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Research Article

Single Cell Protein production by Probiotics Grown on Fruit Waste

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ABSTRACT:
The increasing demand for food production leads to a wide gap in-between demand and supply. To bridge this gap the microbial biomass i.e. single cell protein (SCP) can be considered as an alternative to conventional source of food or feed. In this present work we have attempted for production of SCP by using the probiotics such as Lactobacillus acidophilus, Streptococcus thermophilus and Bacillus coagulans as they don't cause any harm if ingested. These probiotics were grown in medium containing 5g/l $(NH_2)_2SO_4$, 1g/l $MgSO_4.7H_2O$, 0.5g/l NaCl, 0.1g/l $CaCl_2$ and fruit waste as a carbon source and incubated for 7 days. The fruit wastes of Custard apple, Watermelon and Sweet lime were used in this study. The media was then optimized for carbon sources, pH and temperature. The produced biomass was then harvested by filtration. The harvested biomass was analyzed for crude protein by Kjendhal method and protein recovery from fruit waste was calculated. We obtained maximum biomass yield by S. thermophilus where as custard apple was found to serve as best carbon source. The protein recovery by S. thermophilus was more (11.15 g/100g waste) as compared to other two probiotics. The present findings reveal that custard apple waste can be used as an effective alternative carbon source for SCP production and probiotic S. thermophilus was found to be best among used probiotics to recover more protein from waste.

Keywords: Single cell protein, biomass, probiotics, fruit waste, Kjendhal method etc.

INTRODUCTION

The scaring rate of growing population had increased the demand for food production, leading to a wide gap in demand and supply. This gap for protein-rich food and feed has forced mankind to search for alternative protein sources. Since the early fifties of 20^{th} century, intense efforts have been made to explore new, alternate and unconventional proteins that replace conventional sources.

Hence, the focus had shifted in recent times to exploit microbes directly as food sources for fortification of the food supply or for food consumption as a Single Cell Protein (SCP) [1]. Microbial biomass has been considered as alternative to conventional source of food or feed [2]. The term SCP was coined to the dried cells of microorganisms such as algae, actinomycetes, bacteria, yeast, molds and higher fungi grown for use as a protein source in human food or animal feed [3]. SCP is considered as a potential protein source for humans and as feed for animals [4, 1, 5, 6].

The production of SCP from various microbes, particularly fungi and bacteria has received considerable attention.

As compared with algae and fungi, bacteria have the advantages of rapid succession of generations, easily modifiable genetically and high protein content, so it can be good source for SCP [7, 8, 9, 10]. *Cellulomonas, Psuedomonas, Methylomonas, Bacillus* and *Alcaligenes* are the most frequently used bacterial genera as SCP source [11, 12, 13, 14].

Various materials can be used as a substrate for producing SCP as whey, orange peel residue, sweet orange residue, sugar cane bagasse, paper mill waste, rice husks & straw, wheat straw, cassava waste, sugar beet pulp, coconut waste, banana peel, mango waste, grape waste, sweet potato, pea and been vines, corn cobs & stalks and vegetable processing wastes [15, 16, 17, 18, 19].

In this work we intended to investigate the possibility of bioconversion of fruit wastes in to SCP by using three probiotics; *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Bacillus coagulans* grown on media containing fruit wastes.

MATERIALS AND METHODS

Collection and Analysis of Fruit Wastes

By the choice of availability, the wastes of custard apple, watermelon and sweet lime were obtained from local vendors from Solapur city. The wastes were cleaned with distilled water, shade dried and grinded to fine powder. The dried powdered wastes were then analyzed for protein content by Lowery's method [20] and for total carbohydrates by Anthrone method [21].

Collection of Probiotic Microorganisms

The probiotics *Lactobacillus acidophilus, Streptococcus thermophilus* and *Bacillus coagulans* were collected from Laboratory of Microbiology, Walchand College of Arts and Science, Solapur. They were maintained on their respective media. *L. acidophilus*, and *S. thermophilus* requires MRS agar medium (Proteose peptone 10g/l, Beef extract 10g/l, Yeast extract 5g/l, Dextrose 20g/l, Polysorbate 80 1g/l, Ammonium citrate 2g/l, Sodium acetate 5g/l, Magnesium sulphate 0.1g/l, Manganese sulphate 0.05g/l, Dipotassium phosphate 2g/l, Agar 12g/l, Final pH 6.5±0.2) and *B. coagulans* requires nutrient agar medium (Peptic digest of animal tissue 5g/l, Sodium chloride 5g/l, Beef extract 1.5g/l, Yeast extract 1.5g/l, Agar 15g/l, Final pH 7.4±0.2) for their growth and maintenance. They were preserved at 4^0C.

SCP Production

The inoculum for both *L. acidophilus* and *S. thermophilus* were prepared in MRS broth having same composition as MRS agar medium expect agar was absent, whereas for *B. coagulans* nutrient broth was used.

The incubation period was 24 hrs and temperature was 28^0C. The media was designed for SCP production from available literature resources with some modifications [22]. 5 ml inoculums was transferred to 100 ml production medium containing fruit waste 1%, Ammonium sulphate 5g/l, Potassium dihydrogen sulphate 1g/l, Magnesium sulphate 0.5g/l, Sodium chloride 0.1g/l, Calcium chloride 0.1g/l. The pH was adjusted to 6.2. Then incubated at 28^0C for 7 days.

Harvesting of SCP

SCP was harvested from broth through filtration. The biomass (Bacterial biomass + residual waste substrates) was filtered through Whatman filter paper No. 1. The biomasses were washed repeatedly with distilled water to remove any adherence, then dried in an oven at 90^0C till constant weight. The dried filter papers with their content were weighted and the biomass (mg/100 ml broth) was estimated.

Media Optimization for SCP Production

The production media for SCP was further optimized for carbon source, pH and temperature to obtain maximum yield of biomass as SCP. Carbon source was optimized with different fruit waste and then its different concentrations such as 1%, 2%, 3%, 4%, 5%. The second parameter was selected as pH such as 4.5, 5.5, 6.2 and 7.5. Also different temperatures for incubation such as 25^0 C, 28^0 C, 35^0 C and 45^0 C were selected for optimization.

Protein Recovery

After media optimization the harvested biomass was analyzed for nitrogen (crude protein %) content by Kjendhal method [23]. The protein recovery was then calculated according to the following equation:

$$\text{Protein recovery (g/100g substrate)} = \frac{\text{Crude protein \%} \times \text{Biomass yield (g/100g subtrate)}}{100}$$

RESULTS

Analysis of Fruit Wastes

After collecting the fruit wastes they were dried and powdered to fine. The powder was analyzed for total protein contents by Lowery's method and carbohydrate contents by Anthrone method. The results were shown in Table 1.

Fruit Waste	Protein %	Carbohydrate %
Watermelon	0.50	8.50
Sweet lime	2.35	11.20
Custard apple	2.45	18.75

Table: 1. Analysis of fruit waste: Total protein and carbohydrate analysis of dried fruit waste of Watermelon, Sweet lime and Custard apple.

SCP Production

The SCP was produced by *L. acidophilus*, *S. thermophilus* and *B. coagulans*. After incubation of 7 days period at 28^0C the SCP was harvested by filtration method. The obtained SCP content was further weighed and biomass content was determined. The obtained results were shown in Table 2.

Fruit waste	*L. acidophilus*	*L. thermophilus*	*B. Coagulans*
Sweet lime	320 mg	380 mg	180 mg
Watermelon	770 mg	500 mg	530 mg
Custard apple	**890 mg**	**760 mg**	**680 mg**

Table: 2. Biomass production in SCP: Biomass content produced per 100 ml broth by *L. acidophilus, S. thermophilus* and *B. coagulans* when using fruit waste of sweet lime, watermelon and custard apple.

Media Optimization

Maximum biomass was produced for custard apple fruit waste. So it was used as final carbon source. Then the various concentrations of custard apple were used in media such as 1%, 2%, 3%, 4% and 5%. The results obtained for biomass yield were quoted in Table 3.

Custard Apple %	Biomass yield for probiotics (per 100 ml broth)		
	L. acidophilus	*S. thermophilus*	*B. coagulans*
1%	890 mg	760 mg	680 mg
2%	1630 mg	1540 mg	1570 mg
3%	1910 mg	2110 mg	2370 mg
4%	3250 mg	2740 mg	3170 mg
5%	**3500 mg**	**3440 mg**	**3940 mg**

Table: 3. Biomass yield for different concentration of custard apple: All *L. acidophilus, S. thermophilus* and *B. coagulans* gives maximum biomass yield in mg/100 broth when 5% custard apple waste was used.

Then we optimized the pH of medium by varying the different pH such as 4.5, 5.5, 6.2, 7.5 and observed for the maximum yield for biomass production. The results obtained for different pH were quoted in Table 4.

Organism	pH	Biomass (per 100ml broth)
L. acidophilus	**7.5**	**4120 mg**
	6.2	3500 mg
	5.5	2770 mg
	4.5	2650 mg
S. thermophilus	**7.5**	**4500 mg**

	6.2	3440 mg
	5.5	3030 mg
	4.5	2600 mg
B. coagulans	7.5	3740 mg
	6.2	3940 mg
	5.5	**4250 mg**
	4.5	4150 mg

Table: 4. Biomass yield for pH concentration: *L. acidophilus*, *S. thermophilus* and *B. coagulans* gives maximum yield of biomass at pH 7.5, 7.5 and 5.5 respectively.

In media optimization the incubation temperature for SCP production was altered such as 25^0C, 28^0C, 35^0 and 45^0C. The results for the biomass production in each different incubation temperature were given in Table 5.

Organism	Temperature	Biomass
L. acidophilus	25°C	4060 mg
	28°C	4120 mg
	35°C	**4330 mg**
	45°C	3910 mg
S. thermophilus	25°C	4270 mg
	28°C	4500 mg
	35°C	4560 mg
	45°C	**4790 mg**
B. coagulans	**25°C**	**4520 mg**
	28°C	4250 mg
	35°C	3760 mg
	45°C	3430 mg

Table: 5. Biomass yield at different incubation temperature: The maximum biomass yield for *L. acidophilus*, *S. thermophilus* and *B. coagulans* was 35^0C, 45^0C and 25^0C respectively.

Protein Recovery

The crude protein content of biomass obtained from final optimization step was then determined by Kjendhal method. From which protein recovery was determined and the results of this were noted in Table 6

Probiotics	Biomass (mg/100ml broth)	Biomass (g/L)	Biomass (g/100g waste)	Crude Protein (%)	Protein Recovery (g/100g waste)
L. acidophilus	4330	43.3	86.4	9.95	8.59
S. thermophilus	4790	47.9	95.8	11.64	11.15
B. coagulans	4520	45.2	90.4	9.15	8.27

Table: 6. Protein yield and Protein recovery in SCP production: the crude protein content in percentage and protein recovery for *L. acidophilus*, *S. thermophilus* and *B. coagulans* shows maximum protein recovery by *S. thermophilus*. .

DISCUSSION

There is a doubt to be SCP to replace the conventional protein sources due to their high nucleic acid content and slower in digestibility. They also may be considered as foreign material by body, which may subsequently results in allergic reactions [24]. The probiotics are viable microbial dietary supplements that, when introduced in sufficient quantities, beneficially affect human organism through their effects in the intestinal tract. They show much of the health benefits [25]. So it's a good attempt to produce the SCP by using the probiotics.

The maximum biomass was obtained for the custard apple at 5% concentration for 7 days incubation for all three probiotics used. This finding agrees with the observations when single cell protein produced from pineapple waste using yeast and got maximum yield at 5% concentration of waste on 7th day [22]. The optimum pH obtained for the *L. acidophilus* and *S. thermophilus* was 7.5 where as for *B. coagulans* was 5.5. The temperature optima for biomass production for *L. acidophilus* was 35^0C, for *S. thermophilus* is 45^0C and for *B. coagulans* was 25^0C.

The obtained results showed that crude protein content of biomass was 9.15%, 9.95% and 11.64% by *B. coagulans*, *L. acidophilus*, *S. thermophilus* respectively. These findings were in agreement with findings for SCP produced by *Penicillium expansum* on orange peel which gives 9.89 % protein content [26] and *Arachniotus* species when grown on Corn cob waste for SCP production gives 11.20% of protein content [27].

The protein recovery from custard apple waste substrate was 8.27, 8.59 and 11.15g/100g by *B. coagulans*, *L. acidophilus*, *S. thermophilus* respectively suggest that the SCP can be produced by using probiotics when grown on custard apple fruit waste and it may be used as human food or animal feed with health benefit.

CONCLUSION

As a result of the current shortage of protein due to the rapid rise of population, especially in the developing countries, the research for nonconventional protein sources is conducted. Single cell protein (SCP) is suggestive as a supplemental protein source.

The bioconversion effect of fruit wastes into SCP was evaluated using three probiotics namely *L. acidophilus*, *S. thermophilus* and *B. coagulans*. The best production of SCP was observed with the custard apple waste. The increase in biomass contents were observed when there was increase in custard apple waste concentration. The highest biomass content of *L. acidophilus*, *S. thermophilus* and *B. coagulans* was recorded after 7 day fermentation at 5 % concentration. The pH optima for *L. acidophilus*, *S. thermophilus* for SCP production wass 7.5 where as for *B. coagulans* it was 5.5. The highest biomass content was recorded at 35^0C, 45^0C and 25^0C *L. acidophilus*, *S. thermophilus* and *B. coagulans* respectively.

The crude protein content is 9.95%, 11.64% and 9.15 for biomass using *L. acidophilus*, *S. thermophilus* and *B. coagulans* respectively. Whereas the protein recovery in terms of g/100g waste was 8.59, 11.15, 8.27 for *L. acidophilus*, *S. thermophilus* and *B. coagulans* respectively.

The present findings reveal that custard apple waste can be used as effective alternate carbon source for SCP production by using probiotics i.e. *L. acidophilus*, *S. thermophilus* and *B. coagulans*.

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