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Utilization of Wheat Bran as Bed Material in Solid State Bacterial Production of Lactic Acid with Various Nitrogen Sources

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Abstract**—**The present experimental investigation brings about a comparative study of lactic acid production by pure strains of *Lactobacilli* (1) *L. delbreuckii* (NCIM2025), (2) *L. pentosus* (NCIM 2912), (3) *Lactobacillus sp*.(NCIM 2734, (4) *Lactobacillus sp*. (NCIM2084) and coculture of strain-1 and Stain-2 in solid bed of wheat bran, under the influence of different nitrogen sources such as baker's yeast, meat extract and proteose peptone. Among the pure cultures, strain-3 attained lowest pH value of 3.44, hence highest acid formation 46.41 g/L, while the coculture attained an overall maximum value 47.56 g/L lactic acid (pH 3.38) at 15 g/L and 20 g/L level of baker's yeast, respectively.

Keywords**—**Eco-friendly, lactic acid, lactobacilli, wheat bran

I. Introduction

LACTIC acid (2-hydroxypropanoic acid) is a speciality chemical used as preservative, acidulant, flavouring agent and probiotics in food and dairy industries and in production of ecofriendly biodegradable polymer (PLA) having biomedical uses [1], [2]. Major portion of the lactic acid production is through solid state or liquid state fermentation. The global lactic acid production predominantly follows the microbial fermentation pathway, rather than chemical synthesis to avoid mixture of isomers [3]. Apart from lactic acid as the major product, *Lactobacilli* are also known to synthesize several antagonistic and biocontrol products that find application in agricultural food and health protection purposes [4]. Nitrogen sources form an integral part of bacterial nutrition as nitrogen exists as component of important biomolecules such as (nucleic acids) DNA, RNA, enzymes, proteins and amino acids that are essential for the cell growth and metabolism. Apart from nitrogen, the complex nitrogen sources such as meat extract, proteose peptone and dried baker's yeast contain several important amino acids and vitamins that are helpful in the bacterial growth and metabolism. It has been reported that the cost of yeast extract or the pure nitrogen source approximately amount to 38% of the cost of fermentative production of lactic acid, hence the use of cheaper nitrogen sources such as dried baker's yeast has been suggested [5].

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Solid state fermentation technology utilizes the agro-wastes (wheat bran, wheat straw, rice straw and sugarcane Bagasse) and forestry wastes such as pine needles [6] as bed materials in preparation of value added products in an eco-friendly way. Wheat bran constitutes a significant portion of agro-industrial waste produced in the flour mills. The wheat bran is an abundant agro-industrial waste as wheat is the second major food crop in India (about one third of the food grain production), with 26.6 million hectares of cropped land under it, having a productivity of 2,707kg/hectare [7]. The global production of wheat was 585million tones (1999-2001) to which India contributed 12.3% [7]. Wheat bran consists of approximately about 19% starch, 18% crude protein, 58% non starch carbohydrates with 24% cellulose, 70% arabinoxylans and 6% glucans as major non starch polysaccharides and lignin [8]. Wheat bran could be utilized in solid state, liquid state fermentations and animal feeds. Wheat bran contains sugars, nitrogenous substances such as proteins, amino acids and various vitamins (niacin, thiamine, riboflavin, vitamin A, vitamin K, vitamin E and folate etc.) and minerals (potassium, phosphorus, magnesium, calcium), that may be potentially helpful in supporting growth of *Lactobacilli* [9]. Wheat bran, treated with protease has been utilized along with mixed cultures of *L. delbrueckii* and *L. casei*, to produce lactic acid through simultaneous saccharification and fermentation [10]. The amino acids that are essential for growth and existence for lactobacilli consist of arginine, cysteine, tryptophane, histidine, tyrosine, isoleucine, glutamic acid, valine, leucine, methionine, phenyl alanine, proline and threonine etc. Many of these amino acids can be supplied from the wheat bran bed material [11].

In the present experimental studies feasibility of solid state bacterial fermentation of lactic acid through various *Lactobacillus* strains and coculture utilizing a bed of wheat bran and the effect of different nitrogen sources at different doses, on lactic acid production have been investigated. Performances of the *Lactobacillus* strains and coculture in terms of lactic acid production are also compared.

II. Materials and Methods

The chemicals used in these experiments were of Merck and High media make. Pure cultures of *Lactobacilli* (1) *L. delbreuckii* (NCIM2025), (2) *L. pentosus* (NCIM 2912), (3) *Lactobacillus sp*. (NCIM 2734), (4) *Lactobacillus sp*. (NCIM2084) had been acquired from National Chemical Laboratory (NCL) Pune, in form of stab cultures in agar , were

cultured monthly as directed. The inoculum for the *lactobacilli* strains were prepared in MRS (de Mann Rogosa Sharpe) media at 37^0 C, 180 rpm for fourteen hours. Composition of one litre MRS medium is : 10 g proteose peptone, 5 g yeast extract, 10 g beef extract, 20 g dextrose, 1 g tween 80, 2 g ammonium citrate, 5 g sodium acetate, 0.1g $MgSO_4.7H_2O$, 0.05 g $MnSO_4$, 2g K_2HPO_4 in distilled water as solvent. One liter of glucose based synthetic media consists of : 60g glucose, various levels of nitrogen sources (10,12, 15, 20) g/L, 1g sodium acetate, 0.03g $MnSO_4.H_2O$, 0.10 g $MgSO_4.7H_2O$, 0.25 g KH_2PO_4, 0.25 g K_2HPO_4 and 0.03 g $FeSO_4$. Various nitrogen sources were meat extract (ME), proteose peptone (PP) and bake's yeast (BK). An inoculum dose corresponding to 2 g/L cell dry weight was added to sterilized and prewetted 6 g of finely powdered wheat bran bed, by 40mL glucose based production media (pH 6.5) containing 2% NaOH as neutralizer in 250mL Earlenmayer flasks. These were kept at 37^o C for six days incubation. After the incubation, the flasks were added with 50 mL distilled water, shaken and then the product was extracted out by passing through a muslin cloth. The pH drops (hence acid formation) of the extracts were determined with the help of, a digital pH meter.

The lactic acid present in the extracts were quantitatively assayed by Kimberly Taylor method, which utilizes hot concentrated sulphuric acid effects, that include oxidation of lactic acid to acetaldehyde, which subsequently forms a chromogenic complex with p-phenyl phenol in presence of copper. The extracts were centrifuged at 8000g and the supernatants were used for lactic acid estimation. 0.5 mL of supernatant was added with 3mL of 96% sulphuric acid, followed by heating for ten minutes in boiling water bath for ten minutes, then cooling it to room temperature for about 30 minutes. The cool solution was added with 50 µL 4% copper sulphate and 100 µL p- phenyl phenol (prepared by dissolving 1.5% of the reagent in 95% ethyl alcohol) which provided a chromogenic complex. The absorbance for lactic acid is measured in a UV-VIS double beam spectrophotometer at 570nm [12], [13].

III. Results And Discussions

The data observed in Table I reveal that the coculture and the strain-1 attain their highest values of lactic acid concentration 43.75 g/L and 41.83 g/L respectively at a dose of 12 g/L meat extract as nitrogen source. The Table 1 results also indicate that all the strains and the coculture attain their maximum lactic acid production at 12 g/L dose of meat extract. At higher doses of meat extract 15 and 20 g/L , a decline in lactic acid production has been observed probably due to the fact that excess growth of bacterial biomass due to higher dose of the nitrogen source deviates the metabolic activities from acid synthesis towards growth.

TABLE I
LACTIC ACID PRODUCTION WITH VARIOUS DOSES OF MEAT EXTRACT

	ME 12 g/L	ME 15 g/L	ME 20 g/L
Bacterial strains	LA (g/L)	LA (g/L)	LA (g/L)
Strain-1	41.83	29.68	9.89
Strain-2	21.06	13.61	5.81
Coculture	43.75	35.41	10.76
Strain-3	33.67	27.48	7.71
Strain-4	21.46	18.77	4.09

TABLE II
LACTIC ACID PRODUCTION WITH VARIOUS DOSES OF PROTEOSE PEPTONE

	PP 12 g/L	PP 15 g/L	PP 20 g/L
Bacterial strains	LA (g/L)	LA (g/L)	LA (g/L)
Strain-1	42.31	31.44	11.04
Strain-2	22.79	14.37	6.93
Coculture	45.0	36.84	12.52
Strain-3	35.42	2.01	10.61
Strain-4	23.50	17.0	4.86

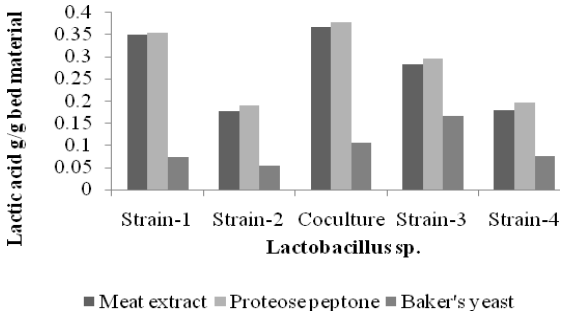
TABLE III
LACTIC ACID PRODUCTION WITH VARIOUS DOSES OF BAKER'S YEAST

	BK 12 g/L	BK 15 g/L	BK 20 g/L
Bacterial strains	LA (g/L)	LA (g/L)	LA (g/L)
Strain-1	8.85	12.29	23.28
Strain-2	6.36	28.31	12.18
Coculture	12.61	15.04	47.56
Strain-3	19.75	46.41	27.37
Strain-4	9.06	10.07	22.08

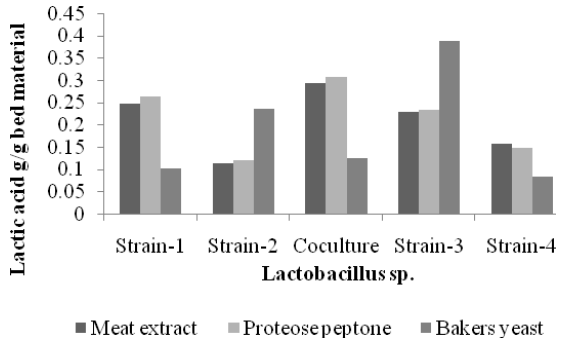
The data in Table II suggests that the proteose peptone nitrogen source provides a higher value of lactic acid concentration for strain-1 and co-culture 42.31 g/L and 45.08 g/L than those in Table 1. Here also the maximum lactic acid production has been attained at 12 g/L dose of proteose peptone and a decline in lactic acid concentration similar to that in Table I has also been witnessed. The dried baker's yeast serves as an inexpensive source of nitrogen applied for the strains under study. The Table 3 data shows that the highest lactic acid production has been attained by the coculture 47.56 g/L (lowest pH 3.38), followed by strain-3, 46.41 g/L at 20 g/L and 15 g/L doses of baker's yeast. The strain-3 achieved its highest lactic acid production 46.41 g/L but at a lower input of (15 g/L) of baker's yeast, indicates lower nitrogen requirement. The baker's yeast (Table III) proved to be reasonably good nitrogen source for the lactobacilli under study, which attained the maximum lactic acid production, 28.31 g/L, 46.41 g/L and 47.56 g/L for strain-2, strain-3 and coculture. The strain-1 and strain-4 attained their maximum value in lactic acid production with proteose peptone as nitrogen source. The coculture emerged to be the highest lactic acid producer among all the strains under study that too with the cheaper nitrogen source baker's yeast, hence the coculture may play an important role in fermentation

industries for lactic acid production. The wheat bran showed its potential as a good bed material that can support the bacterial growth through its amino acids and proteins and can provide important pentose or hexose sugars significant in lactic acid production.

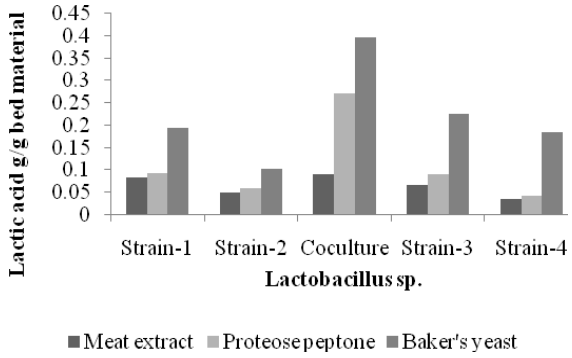
Fig. 1 shows the highest lactic acid produced by the strain-1, was 0.3526 g/g bed material with 12 g/L dose of proteose peptone, while the strain-2 attains maximum weight 0.2359 g/g of bed material with 15 g/L baker's yeast in Fig. 1(b). Fig. 1(a) shows that coculture liberated high amounts of lactic acid, 0.3646 g and 0.3757 g per unit weight of bed material with 12 g/L dose of ME and PP, respectively.



(a) Nitrogen Source 12 g/L



(b) Nitrogen Source 15 g/L



(c) Nitrogen source 20 g/L

Fig. 1 Comparison of production of lactic acid by different species with various doses of nitrogen sources

In Fig. 1(b) the strain-3 showed the highest weight of 0.3867 g/g bed material at 15g/L dose of baker's yeast. As seen from Fig. 1(c), the coculture produced the overall highest lactic acid 0.3963 g/g bed material at 20 g/L dose of baker's yeast. Thus for the production of lactic acid, combination of coculture and baker's yeast (cheap nitrogen source) with wheat bran as bed may prove beneficial for the fermentation industries.

IV. Conclusions

The lower dose 12g/l of the synthetic nitrogen sources serve most suitable for lactic acid production. The superior performance of the coculture is evident in terms of lactic acid production with higher doses of baker's yeast (20 g/L). The meat extract and proteose peptone showed better compatibility for production with strain-1, coculture and strain-4. The co-culture showed higher acid production than its constituent strains-1 and 2. The baker's yeast provides reasonably good help as low cost nitrogen source, in terms of acid production. The wheat bran showed its potential as a good bed material that can support the growth and production of lactic acid by the different strains of lactobacilli through solid state fermentation technology. This presents an amicable solution to the problem of solid agro-waste generation during processing.

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