International Journal of Engineering Research & Technology (IJERT)

Studies on Encapsulation of Curcumin in Plasmolysed Cells of Saccharomyces Cerevisiae

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***Abstract*— Microencapsulation is a potential technique to load and deliver variety of therapeutic agents. Curcumin is hydrophobic bioactive molecule which has fast serum clearance rate after delivered into the human body. In the present study, Curcumin is encapsulated in plasmolysed cells of *S.cerevisiae* prepared using NaCl. Fourier Transform Infrared Spectroscopy (FTIR) was used to characterize the identify the action of curcumin with the plasmolysed cells. The encapsulation efficiency of curcumin in plasmolysed *S.cerevisiae* cells was found to be 0.6%(w/w). The antioxidant activity of Curcumin released from the encapsulated cells was determined by employing DPPH radical scavenging assay. Microcapsuled *S.cerevisiae* cells were further subjected to curcumin release and kinetic studies. Different mathematical models were used to know the type of release. Curcumin encapsulated in the cells of *S. cerevisiae* and showed controlled release and it could be used for various therapeutic applications.**

Keywords—Curcumin, Plasmolysed cells, Encapsulation

I. INTRODUCTION

Bioactive compounds present in various medicinal plants play a significant role in human health care applications. Since ancient times, various herbals plants with therapeutic properties are used in daily preparation of foods. Among them, turmeric, scientifically *Curcuma longa L*., is one such important ingredient added in Indian cuisines which has a potential active ingredient called curcumin. Owing to its numerous medicinal properties, *C.longa* is called yellow gold. It is been proved by many research findings that curcumin has exceptional properties like antimicrobial, antioxidant, anti-inflammatory and anticancer activities [1]. Hence, it is used in treatment of the common chronic diseases like arthritis, autoimmune disease, cardiovascular disease, hypertension [2].

Curcumin is extracted from rhizome of *C.longa* and it is a natural polyphenolic compound and named as diferuloylmethane [3]. Experimental evidences and molecular docking studies showed that curcumin binds with multiple targets in the human body and exerts its functions. Despite its many proven health beneficial effects, full potential of curcumin is notshown inside the human body. This is mainly due to its poor bioavailability in the human body [4] . Curcumin shows poor absorption, fast metabolism, quicker elimination from the body [5].

Some of the bioactive molecules are susceptible to various factors like pH, temperature, solvents etc. Because of their susceptible nature, it is difficult to harvest full therapeutic effect. Encapsulation of bioactive compounds in a physical enclosure protects the bioactive compounds from potential harsh environment. Curcumin is one such bioactive compound limited by its highly susceptible nature [6].

Many methods such as combining with other bioactive compounds like piperin, nanomicelle formulation, encapsulation in various polymers, chemical modification of curcumin structure etc., were done to improve the bioavailability of the curcumin [7]. Slow release of curcumin and use of adjuvants that delay the metabolism of curcumin are the most promising methods.

Saccharomyces cerevisiae (yeast) is a single cell eukaryotic organism belongs to group of basidiomycetes under fungi. Yeast is best known for its fermentative properties and has been used in fermentation of foods and production of many industrial products [8]. Owing to its non-pathogenic nature, *S.cerevisiae* is grouped under GRAS status organism [9]. Encapsulation of bioactive molecules in *S.cerevisiae* cells is found to be a viable method as it is a low cost and safe. In the present study, curcumin is encapsulated in plasmolysed cells of *S.cerevisiae* and release kinetics study was carried out.

II. MATERIALS AND METHODS

A. Samples and Chemicals

Saccharomyces cerevisiae (MTCC No.3821) was purchased from IMTECH, Chandigrah. Curcumin was purchased from Sigma Aldrich, USA. Malt extract agar, 2,2’ diphenyl-1-picrylhydrazyl (DPPH), Sodium Chloride and other chemicals used in this study were of analytical grade and procured from Himedia, Mumbai, India.

B. Culture Preparation

The yeast culture was maintained on malt extract agar media. Subculturing was done after every 5 days. The culture was grown in malt extract liquid media by incubating for 48 hours at 30°C and thereafter storing in a refrigerator at 4°C till further use.

C. Plasmolysis of S.cerevisiae using NaCl

Yeast cells were grown in malt extract liquid media for 24 hours at 30°C in an orbital shaker. Then the cells were harvested after centrifugation and washed with sterile PBS buffer for three times. The collected cell pellet was resuspended in different concentrations of sterile sodium

Published by, www.ijert.org

ISSN: 2278-0181

chloride solution (10%, 20%, 30% NaCl(w/v)) and stirred at 200 rpm for 2 hours at room temperature. The pellet was collected, washed with sterile water to remove impurities. The resultant pellet was freeze dried and stored at 4°C for further use.

D. Encapsullation of curcumin in plasmolysed S.cerevisiae cells

The freeze dried plasmolysed cells of *S.cerevisiae* were suspended in PBS buffer and to this solution, a known concentration of curcumin dissolved in methanol was added. Then the tube containing the above mixture was kept at room temperature for 2 hours at 200 rpm shaking. The cells were harvested by centrifugation at 6000 rpm. The cell pellet was washed for three times using sterile distilled water to remove the free curcumin. The cells with encapsulated curcumin were freeze dried and stored for further analysis.

E. Encapsulation Efficiency of Curcumin

The encapsulated cells were resuspended in deionized water and methanol in the ratio of 1:4 and stirred at room temperature for 48 hours. Then the supernatant was collected after centrifugation. The amount of curcumin was quantified spectrophometeric method and standard graph was used for calculating amount of curcumin. The percent encapsulation efficiency (EE) was calculated using the following formula:

$$\%EE = \frac{\text{Mass of encapsulated curcumin}}{\text{Mass of the encapsulated cell}} \times 100$$

F. FTIR spectrocopy

Curcumin encapsulated plasmolysed cells were subjected to Fourier Transform Infrared Spectroscopy (FTIR) analysis. The spectrum was recorded from 4000cm^{-1} to 400 cm^{-1} and per cent transmission was recorded.

G. Curcumin release kinetics

The curcumin encapsulated plasmolysed *S.cerevisiae* cells (20 mg) were suspended in PBS buffer (5 ml) and stirred at room temperature. After every 30 minutes, 3ml was removed and centrifuged to obtain released curcumin. From the supernatant, curcumin released was measured using spectrophotometer at 413 nm. The absorbance values were tabulated to study the release pattern.

H. Antioxidant Assay

The antioxidant activity of the curcumin was determined using 2,2' diphenyl-1-picrylhydrazyl (DPPH) as free radical source [10]. 0.1mM DPPH solution in methanol was mixed with the supernatant collected from the plasmolysed *S.cerevisiae* cells containing curcumin and incubated for 30 min in dark. Absorbance was measured at 517 nm and methanol was used as blank. The per cent free radical scavenging (inhibition) was calculated using the formula:

$$\% \text{ of inhibition} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

III. RESULTS AND DISCUSSION

S.cerevisiae cells were cultured in malt extract liquid media and collected after 24 hours. The cells collected were divided into two groups, one group was retained as non-plasmolysed cells and the other groups was subjected to plasmolysis using NaCl solution. After plasmolysis, the cells were collected and freeze dried for further use. Plamsolysis of cells in hypertonic solution of sodium chloride results in leakage of water from the cells and shrinkage. The space created inside the cell is available to accommodate other molecules like hydrophobic drugs or bioactive compounds [10].

The encapsulation efficiency of plasmolysed and non-plasmolysed cells was found to be 06% and 0.06 % respectively. These results indicated that there is an improvement in the encapsulation when the cells are plasmolysed. The FTIR spectrum analysis of curcumin encapsulated *S.cerevisiae* cells showed shift in the characteristics functional groups at 3501cm^{-1} which indicates the interaction of curcumin with yeast cells components (Figure 1).

Figure 1: FTIR spectrum of curcumin encapsulated plasmolysed cells of *S.cerevisiae.*

The study on release of curcumin from plasmolysed cells of *S. cerevisiae* was carried out in PBS buffer at fixed interval of time and the released curcumin was measured using UV-visible spectrophotometer at 423 nm. The amount of curcumin released from plasmolysed cells was calculated using standard graph. The amount of curcumin released from plasmolysed *S.cerevisiae* cells was fitted in four major mathematical models:

(i) First- order kinetics (ii) Zero –order kinetics (iii) Hixson – Crowell model (iv) Higuchi model. Based on the release coefficient (R^2), the curcumin released from the plasmolysed cells followed zero order kinetics (Table1).

Table 1 The curcumin release kinetics parameters of different mathematical models

MODEL	R^2	K
Zero order	0.95	0.038
First order	0.69	0.002
Higuchi model	0.68	0.223
Hixson Crowell	0.60	0.005

The released curcumin over period of time from plasmolysed *S.cerevisiae* cells was recorded and plotted as graph (Figure 2). From the graph, it is inferred that the curcumin release from the cells is slow and increases with time. The *S.cerevisiae* cell is made up of beta-glucan network and chitin and is associated with mannoprotein layer. The composition and characteristics of yeast cell wall help to load variety of molecules and slow release [12].

Figure 2: Release of curcumin from plasmolysed cells of *S.cerevisiae*

Antioxidant capacity of curcumin released from plasmolysed and non-plasmolysed yeast cells was carried out using DPPH based free radical scavenging assay. The result showed that curcumin encapsulated in plasmolysed yeast cells showed higher free radical scavenging capacity compared to the non-plasmolysed cells (Figure 3). The slight increase in antioxidant capacity may be due to the protection given by the plasmolysed cells than non-plasmolysed cells. Many reports are available showing that the encapsulated curcumin show better activity than free curcumin [13,14].

Figure 3. Antioxidant capacity of curcumin released from plasmolysed and non plasmolysed cells of *S. cerevisiae.*

IV. CONLCUSION

Curcumin was encapsulated in plasmolysed cells of *S.cerevisiae* showed good encapsulation percentage. The shift in the functional groups of curcumin in the FTIR spectrum indicated the functional group interaction between plasmolysed cells of *S.cerevisiae* and curcumin. Encapsulation efficiency was found to be more in plasmolysed cells than nonplasmolysed cells. Plasmolysis helped the cells to take up more curcumin. Based the curcumin release data, it is concluded that curcumin is released from plasmolysed cells of *S.cerevisiae* slowly and followed zero order kinetics. Hence, curcumin encapsulated in plasmolysed cell can be used for various therapeutic and food applications instead of free curcumin.

ACKNOWLEDGMENT

The authors thank Kumaraguru College of Technology, Coimbatore for supporting to carry out this research work.

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