Table 1: Phenotypic characterization of *Bacillus tequilensis* NCS-3

Traits	*Results*
Colony morphology	
Colour	Yellowish
Shape	Circular
Elevation	Raised
Margin	Entire
Transparency	Opaque
Surface	Smooth
Gram's test	+
Motility	+
Anaerobic growth	-
Growth at ^0C	
30	+
35	+
40	+
Growth at pH	
6	+
7	+
8	+
Catalase	+
Oxidase	+
Starch hydrolysis	+
Citrate Utilization	+
Nitrate reduction	+

+ = positive, - = negative

3.3 Effect of different carbon sources, nitrogen sources, pH and temperature on PHB accumulation

In this investigation, the culture was grown for 48 h at 30^0C and the effect of carbon sources (glucose, mannitol, fructose, sucrose and starch) at a fixed concentration of 4g/l, on the production of PHB by isolate 3 were evaluated. Of the various carbon sources, fructose positively affected PHB production with a concentration of 5.55g/l when it was the sole carbon source. Moreover, the isolate 3 was also able to take up some of the other carbon sources (like starch and sucrose) as nutrients for producing PHB. However, low PHB production and low cell growth were observed when the carbon source was glucose and mannitol (Table 2). Therefore, isolate 3 adapted to use fructose as carbon source resulting in greater PHB production than using other carbon sources.

PHB production was also significantly affected when different nitrogen sources were added to the production medium as also shown in Table 2. The nitrogen sources were taken at a fixed concentration of 1 g/l and the isolate was grown for 48 h at 30^0C in the DM9 medium. According to the results obtained, almost all the nitrogen sources except urea enhanced PHB production and tryptone had the largest effect producing 7.65 g/l PHB concentration from 10.02 g/l DCW. However, urea did not show any PHB production, this may indicate that isolate 3 can't take up urea as nitrogen source for PHB production.

The influence of pH on PHB production by the isolate 3 was studied and highest production of 5.75 g/l PHB and 4.81 g/l PHB was obtained at pH 6 and 7 respectively. The result of PHB yields at different temperature conditions (20^0C, 25^0C, 30^0C, 35^0C and 40^0C) was also examined. Table 2 shows that the optimal temperature was 30^0C producing 5.72g/l PHB from 8.21g/l DCW.

Table 2: PHB production at different carbon sources, nitrogen sources, pH and temperatures

Parameters		*DCW (g/l)*	*PHB concentration (g/l)*	*PHB content (%)*
Carbon sources	Glucose	1.06	0.35	33.0
	Fructose	12.23	5.55	45.3
	Mannitol	1.01	0.42	41.5
	Sucrose	8.21	2.45	29.8
	Starch	9.03	3.20	35.4
Nitrogen sources	Peptone	9.20	4.51	49.0
	Yeast extract	7.01	3.92	55.9
	Tryptone	10.02	7.65	76.3
	Urea	5.12	*ND	*ND
	Ammonium chloride	7.81	5.25	67.2
pH	5	9.20	4.01	43.5
	6	7.65	5.75	75.1
	7	7.25	4.81	66.3
	8	5.03	2.55	50.6
	9	9.71	4.65	47.8
Temperatures	20^0C	10.11	5.45	53.9
	25^0C	7.65	3.87	50.5
	30^0C	8.21	5.72	69.6
	35^0C	6.09	2.34	38.4
	40^0C	8.15	2.01	24.6

*ND=Not Detected

4. Discussion and Conclusion

Concern over petrochemical plastics in the environment, has created a renewed interest in biologically derived polymers. PHAs can serve as an efficient alternative to petroleum based non-degradable polymers [15]. Many bacterial strains which have potential to produce PHA have been isolated and identified from different origins but, still screening of a novel bacterium remains untapped [16]. The sheer diversity of the microbial community calls for the identification of bacteria capable of producing large amounts of PHB utilizing cheap nutrient sources. This requires careful optimization and analyses of conditions under which PHB synthesis is maximized.

In this study, a new potential PHB producer was isolated from the municipal waste areas of Silchar, Assam and its culture conditions were optimized for efficient PHB production. On the basis of morphological information, cultural studies, biochemical data and molecular analysis, the new bacterium isolated, clearly belonged to the genus *Bacillus*. Phylogenetic analyses of 16S rRNA demonstrated that this bacterium grouped with *B. tequilensis* shows a well-defined taxon that deserves the rank of species. The isolate was termed as *B. tequilensis* NCS-3 and found to be aerobic, gram positive, motile, rod shaped, spore forming, oxidase positive, catalase positive and positive for citrate utilization test. This bacterium can also reduce nitrate to nitrite and can hydrolyse starch too. Production of PHB has also been reported in different *Bacillus* species i.e, *B. megaterium* [17], *B. subtilis* [18], *Bacillus* species [19, 20], *B. mycoides* [21], etc.

PHB production is largely dependent on the type of the carbon source utilized by the bacteria. The course of PHB concentration and PHB formation rate was shown in Table 2. The maximum PHB production was attained (45 % DCW) when fructose was used as a sole carbon source. The PHB production was clearly decreased when fructose was replaced by glucose, sucrose, mannitol and starch that were 33, 29, 41 and 35 % DCW, respectively. Similar results were observed in *Bacillus megaterium* and other *Bacillus* sp. [22]. Reports were also there which showed fructose as an effective nutrient for producing PHB [23]. Using sugars as carbon source for PHB production can also be useful for saving energy required for liquefaction and saccharification.

Supplementation of different nitrogen sources in DM9 medium clearly shows the influence of nitrogen in the production of PHB. The cell biomass varied from 5.12g/l to 10.02g/l and maximum biomass was obtained with tryptone as shown in Table 2. Previous reports suggested that complex nitrogen sources increased the yield of PHB [19] whereas better yield of PHB was obtained by *Bacillus*, *Staphylococcus* and *Pseudomonas* using ammonium sulphate and ammonium phosphate as nitrogen sources than that of yeast extract [21]. Peptone is also a good source of nitrogen which favours the growth and PHB production by *Azotobacter chroococcum* [24]. In the present study, *Bacillus tequilensis* strain showed substantially higher biomass (10.02g/l) in turn, high PHB accumulation (67.3%) when compared with other *Bacillus sp* strains like *Bacillus sp* INT005 (35.30%) [25], *Bacillus cereus* SPV (41.90%) [8], *Bacillus cereus* CFR06 (46.0%) [26] reported so far.

Temperature and pH also plays an important role in the production of PHB and therefore its influence was studied in optimizing the culture conditions of the bacteria. PHB production by *Bacillus tequilensis* NCS-3 was examined at 20^0C, 25^0C, 30^0C, 35^0C and 40^0C. The optimal temperature found was 30^0C producing 5.72g/l PHB from 8.21g/l DCW. Similar results were obtained by other *Bacillus sp.* which showed that 30^0C was a favourable temperature for PHB production [23, 27]. The influence of pH on PHB production by *Bacillus tequilensis* NCS-3 strain was also optimized and highest PHB production of 5.75g/l and 4.81g/l was obtained at pH 6 and 7. It has been reported that pH value ranging from 6.0-7.5 is optimum for PHB production by *Alcaligenes latus* [27]. PHB production occurs at pH 6.4 and that the lack of polymer accumulation at higher pH value may be due to an effect on the degenerative enzymes of polymer breakdown, so that the PHB is utilized at the rate almost equal to the rate of its synthesis [28].

Based on the above results, it could be concluded that optimum culture conditions for effective PHB production by *Bacillus tequilensis* NCS-3 was pH 6 at 30^0C by utilizing fructose and tryptone as carbon and nitrogen sources respectively. Before optimizing the culture conditions, it produces 1.0g/l PHB from 1.92g/l DCW but after optimization, it produces 1.75g/l PHB from 2.01g/l DCW i.e PHB production increases from 52% to 87% of DCW. Therefore, selection of efficient PHB producing bacteria and on optimizing the most favourable conditions for PHB production is very important for successful production of biodegradable plastics.

5. Future Scope

This study suggests that *Bacillus tequilensis* NCS-3 is a potential PHB producer that could be utilized for industrial production of bioplastic and by utilizing its optimized culture conditions; we could somehow reduce the high cost of PHB production.

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