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Production, Estimation and Protein Network Studies of Bioethanol Fermented Media from *Maduca Longifolia* Produced by Surface Fermentation using *Candida Albicans*

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Abstract

Candida albicans is a fungus that is used as industrially important species in fermentation productions from past several decades. In the present experimentation, Madhuca longifolia dried flowers with Candida albicans, *Saccharomyces cerevisiae, and Pseudomonas aeruginosa respectively are used as cultures and was analyzed for bioethanol production. Bioethanol produced in M. longifolia+yeast with about 40% was more compared with other samples. M. longifolia+ Pseudomonas showed 20% ethanol, M. longifolia+ candida 14% and M. longifolia fermentation, only 5%. There is a good interaction in orange, strawberry, cucumber, apple, cabbage and papaya compared with other samples like grapes, general spinach, red spinach and fenugreek. There is a presence of 24 mg/ml proteins in fermented product of M. longifolia with Candida. The samples have band observation at same distance with standard. Hence the standard marker (Albumin) has 66.5 kD, the fermented product may also have protein with molecular weight of 66.5 kD. The protein interaction studies of albumin with several other proteins of ethanol production showed network with AHSG (alpha-2-HS-glycoprotein), APOA1 (Apolipoprotein A-I), EGF (Epidermal growth factor), GSR (Glutathione reductase), PROS1 (Protein S (alpha)), IGF1 (Insulin-like growth factor 1 (somatomedin C)) etc. All these proteins are involved in body factors in the alcohol production and metabolism.*

Keywords: *Bioethanol production, protein network, Candida albicans, Saccharomyces cerevisiae, Pseudomonas aeruginosa*

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INTRODUCTION

Candida albicans is an opportunistic pathogenic fungus and also an industrially important species in the industrial microbiology [1, 2]. Apart from Candida, the other important species used in the alcohol fermentation is yeast (*Saccharomyces cerevisiae*) [3]. *Madhuca longifolia* (Common Name in Hindi: Mahua) is an important medicinal plant from *Sapotaceae* where the dried flowers are used in the alcohol production in the states like Andhra Pradesh, Chhattisgarh, Maharashtra and some tribal communities [4, 5]. In the fermentation process, the production of bioethanol is been isolated from *Madhuca longifolia* flowers by using *Saccharomyces cerevisiae* as microbial species. The experimentation by Gedela *et al*. showed that test 1 (*S.cerevisiae* + Mahua flowers + Media), test 2 (Mahua flowers + Media) and control (media) showed production levels of bioethanol to about 46.6, 24.6 and 0% respectively [6].

Madhuca longifolia J.F. Macbr. (Sapotaceae) is an Indian tropical tree used for nutritive and medicinal products [7, 8]. The flower of *Madhuca longifolia* is a suitable alternative media due to cheaper carbohydrate source with 40 to 70% sugar content which is suitable for the growth of several microbial species like *Candidaalbicans, Saccharomyces cerevisiae, Pseudomonas aeruginosa* etc. [9]. In the present work, the dried flower of plant species like *Maduca longifolia* is selected for the study production, estimation and protein network studies of bioethanol fermented media produced by surface fermentation using *Candida albicans.*

MATERIALS AND METHODS

Ethanol Production, Estimation and Protein Studies by *Madhuca longifolia* in Cultures

About 10 g of *Madhuca longifolia* dried flowers has to be taken in four different bottles. About 100 ml of the sterile and double distilled water has to be added in the bottles and 10% of the microbial cultures (*Candida albicans, Saccharomyces cerevisiae, Pseudomonas aeruginosa)* are added and one is kept as blank. The inoculated bottles are kept for fermentation process for about 48 h at 25°C [10]. The production of ethanol is calculated by potassium dichromate method and the protein present in the fermented media is estimated using ninhydrin method. The centrifuged sample should be further separated with column chromatography, and all 12 eluents were quantified using SDS PAGE.

Protein Interactions Using X-Ray Analyses

An X-ray film is to be taken for the conduct of experiment and the process has to be conducted in a dark room. On a clean tile, mix one drop of aqueous extract of selected fruits and vegetables (Apple, straw berry, cucumber, grape, orange, papaya, normal spinach, red spinach, fenugreek and cabbage respectively) with one drop of purified protein sample of fermented product of *Madhuca longifolia +Candida albicans.* Place one drop of mixed sample on X-ray film and leave for about 10 min. Finally, wash the X-film with water gently without touching with hands and observe the film for interaction [11].

Protein-Protein interaction was analyzed with KEGG and String database [12] (Figure 1).

RESULTS AND DISCUSSION

The production of ethanol by *M. longifolia* using production test samples like test 1 (*M. longifolia)*, test 2 (*M. longifolia*+ candida), test 3 (*M. longifolia*+ Pseudomonas) and test 4 (*M. longifolia*+yeast) has been conducted in the present experimentation. Test 4 was observed in more number of bubbles followed by test 3, test 2 and test 1 after 2 days.

Mahua flowers which contain sugars were used as a substrate for production of citric acid from Aspergillus niger NCIM-545 and NCIM-595 which is involved in ethanol production mechanism [13]. In the ethanol fermentation technology, a bacterial species *Zymomonas mobilis*, and an ancient and traditional yeast *Saccharomyces cerevisiae*, are being widely used [14]. The present studies showed that *Candida* and *Pseudomonas* can produce bioethanol.

Bioethanol estimation has been conducted using potassium dichromate method. Table 1 and Figure 2 show the standard reading for different concentrations of ethanol and the experimental samples. Based on the standard graphs and the sample cutting, most of the bioethanol was produced in *M. longifolia*+yeast with about 40% was more compared with other selected samples. *M. longifolia*+ Pseudomonas showed 20% ethanol, *M. longifolia*+ candida 14% and only *M. longifolia* fermentation 5%. *Pseudomonas cepacia* has shown highest yield of alkyl esters in immobilized form for biodiesel fuel production [15]. Some of the toxins like phenazines produced by *P. aeruginosa* interact with *C. albicans* to promote ethanol production [16]. Hence the fermentation produced better bioethanol in presence of *M. longifolia* in several microbes.

Figure 3 shows the presence of interactions in the isolated proteins of fermented product from *M. longifolia*+ Candida. There is a good interaction in orange, strawberry, cucumber, apple, cabbage and papaya compared with other samples like grapes, general spinach, red spinach and fenugreek. The protein-engineered $NADP^+$-dependent XDH-expressing strains decrease in xylitol excretion and increase in ethanol production [17].

Table 1: *Estimation of Ethanol Concentration in M. longifolia (M).*

Ethanol Concentration	Optical Density
0	0
10	0.05
20	0.06
30	0.08
40	0.1
50	0.11
60	0.11
70	0.12
80	0.12
90	0.13
100	0.14
M	0.04
M +Candida	0.05
M +Pseudomonas	0.06
M +Yeast	0.08

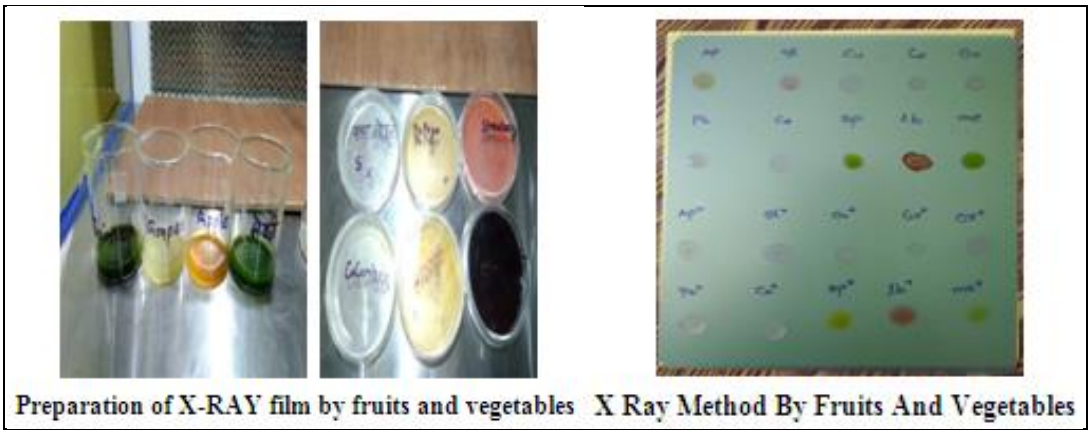

Fig. 1: *Samples and Blotting on X-Ray Film.*

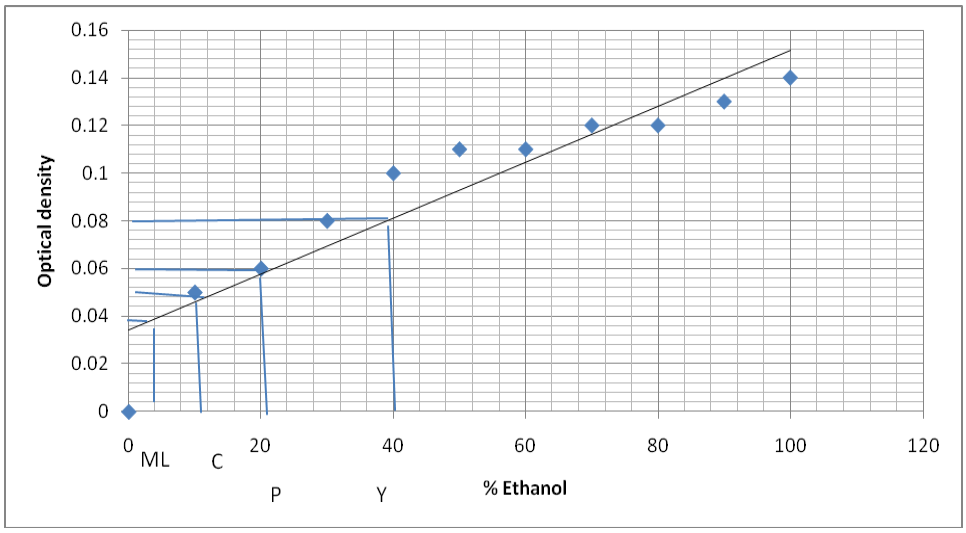

Fig. 2: *Graph Plot for Selected Samples.*

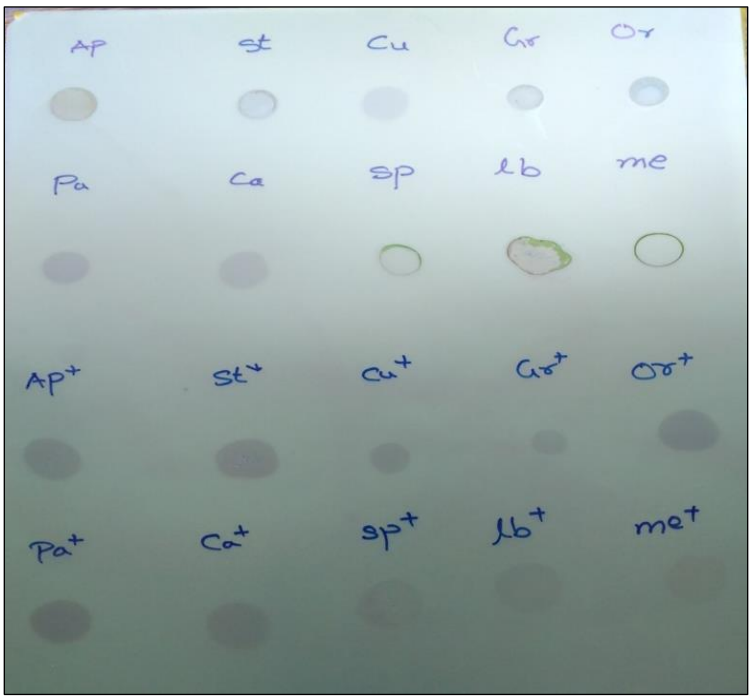

Fig. 3: *X-Ray Film Method is used by Fruits and Vegetables for Protein-Protein Interaction.*

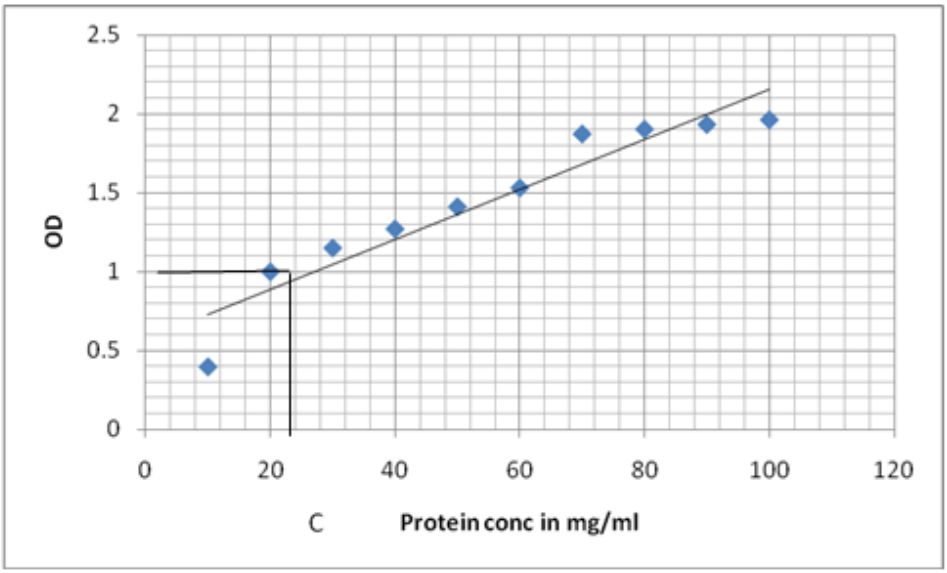

Fig. 4: *Graph Plot for Protein.*

Table 2: *Estimation of Protein Concentration.*

Protein Conc. (mg/ml)	OD
100	1.96
90	1.93
80	1.90
70	1.87
60	1.53
50	1.41
40	1.27
30	1.15
20	1.00
10	0.4
M. longifolia +Candida	1

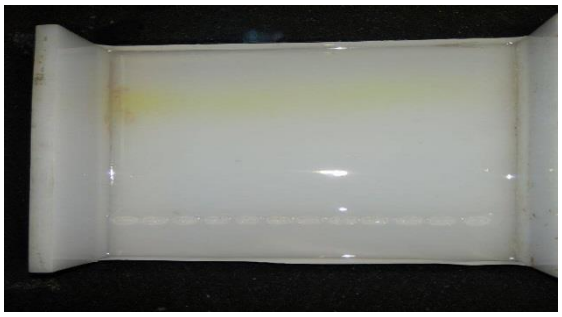

Fig. 5: *Quantification Using Electrophoresis Technique.*

Table 2 and Figure 4 show presence of 24 mg/ml proteins in fermented product of *M. longifolia* with *Candida.*

Figure 5 shows the purification and quantification for the *M. longifolia* with *Candida* fermentation using albumin as standard. The samples have band observation at same distance with standard. Hence the marker (Albumin) has 66.5 kD, the fermented product may also have 66.5 kD.

Submission of the Following Proteins with String Showed Network for the Production of Ethanol

- Glutathione Peroxidase 4,
- Phospholipid Hydroperoxidase,
- EC 1.11.1.12,
- GSHPx-4,
- GPx-4,
- PHGPx,
- Phospholipid Hydroperoxide Glutathione Peroxidase, Mitochondrial,
- Glutathione Peroxidase 4 (Phospholipid Hydroperoxidase), and
- Albumin.

Figure 6 shows the presence of interaction of albumin with several other proteins like AHSG (alpha-2-HS-glycoprotein), APOA1 (Apolipoprotein A-I), EGF (Epidermal growth factor), GSR (Glutathione reductase), PROS1 (Protein S (alpha)), IGF1 (Insulin-like growth factor 1 (somatomedin C)) etc. All these proteins are involved in body factors in the alcohol production and metabolism. Albumin administered to humans can disrupt genes in presence of microbes and coverts molecules like β-glucan to ethanol within the system [18].

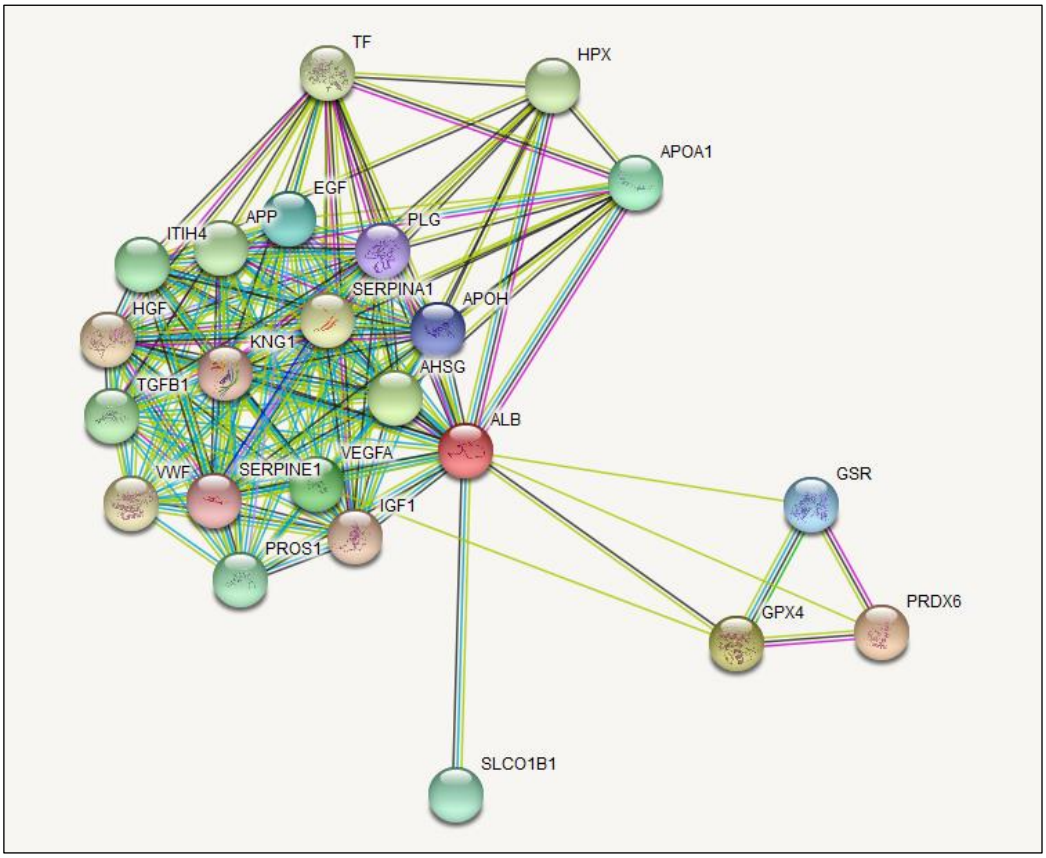

Fig. 6: *Protein Networking in Bioethanol.*

Production of bioethanol production and expansion through microbes has huge potential impact in fuel technology [19]. Several microorganisms like *Escherichia coli*, *Klebsiella oxytoca*, and *Zymomonas mobilis* have been engineered for converting biomass into fuel ethanol at high yields [20]. The present work has shown that microbes like yeast, candida and pseudomonas in presence of *Madhuca longifolia* dried flowers has produced efficient bioethanol.

CONCLUSION

Several microbes in presence of *Madhuca longifolia* dried flowers produce efficient bioethanol. Further studies in microbial species have to be conducted for efficient production of bioethanol.

CONFLICT OF INTEREST

No conflict of interest.

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