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Full Length Research Paper

Effect of temperature and pH on ethanol production by a *Blastomyces* species isolated from the intestine of oil palm weevil (*Rhynchophorus palmarum*, coleoptera)

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The effects of pH (4.5, 5.0, 5.5, 6.0, 6.5 and 7.0), temperature (20, 25, 30, 35, 40 and 45°C) and combinations of the two (pH 4.5, 20°C; pH 5.0, 25°C; pH 5.5, 30°C; pH 6.0, 35°C; pH 6.5, 40°C; pH 7.0, 45°C) on ethanol production by a putative *Blastomyces* species isolated from the intestine of oil palm weevil larva were studied. Peptone water was used as the growth medium and granulated sugar (sucrose) was used as the fermentation substrate. Ethanol contents were determined gravimetrically and the percentage of ethanol was determined as volume estimated from an alcoholometry table. Results show that the highest amount of ethanol (10.50%) was produced at a temperature of 35°C and the lowest (3.15%), at 20°C. Similarly, the highest amount (4.95%) was produced at a pH of 5.0 while the lowest (3.03%) was at pH 7.0. When pH and temperature were taken together, the highest amount of ethanol (6.0%) was produced at temperature 30°C and pH 5.5. Appropriate adjustments in pH and temperature of the fermenting medium are, therefore, necessary for maximum ethanol production by the organism.

Key words: Temperature, *Blastomyces*, oil palm weevil, ethanol.

INTRODUCTION

Ethanol is made by the fermentation of sugary substrates by certain microorganisms. For thousands of years, ethanol has played a role in the life of humans. It has been known as a constituent of beer and wine, and of other kinds of alcoholic beverages with varieties of names and containing varying higher concentrations of alcohol. It is used as solvent, extractant and antifreeze, a substrate for the synthesis of many other solvents of dyes, in pharmaceuticals, detergents, plasticizers, etc (Sasson, 1984; Dubey, 2006). As the world advances more and more in science and technology, the uses and, therefore, demand for, ethanol become correspondingly increased.

Blastomyces species are saprobes of the soil. They commonly inhabit decaying wood material. In particular, *Blastomyces dermatitis* (syn. *Ajellomyces dermatitis*) is a fungus that grows as a budding yeast in humans but as a mold on culture media and in the environment (Prescott et al., 1999). It is the causative agent of Blastomycosis in humans (Talaro and Talaro, 2002). In a preliminary investigation, a *Blastomyces* species isolated from the intestine of oil palm weevil was shown to produce ethanol. This is not unlikely since many fungi are known to be capable of ethanol production. Aside yeast species, fungi known to produce ethanol include *Aspergillus niger, Endomyces lactis, Kloeckera* sp., *Kluyreromyces fragilis, Mucor* sp., *Neurospora crassa* and *Rhizopus* species (Dubey, 2006).

Environmental factors are known to greatly influence the growth and metabolism of microorganisms. The study of such environmental factors is essential since it contributes to understanding the control of metabolic activities and the optimization of yields of metabolic end-products of interest (Ogbonda et al., 2007). Such environmental factors include pH and temperature. pH

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alters the solubility of ions and influences the dissociation of molecules, thus determining their availability to the microorganism. At the correct pH, therefore, ions and nutrients needed by the microorganism are readily used by the microorganism, then growth rate increases and yields of metabolic product is enhanced (Hawker and Linton, 1972). In like manner and in a given growth medium, a microorganism grows most rapidly at a particular temperature or small range of temperatures. This often increases the production of certain metabolic end-products (Patchy and Rose, 1970).

In a world where innovation and versatility are the driving forces of technology, information on the various organisms that can produce a particular metabolite, aside from the conventional ones, and the affective environmental factors, is necessary. The number and diversity of the organisms that can produce ethanol is, therefore, of value. The aim of this study was therefore, to investigate the effect of pH and temperature on ethanol production by a *Blastomyces* species isolated from the intestine of oil palm weevil. Results will provide guidance as to the best pH and temperature requirements for the optimization of ethanol production by the organism.

MATERIALS AND METHODS

Test organism

Blastomyces species previously isolated from the intestine of the larva of oil palm weevil was used in this study.

Maintenance of test organism

The *Blastomyces* species was maintained in Sabouraud's agar slants in McCartney bottles. The Sabouraud's agar was composed of: glucose, 40.0 g; peptone, 10.0 g; agar, 15.0 g; distilled water, 1000 ml.

Growth medium

The medium used in the study was peptone water (lab 104), which was composed of peptone, 5.0 g/l; tryptone, 5.0 g/l; and sodium chloride, 5.0 g/l.

Preparation of fermentation medium

Fifteen grams (15.0 g) of the peptone water powder was dissolved in 1 L of sterile distilled water and the solution was dispensed into final containers. Into each of several 250-ml conical flasks was pipetted 200 ml of the growth medium. One gram (1.0 g) of granulated sugar (sucrose) was added into the medium in each of the conical flasks. The flasks were sterilized in the autoclave at a temperature of 121°C (1.02 bar) for 15 min.

Inoculation of test organism

The *Blastomyces* species was inoculated into the growth medium in each of the conical flasks using a sterile inoculation loop. Each inoculum contained 10 to 15 fungal cells. Vaspar (Prepared as 50:50 paraffin and vaseline, and sterilized) was poured into each of the conical flasks to provide an anaerobic condition.

Determination of the effects of temperature and pH

To determine the effect of temperature, the inoculated flasks were incubated in thermostatically controlled water baths (Cole-Medical, England; serial No. 0606058) maintained at the appropriate temperature (20, 25, 30, 35, 40 and 45°C). To determine the effect of pH, the pH of the medium in the inoculated flasks was adjusted appropriately (4.5, 5.0, 5.5, 6.0, 6.5 and 7.0).

Interaction between temperature and pH

The interaction between temperature and pH on ethanol production by the *Blastomyces* species was studied using the following combinations of temperature and pH: 20°C, pH 4.5; 25°C, pH 5.0; 30°C, pH 5.5; 35°C, pH 6.0; 40°C, pH 6.5; and 45°C, pH 7.0.

Fermentation

Incubation at the appropriate temperature was for 14 days.

Determination of alcohol contents of fermented solutions

The alcoholic strengths of the fermented solutions were determined by gravimetric method using double distillation. The specific gravity of the distillates was measured at $20^0C/20^0C$ using a 50-ml pycnometer bottle. The alcohol contents as percentage by volume were estimated from the laboratory tables (alcoholometry).

RESULTS AND DISCUSSION

Effect of pH

Figure 2 shows the effect of pH on ethanol production by the *Blastomyces* species. Higher amounts of ethanol were produced at higher acidic (lower pH) conditions with the highest amount (4.95%) being produced at pH 5.0. As acidity decreased (higher pH), the amounts of ethanol produced decreased with the least (3.03%) at pH 7.0.

Blastomyces species is known to share the characteristics of budding yeast (Prescott et al., 1999). As a mold, its range of growth pH is 2.0 to 9.0 (Bilgrami and Verma, 1981). Behaving as a yeast, the pH range for growth can vary from 4 to 6 (Narendranath, 2001). The range at which it produced the highest amounts of ethanol in this study is shown in Figure 2.

Effect of temperature

The effect of temperature on ethanol production by the *Blastomyces* species is presented in Figure 1. The highest amounts of ethanol were produced at a temperature range of 30 to 40°C, with the highest amount (10.50%) being produced at 35°C. Below and above this

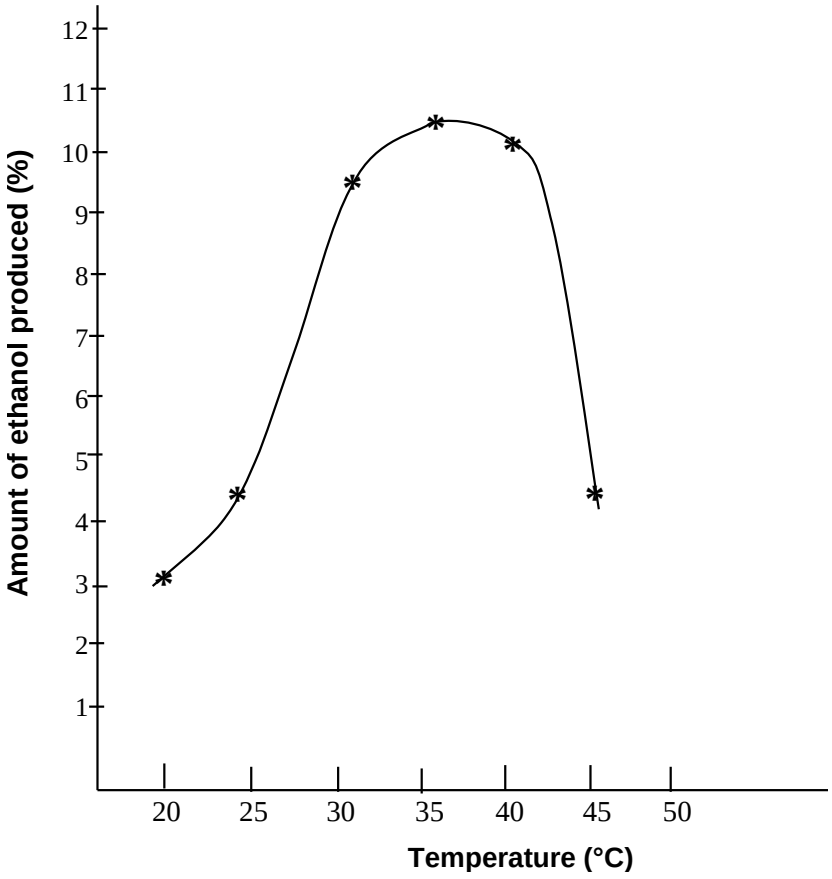

Figure 1. The amounts of ethanol produced at various temperature levels.

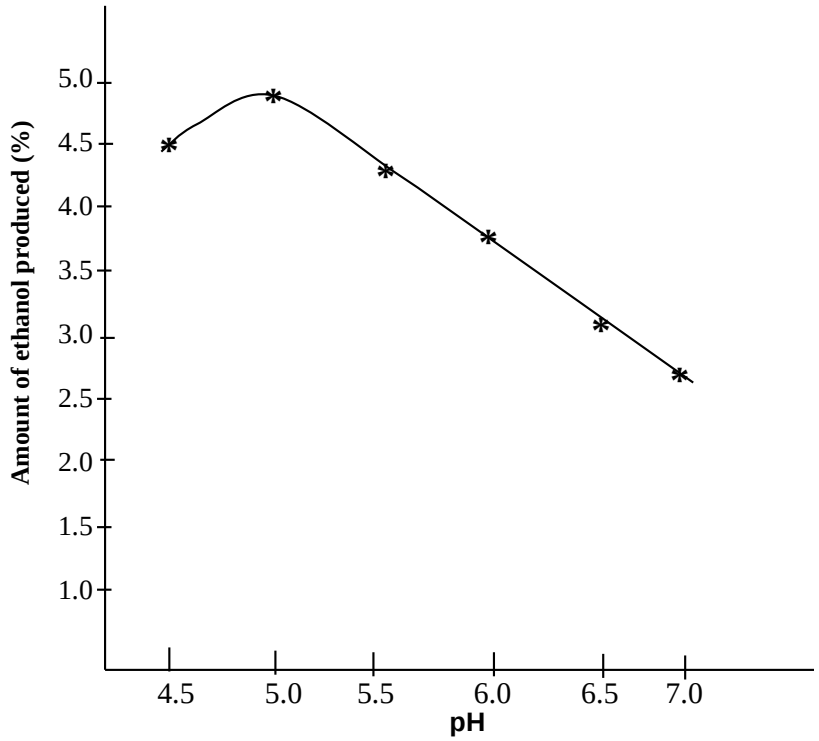

Figure 2. The amounts of ethanol produced at various pH levels.

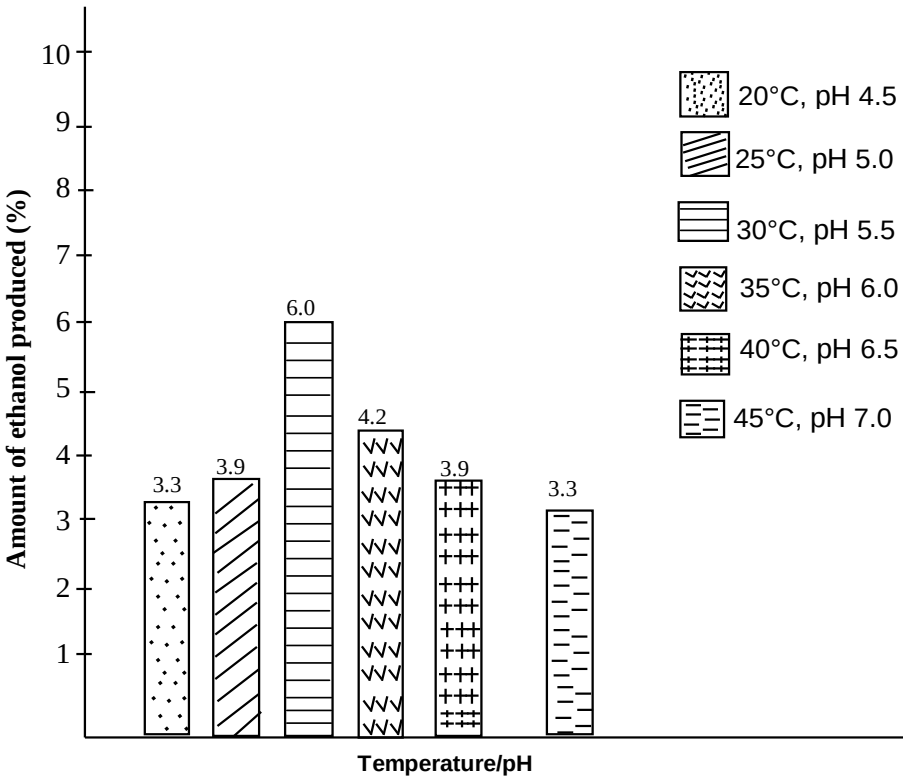

Figure 3. The amounts of ethanol produced at various temperature and pH values.

temperature value, the amounts of ethanol produced decreased, with the least being produced at 20°C (3.15%) and 45°C (4.56%). Slavikova and Nadketrova (2003) reported that yeast generally, will grow over an optimum temperature range of 30 to 37°C. This range of temperature agrees with the range at which the highest amounts of ethanol were produced in the present study.

Interaction of temperature and pH

The result of the effect of temperature and pH combined on ethanol production by the *Blastomyces* species is presented in Figure 3. The highest amount of ethanol (6.0%) was produced at a temperature of 30°C and pH 5.5. Recall that the temperature and pH values (each taken singly) at which the highest amounts of ethanol were produced are different from those at which the highest amount of ethanol was produced when both were combined. This clearly showed that each parameter (temperature or pH) has a modulating effect on the other. It would, therefore, be necessary to adjust the pH and temperature of the growth (fermentation) medium appropriately in order to maximize ethanol production by the organism.

Conclusion

To maximize ethanol production by the *Blastomyces* species would require adjusting the pH and temperature of the growth medium appropriately.

REFERENCES

Bilgrami KS, Verma RN (1981). Physiology of fungi. 2nd Revised edition. Vikas Publishing House PVT Ltd. p. 3.

Dubey RC (2006). A textbook of Biotechnology. S. Chand & Company Ltd. Ram Nagar, New Delhi. pp. 386-411.

Hawker LE, Linton AH (1972). Microorganisms: function, form and environment. Edward Arnold (publishers) Ltd. p. 727.

Narendranath NV (2001). Acetic and Lactic acid inhibition of growth of *Saccharomyces cerevisiae* by different mechanisms. J. Am. Soc. Brew. Chem. 59:157-194.

Ogbonda KH, Aminigo RE, Abu GO (2007). Influence of temperature and pH on biomass production and protein biosynthesis in putative *Spirilina* species. Bioresour. Technol. 98: 2207-2711.

Patchy JW, Rose AH (1970). In Noris JR and Ribbons DW (eds.). Methods Microbiol. Academic Press, London. 2:23-24.

Prescott LM, Harley JP, Kleim D (1999). Microbiology. Fourth edition. WCB McGraw-Hill, p. 816.

Sasson A (1984). Biotechnology: Challenges and Promises: UNESCO, Paris.

Slavikova E, Vadketrova R (2003). The diversity of yeast in agricultural soil. J. Basic Microbiol. 43(5):430-436.

Talaro KP, Talaro A (2002). Foundation in Microbiology. Fourth edition. McGraw-Hill Book Company, New York. p. 819.