion assay method in Muller Hinton agar plates. After the incubation period, the zone of inhibition (ZOI) was measured and noted.

RESULTS AND DISCUSSION

Generally the release of bioactive peptides from milk proteins in the gastrointestinal tract results from the action of digestive enzymes such as pepsin and pancreatic enzymes (trypsin,chymotrypsin, carboxy- and aminopeptidases). The efficiency of physiological activity of biopeptides depends on their ability to maintain integral state during transport to the various functional systems of the body [8,9]. Many well-known bioactive peptides have been generated *in vitro* by the action of digestive enzymes, mostly pepsin and chymotrypsin. Other digestive enzymes and combinations of proteinases (alcalase, chymotrypsin, pancreatin, pepsin, thermolysin) as well as enzymes derived from bacteria and fungi are also used for the production of bioactive peptides from various sources. In this study, the whey protein concentration was estimated by Bradford assay method. From the standard curve plotted, the protein content was estimated to be 4 mg/ml, the TLC showed a clear band (Fig 1) and the molecular weight of the protein was found as 18 KDA (Fig 2) The antimicrobial activity results of our study also clearly demonstrated the effect of whey protein, by the maximum and minimum zone of inhibition (ZOI) against some common bacterial and fungal pathogens (Table 1).

Table 1. Antimicrobial activity of whey protein against test pathogens

Test Pathogens	Zone of Inhibition (ZOI) in mm
Bacteria	
Escherichia coli	++
Klebsiella pneumonia	+
Staphylococcus aureus	+++
Pseudomonas aeroginosa	++
Bacillus subtilis	++
Fungi	
Aspergillus niger	+++
Aspergillus flavus	++
Candida albicans	+

\+ : 2mm ++ : 3mm
+++ : 4mm - : No Zone

Figure 1. Zone of inhibition of whey protein against bacterial pathogens

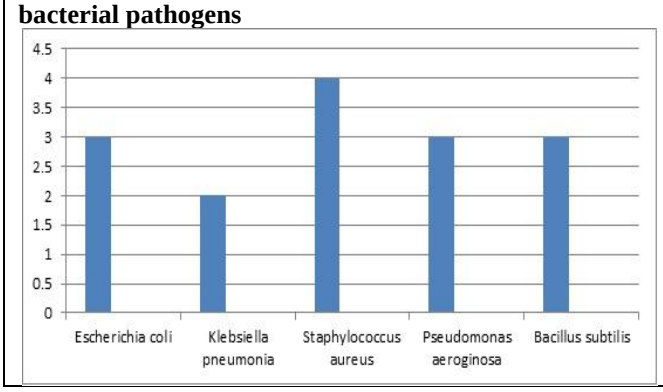


Figure 2. Zone of inhibition of whey protein against fungal pathogens

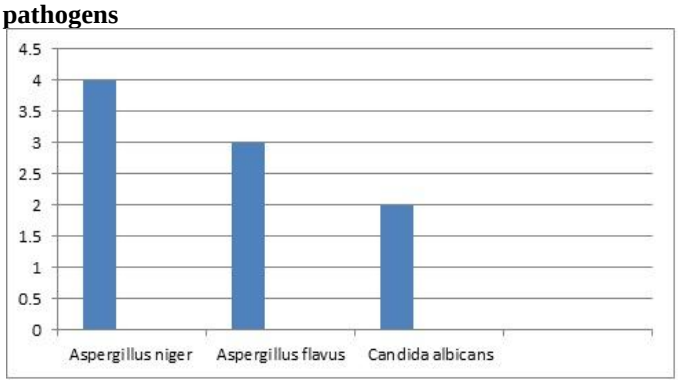


Figure 3. Thin Layer Chromatography analysis

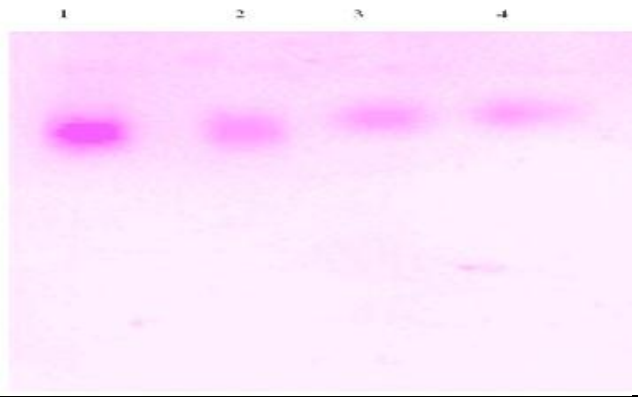
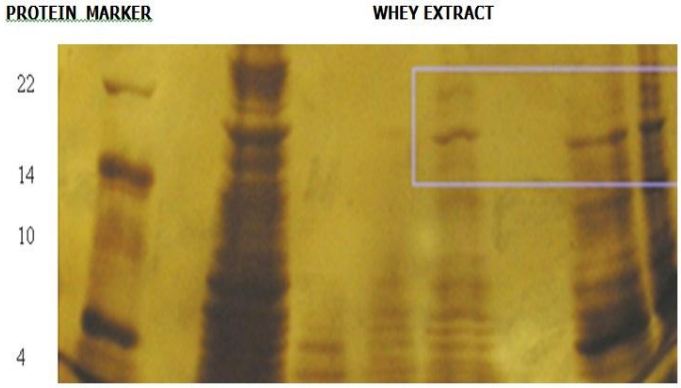


Figure 4. SDS PAGE – (Molecular weight estimation)



Among the pathogens the maximum zone was observed against to *Staphylococcus aureus* and *Aspergillus niger*. The results were very similar to the authors Previous study results also reported that the whey protein from buffalo milk showed highest zone of inhibition against *E.coli*, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae* [10]. The antimicrobial effect of the whey protein may be due to the presence of protieaneous substances, because It is well established that the capacity of the peptide to bind to the surface of Gram-negative bacteria results in the release of lipopolysaccharide (LPS) from the bacterial cell wall, which causes damage to cell walls and other morphological changes [11].

CONCLUSION

The study as well demonstrated the effect of whey protein from milk sample have potent antibacterial activity against some bacterial and fungal pathogens and the study revealed that whey protein from milk can be used for production of antibacterial peptides and can be used for treating bacterial and fungal infections in mammals by after evaluating their antimicrobial activity under *invivo* condition.

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