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GROWTH PERFORMANCE OF *STENOTROPHOMONAS MALTOPHILIA* (SB16) IN DIFFERENT CULTURE MEDIA

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Abstract

An *in vitro* experiment was conducted to test the growth of *Stenotrophomonas maltophilia* (Sb16) in different media in laboratory. The treatments for different media were made by adding molasses (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0 g w/v) as carbon sources compared to commonly used Nutrient Broth and Tryptic Soy Broth. Dry cell mass of *S. maltophilia* (Sb16), total reducing sugar and total N in culture media were determined. The highest growth of *S. maltophilia* (14.84 mg/ml) was observed at sugarcane molasses concentration 20% w/v after 24 hrs of incubation. Total reducing sugar increased with increasing molass concentration. High total N (505 mg/l) was ploduced in 7.5% w/v molasses concentration.

Introduction

Stenotrophomonas maltophilia (Sb16) is an endophytic N_2-fixing bacteria isolated from rhizophere rice soil in Tanjung Karang, Selangor was able to consume several sugars such as glucose, sucrose, fructose and arabinose as its carbon sources for growth (Naher *et al.* 2009). Sugarcane molasses is high in nutrients including B vitamins, iron, calcium, magnesium, and potassium. Sugarcane molasses usually has 17 - 25% water content, 45 - 50% sugar content (sucrose, glucose and fructose) and 25% polysaccharides (dextrin, pentosans, polyuronic acids) (Najafpour and Shan 2003).

Molasses can be used as nutrient medium as it is relatively inexpensive and can serve as an economic alternative to synthetic medium for the production of some other bio-products such as ethanol, biodegradable plastic or polyhydroxyalkanoates (PHA), lactic acid and many more (Nighat and Nazia 2014). Earlier researchers used it as a cheap carbon and energy source to cultivate microorganisms and also to obtain valuable products (El-Enshasy *et al.* 2008). The objective of this study was to determine the effect of different concentrations of molasses on growth of *S. maltophilia*, total reducing sugar and N content in culture media.

Materials and Methods

Stenotrophomonas maltophilia (Sb16) was previously isolated from rice rhizosphere and used as an inoculant in MR 219 rice (Naher *et al.* 2008) with cellulolytic enzyme activity, high IAA and nitrogenase activity (Naher *et al.* 2009, 2011). *S. maltophilia* (Sb16) prior culture was obtained from Microbiology Laboratory, Faculty of Agriculture, Universiti Putra Malaysia (UPM). The bacterial strain was grown in 500 ml Erlenmeyer flasks containing 200 ml Jensen's N-free liquid medium (Jensen 1942) and shaken on the orbital shaker at 100 rpm, 30 ± 1°C for 72 hrs. The bacterial cells were then harvested by centrifugation at 4000 rpm for 40 min and washed with 0.85% sterilized phosphate buffer solution (PBS).

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Approximately 1.0 ml 1.0 × 10^4 of live washed cells were inoculated to the different molasses concentrations solution (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0 g w/v), Nutrient Broth (NB) and Tryptic Soy Broth (TSB). The cultures were shaken at 100 rpm for 24 hours. Dry cell mass (DCM) of *S. maltophilia*, total N and total reducing sugar in growth media were determined. The bacterial cells were harvested by centrifuging at 9000 rpm for 20 minutes. The supernatant of sample was kept for determination of total reducing sugar and total N. The cell pellet was washed with sterilized 0.85% phosphate buffer solution and centrifuged. After that the cell pellet was dried in oven at 70 ± 2 °C until constant weight was obtained and the DCM was determined. DCM was for determination of optimum growth condition. DCM was calculated with the following equation: Dry cell mass (DCM) = DCM with centrifuge tube – empty centrifuge tube (Magdi *et al.* 2010).

One ml of supernatant sample was added to 5 ml of distilled water in 50 ml falcon tube. Two ml of reagent 1 (Somogyi 1945) was added and the flask was capped with glass ball and boiled in water bath at 100 ± 2 °C for 20 minutes. The samples were then rapidly cooled in ice water, and 2 ml of reagent 2 (Nelson 1944) was added to the sample. The color absorbance was determined after 30 minutes using spectrophotometer (CE 1011 1000 Series, Cecil Instruments, Cambridge, United Kingdom) at 710 nm wavelength. The result was compared with the standard graph. The concentration of total reducing sugars in the supernatant was calculated from glucose standard graph. One ml of supernatant sample was added to the 5 ml of concentrated H_2SO_4 and placed on digestion block at 200 °C for an hour. A few drops of 35% H_2O_2 were added to the sample until the sample solution became clear and was heated again on the digestion block at 350 ºC for an hour. Then the samples were left to cool down and the volume in the flask was made up with 100 ml distilled water and filtered, and read for total N by autoanalyzer (Quickchem FIAT 8000, Lanchat Instrument, Loveland, Colorado, USA). All the data collected were subjected to the analysis of variance (ANOVA) appropriate to one way experiments in complete randomized design (CRD) using SAS statistical program version 9.3. Mean separation was carried out using Tukey's Studentized Range (HSD) at the 5% level of probability.

Results and Discussion

Sugarcane molasses positively affected the growth of *S. maltophilia.* The bacterial dry cell mass differed significantly among the treatments. Highest dry cell mass was produced at 20% (w/v) molasses concentration (Fig. 1). The cell mass increased with increased in molasses concentration. This is probably due to sugarcane molasses containing high sugars such as sucrose, glucose and fructose that support the bacterial growth. Sugarcane molasses has been used in fermentation industry as carbon source for the microorganisms (Najafpour and Shan 2003).

Presence of high sugars like sucrose, fructose, glucose and vitamins in molasses, can serve as a source of growth factors to replace the use of glucose in many microbiological production processes such as bio-product production (Albuquerque *et al.* 2007, Malathi and Chakraborty 1991). The requirement of sugars by *S. maltophilia* for growth was also observed by Naher *et al.* (2009) in which about 94 and 90% of applied fructose and glucose, respectively were consumed for growth and N_2 fixation. In this study the growth of *S. maltophilia* in commercialised Nutrient Broth and Tryptic Soy Broth, was lower than in the lowest concentration (2.5%) of sugarcane molasses. This indicates that sugarcane molasses can be an alternative media for bacterial production.

Total reducing sugar in growth media increased with increasing molasses concentration, with values significantly higher than in NB and TSB (Fig. 2). The increased reducing sugar could be due to enzymatic hydrolysis of molasses by bacteria for carbon and energy metabolism. It has

been shown that glucomylase enzyme could be involved in the hydrolysis of molasses to release glucose (Najafpour and Shan 2003).

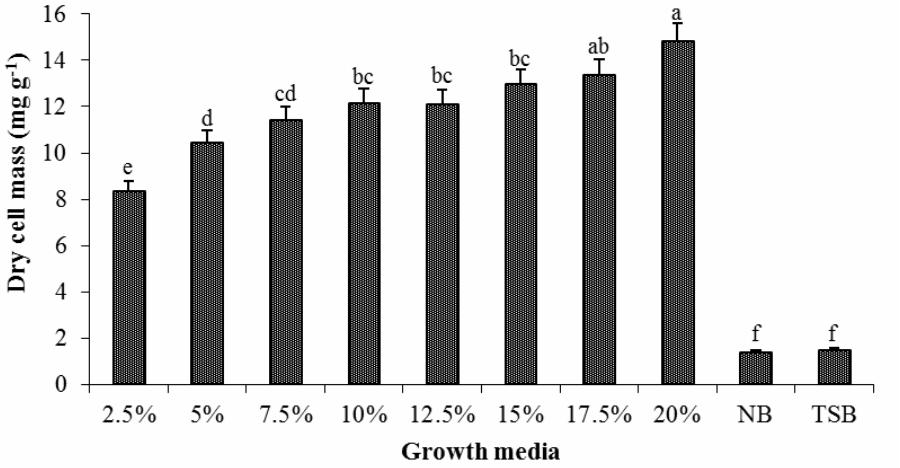

Fig. 1. Cell biomass of *S. maltophilia* (Sb16) in growth media with different concentrations of molasses, Nutrient Broth (NB) and Tryptic Soy Broth (TSB) after 24 hrs of incubation. Means with same letter indicate not significantly different at $p \geq 0.05$ based on Tukey's test. Bars indicates standard error, n = 3.

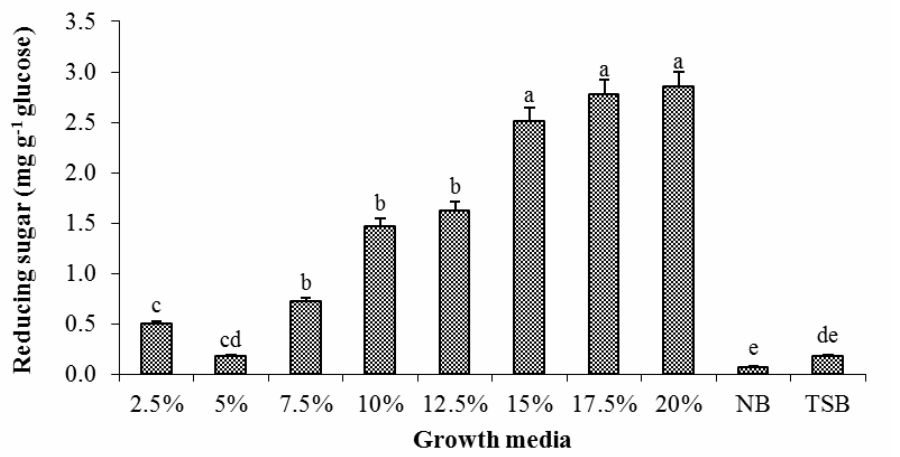

Fig. 2. Total reducing sugar in growth media with different molasses concentration, Nutrient Broth (NB) and Tryptic Soy Broth (TSB) after 24 hrs of incubation. Means with same letter indicate not significantly different at $p \geq 0.05$ based on Tukey's test. Bar indicates standard error n = 3.

During incubation molasses containing high amount of sucrose (non-reducing sugar), was hydrolyzed to reducing sugar. This increased the total reducing sugar with increasing molasses concentration. The amount of reducing sugar in NB and TSB media consistent with less bacterial growth.

The total nitrogen in growth media was affected by the source of carbon applied. The highest total nitrogen was observed at 7.5% molasses after 24 hrs of incubation (Fig. 3). The increase in total N could be due to the accumulation of ammonia a product of nitrogen fixation by *S.*

maltophilia. Rapid growth of *S. maltophilia* in molasses compared to commonly use Nutrient Broth and Tryptic Soy Broth indicated presence of available carbon compounds such as glucose and fructose required by the bacteria. Previous work by Naher *et al* (2009) showed that *S. maltophilia* has high affinity for fructose.

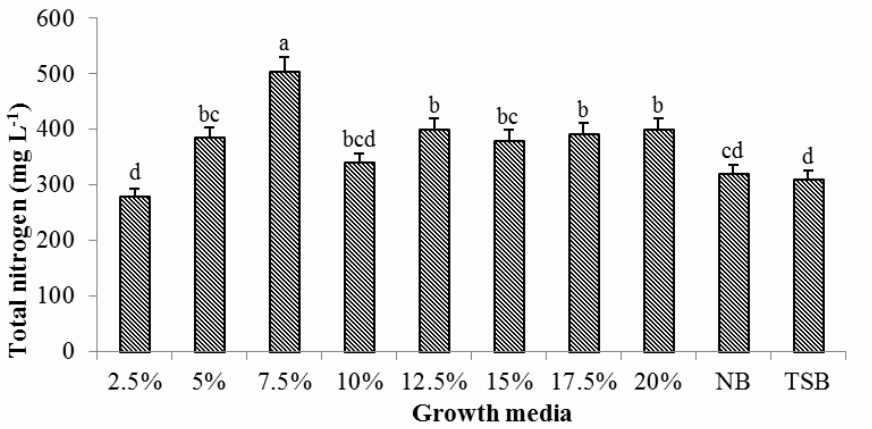

Fig. 3. Total nitrogen in growth media with different molasses concentration, Nutrient Broth (NB) and Tryptic Soy Broth (TSB) after 24 hrs of incubation. Means with same letter do not indicate significantly different at $p \geq 0.05$ based on Tukey's test. Bars indicates standard error, n = 3.

The study showed that dry cell mass, total reducing sugar and total N of *Stenotrophomonas maltophilia* increased in growth media amended with sugarcane molasses. This indicates the potential of molasses as a cheaper source of carbon and energy for mass culturing of the bacteria.

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