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Optimization of Fermentation Conditions for Nata-de-Coco Production

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In this study the optimum fermentation conditions for the production of Nata-de-Coco from Nata isolates were determined in tender coconut milk broth. Among different isolates, used UASB-A (1.67g/5ml) produced highest Nata yield. Optimization of nutritional and environmental factors were carried out by using all four Nata producing isolates. Among the different sugars used as carbon source, at 10 % sucrose level produced (1.99g/5ml) maximum Nata production. Organic sources of nitrogen found to be ideal over inorganic forms of nitrogen. At 1% peptone level, the highest Nata yield (1.88g/5ml) was obtained. The pH level of 4.0 (1.80g/5ml) and temperature of 30^0 C (1.71g/5ml) were found to be optimum for the production of Nata under static culture condition.

Key words: Optimization, Nata-de-Coco, *Gluconoacetobacter xylinum*.

The word "Nata" is derived from the Spanish word "Natare" which means float (Crisostomo, 1983). Nata-de-coco is a product of coconut water fermentation by using *Gluconoacetobacter xylinum* (formerly identified as *Acetobacter xylinum*). Chemically, the fiber contained in Nata-de-coco is a cellulose fiber, known as bacterial cellulose (Piluharto, 2003). Nata-de-Coco is a favorite delicacy native to the Philippines and produced mainly in coconut growing regions of Laguna and Quezon provinces (Embuscado *et al*., 1994). Nata-de-coco is a traditional Philippine dessert, which is "coconut gel-product from coconut water prepared by bacterial fermentation". It is a tough substance produced by growing a species of vinegar group of bacteria in sugared coconut water or coconut milk. Nata de coco is rich in fiber, good for the digestive system and it is low in calories and contains no cholesterol (Metcalfe, 1994). In 1886 Brown first reported that the pellicle produced by *Gluconoacetobacter xylinum* was of pure bacterial cellulose. Its structural features and physical properties are unique and differ considerably from plant cellulose.

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MATERIALS AND METHODS

Microorganism

The *Gluconoacetobacter* isolated from different fruit sources designated as UASB-A (Apple), UASB-B (Banana), UASB-O (Orange) and UASB-P (Pineapple) were used in this study. The reference strain *Gluconoacetobacter xylinum* (GXD5) was obtained from Dept. of Agril. Microbiology UAS, GKVK, Bengaluru was used as a reference culture.

Preparation of Tender coconut milk broth

Tender coconut milk broth was prepared from using grated tender nuts purchased locally from Bengaluru city, India. Tender coconut milk broth was supplemented with sucrose 5%, ammonium dihydrogen orthophosphate 0.5%, glacial acetic acid 2% and pineapple juice 10%.

The 5 ml of prepared coconut milk broth was dispersed in screw capped tubes and sterilized by autoclaving.

Effect of pH on Nata production

Nata production was tested at varied pH levels of 3.5, 4.0, 4.5, 5.0 and 5.5 in 5 ml tender coconut milk broth. pH of the medium was maintained by adding suitable acid as per the procedure followed by Embuscado *et al*. (1994).

Effect of temperature

Effect of temperature on the production of Nata by different isolates were studied by inoculating each isolate to a basal medium and incubated at varied temperature levels of 25^0 C, 30^0 C and 35^0 C. The fresh weight of the Nata was recorded at 15 days after incubation period.

Effect of different carbon sources at different concentrations on Nata production

The effect of different carbon sources on Nata production was tested by growing the effective Nata producing isolate in 5ml of tender coconut milk broth with different sugars at different concentrations as a sole source of carbon. The final fresh weight of Nata was recorded at 15 days after incubation. The incubation was done at room temperature.

Effect of different nitrogen sources at different concentrations on Nata production

Effect of different nitrogen sources on the yield of Nata was studied by using three different organic sources of nitrogen (Peptone, Yeast extract and Tryptone) at different levels of 0.5, 1.0, 1.5 and 2.0% and three different inorganic sources (ammonium sulphate, ammonium di hydrogen orthophosphate and ammonium phosphate) at levels of 0.25, 0.50, 0.75 and 1.0%.

EXPERIMENTAL

All data obtained from the study were subjected to statistical analysis to evaluate treatment effects. Analysis was done by using completely randomized design (CRD). CD values were used to locate significant mean differences (Duncan, 1995).

RESULTS AND DISCUSSION

The maximum Nata production was observed at pH 4.0 (Jaganath *et al.,* 2008 and Suwannapinunt *et al*., 2007) by all four isolates and including reference strain GXD5 followed by pH 4.5, 3.5 and 5.0. At initial pH values of 4.0 and 5.0 is in good agreement with results of earlier studies (Masaoka *et al*., 1993). Further the yield of Nata was significantly low at 5.5. At pH 4.0 reference strain GXD5 yielded about 1.80 g of Nata per 5 ml compared to Nata production at pH 4.5 (1.73 g), 3.5 (1.05 g) and 5.0 (0.85 g) with minimum of 0.80 g of Nata per 5 ml at pH 5.5. Lowest yield was obtained at pH 3.5 (Coban and Biyik., 2011). *Gluconoacetobacter* sp. had shown to be distinctly acid tolerable capable of growing at pH as low as 3.5 (Lapuz *et al.,* 1969). Verschren *et al*. (2000) reported pH 4.0 and 5.0 was ideal for the development of Nata. Therefore, it is important to control the pH within the optimal range (Table 1).

As shown in Table 2, the effect of various temperatures between 25-35°C was examined using tender coconut milk broth. The optimum temperature for highest Nata production was obtained with reference strain GXD5 (1.71 g) at 30° C followed by 25^0 C (1.33 g), 35^0 C (1.10 g) (Son *et*

Table 1. Effect of pH on Nata production

Isolates	Fresh weight g/5ml pH				
	3.5	4.0	4.5	5.0	5.5
UASB-A	1.01	1.78	1.73	0.85	0.78
UASB-B	0.86	0.95	1.05	0.68	0.70
UASB-O	0.93	1.16	1.11	0.83	0.71
UASB-P	1.05	1.46	1.56	0.80	0.80
Reference GXD5	1.03	1.80	1.53	0.76	0.76
S.Em±	0.02	0.007	0.02	0.03	0.03
CD at 5%	0.09	0.024	0.09	0.08	NS

al., 2001 and Hungund *et al*., 2010). In high temperature, the cell component such as nucleic acid and protein was denatured. However, Nata production decreased above 30^0 C. Therefore, temperature is an important factor that will give a large impact for microbial growth (Krystynowicz *et al.,* 2005). These findings are also supported by the reports of Jagannath *et al.* (2008) (Table 2).

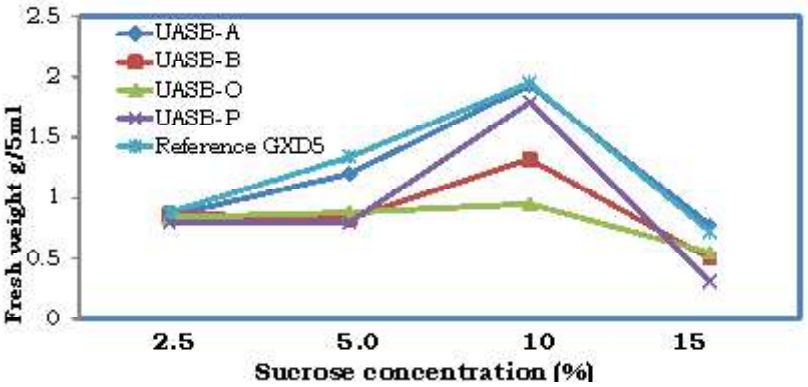

Fig. 1. Effect of different concentration of Sucrose on the yield of Nata by different Nata isolates in tender coconut milk broth

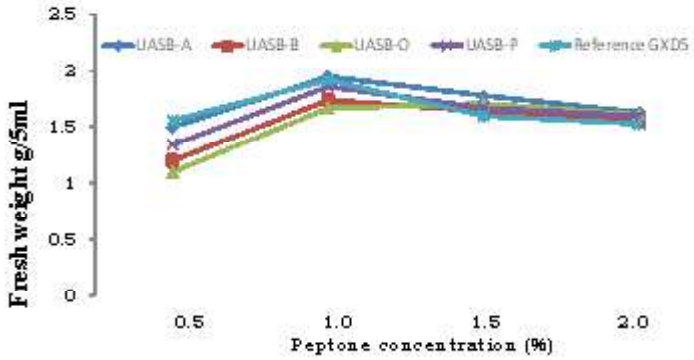

Fig. 2. Effect of different concentration of Peptone on the yield of Nata by different Nata isolates in tender coconut milk broth

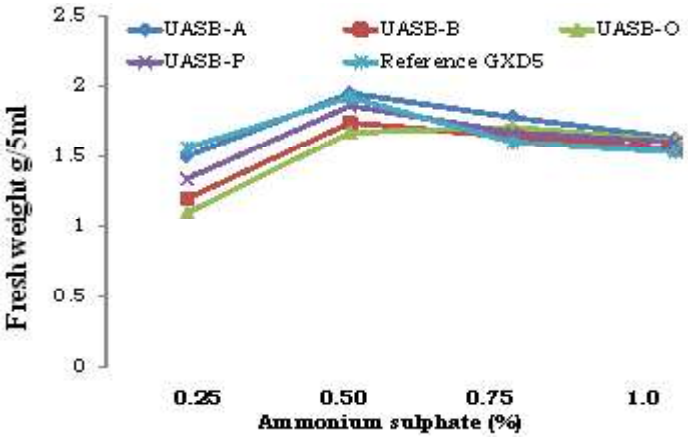

Fig. 3. Effect of different concentration of ammonium sulphate on the yield of Nata by different Nata isolates in tender coconut milk broth

As shown in Table 3, the production of Nata was significantly influenced by different carbon sources (Keshk and Sameshima 2005). All isolates effectively utilized glucose, lactose, sucrose for Nata production. The highest Nata production (1.99g/5ml) was obtained with reference strain GXD5 isolate at 10% sucrose concentration (Jagannath *et al*., 2008). The concentration of sucrose present in tender coconut milk broth affects the synthesis of bacterial cellulose and these results are similar to the previous report by Masaoka *et al.* (1993). However, Tajima *et al.* (1995) have succeeded in enhancing Nata production from sucrose by the co-cultivation of two different types of *Gluconoacetobacter xylinum*. Further increasing the sucrose concentration from 10% to 15% produced gradual decrease in the yield (Fig.1).

Sources of nitrogen were important for the production of Nata. Various nitrogen sources were added separately to the tender coconut milk broth to assess their effects on Nata production. The preliminary search for the nitrogen source for the Nata production revealed that organic nitrogen source gave higher yield than inorganic sources (Ross *et al*., 1991; Embuscado *et al*., 1994; Ramana *et al*., 2000; Hungund and Gupta, 2010). The peptone at 1% concentration gave highest yield was obtained with UASB-A isolate (1.88 g/5ml) compared to other organic source of nitrogen (Table 4 and Fig 2). Whereas Son *et al*. (2001) studied the cells grown on the media containing corn step liquor with peptone or yeast extract as nitrogen source produced a significantly high level of Nata.

The effect of different kinds of inorganic nitrogen sources on Nata production after 15 days of incubation period showed maximum amount of Nata production was obtained with reference strain

Table 2. Effect of temperature on Nata production

Isolates	Fresh weight g/5ml Temperature (°C)		
	25	30	35
UASB-A	1.33	1.68	1.10
UASB-B	1.03	1.10	1.01
UASB-O	1.11	1.43	1.03
UASB-P	1.28	1.60	1.05
Reference GXD5	1.25	1.71	1.01
S.Em±	0.03	0.06	0.02
CD at 5%	0.11	0.18	0.07

Table 3. Effect of different concentration of carbon source on the yield of Nata

Isolates	Fresh weight g/5ml											
	Sucrose (%)				Lactose (%)				Glucose (%)			
	2.5	5.0	10	15	2.5	5.0	10	15	2.5	5.0	10	15
UASB-A	0.85	1.21	1.95	0.78	1.25	1.45	1.77	1.16	0.86	0.90	1.11	0.76
UASB-B	0.87	0.70	1.14	0.50	0.37	1.25	0.58	1.08	0.79	0.83	0.86	0.62
UASB-O	0.84	0.88	0.95	0.55	0.65	1.11	1.54	0.96	0.82	0.81	0.79	0.73
UASB-P	0.80	1.0	1.79	0.31	1.14	1.31	1.35	1.10	0.84	0.87	1.03	0.66
Reference GXD5	0.88	1.36	1.99	0.72	1.32	1.56	1.84	1.11	0.88	0.94	1.08	0.70
S.Em±	0.01	0.07	0.08	0.01	0.03	0.10	0.52	0.02	0.04	0.06	0.03	0.03
CD at 5%	0.04	0.22	0.25	0.03	0.08	0.32	NS	0.07	NS	NS	0.11	0.08

Table 4. Effect of different concentration of organic nitrogen sources on the yield of Nata

Isolates	Fresh weight g/5ml											
	Peptone (%)				Yeast extract (%)				Tryptone (%)			
	2.5	5.0	10	15	2.5	5.0	10	15	2.5	5.0	10	15
UASB-A	1.42	1.88	1.55	1.4	0.76	0.97	0.58	0.66	1.43	1.53	1.65	1.40
UASB-B	1.04	1.41	1.12	0.99	0.66	0.83	0.48	0.65	1.15	1.35	1.16	1.10
UASB-O	1.16	1.41	1.18	0.93	0.64	0.80	0.60	0.58	1.07	1.37	1.25	1.11
UASB-P	1.35	1.76	1.60	1.40	0.75	0.81	0.64	0.66	1.34	1.65	1.60	1.33
Reference GXD5	1.46	1.84	1.61	1.46	0.73	1.02	0.85	0.60	1.64	1.67	1.76	1.28
S.Em±	0.03	0.04	0.03	0.07	0.02	0.08	0.05	0.03	0.02	0.06	0.05	0.05
CD at 5%	0.08	0.12	0.11	0.23	0.07	NS	0.18	NS	0.06	0.21	0.18	0.14

Table 5. Effect of different concentration of inorganic nitrogen sources on the yield of Nata

Isolates	Fresh weight g/5ml											
	Ammonium sulphate (%)				Ammonium dihydrogen orthophosphate (%)				Ammonium phosphate (%)			
	2.5	5.0	10	15	2.5	5.0	10	15	2.5	5.0	10	15
UASB-A	1.76	1.92	1.78	1.63	1.60	1.86	1.71	1.56	1.38	1.65	1.60	1.51
UASB-B	1.56	1.74	1.64	1.55	1.34	1.38	1.43	1.30	1.35	1.40	1.21	1.20
UASB-O	1.60	1.67	1.71	1.61	1.30	1.30	1.44	1.40	1.31	1.43	1.35	1.21
UASB-P	1.64	1.86	1.67	1.60	1.51	1.74	1.66	1.55	1.33	1.56	1.55	1.46
Reference GXD5	1.80	1.95	1.60	1.53	1.64	1.89	1.75	1.53	1.25	1.73	1.50	1.41
S.Em±	0.05	0.03	0.04	0.03	0.02	0.01	0.007	0.06	0.02	0.04	0.02	0.03
CD at 5%	0.17	0.09	0.13	0.10	0.06	0.05	0.02	NS	0.07	0.12	0.08	0.08

GXD5 isolate at 0.5% (1.95g/5ml) ammonium sulphate concentration (Table 5 and Fig 3). These results similar to earlier reports published by Jagannath *et al*. (2008).

CONCLUSION

It is concluded that UASB-A isolate offer great potential for the production of Nata yield. Among the different sugars sucrose produced maximum Nata production. Organic sources of nitrogen found to be ideal over inorganic forms of nitrogen. The viability of UASB-A isolate and the stability of this product could be maintained at 30ºC for 15 days storage.

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