Effect of aeration and method of addition of glucose sugar to culture medium on growth and sporulation of some *Bacillus thuringiensis* isolates from Sudan soils

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ABSTRACT

An experiment was conducted in the microbiology laboratory at the Faculty of Agriculture, University of Khartoum in the year 2003 to study the effect of aeration and method of addition of glucose sugar on growth and sporulation of three selected *B. thuringiensis* isolates (BR12, N10 and EA3) derived from Sudan soils. The isolates responded differently to air supply. Spore counts of isolate BR12 and EA3 increased with an increase in air supply. The highest count was produced at 19:1 air-to-medium ratio (v/v) and the lowest count at 3:1 ratio, whereas the highest spore count by isolate N10 was produced at 4:1 air-to-medium ratio (v/v) and any increase in aeration levels resulted in lower counts. On the other hand, method of addition of glucose sugar was found to affect both pH pattern during incubation period and spore count in the final culture for all selected isolates. Fed-batch addition of glucose resulted in survival of many growing cells which would have been killed in case of batch addition, with subsequent better sporulation. The effect of method of addition of glucose sugar on growth and sporulation of the tested isolates was found to be significant at 1% level.

INTRODUCTION

Oxygen plays an important role in the process of bacterial growth.Oxygen availability may profoundly influence metabolic events and consequently the product obtained (Emanuilova and Kambourova, 1992; Marty-Teysset *et al*., 2000; Talwalkar and Kailasapathy, 2003). Limited supply of oxygen in the bacterial culture restricts the yield (Lockhart and Squires, 1963). However, excessive supply of oxygen may exert deleterious effects (Finn, 1954).

It has been shown that, for many species of the genus *Bacillus*, sporulation is highly related to oxygen supply (Yousten and Wallis, 1987).

Many investigations have indicated that oxygen is essential for sporulation and delta–endotoxin production of various strains of *B. thuringiensis* (Avignone Rossa *et al.,* 1992).

On the other hand, it has been well established that most organisms grow best at pH values around neutrality (pH 6.6-7.5). Most bacteria are neutrophiles (pH 5.5–8.0). However, a few of them prefer extreme values (Bhadsalve *et al.,* 1972). Adverse pH affects at least two aspects of respiring microbial cells. First, it affects the functioning of their enzymes. Secondly, it affects the transport of nutrients into cells. This is because the pH might alter the ionization of nutrient molecules. For this reason and when shift in pH values is great, further growth of the organism will eventually be inhibited (Prescott *et al.,* 1999). The method by which glucose sugar is added to the growth medium of *B. thuringiensis* has an influence on both growth and sporulation. This is mainly because of the fact that the bacterium metabolizes glucose and produces acid which in turn, has a deleterious effect on the growing bacterial cells (Nickerson and Bulla, 1974). Therefore, the aim of this study was to investigate the effect of aeration and method of addition of glucose sugar on growth and sporulation of selected *B. thuringiensis* isolates derived from Sudan soils.

MATERIALS AND METHODS

Preparation of aeration rates

A method described by Foda *et al*. (1985) was used. In 500-ml Erlenmeyer conical flasks, 25, 50, 100 and 200 ml of the medium used by Avignone Rossa *et al*. (1990) were prepared. Thus 19:1, 9:1, 4:1 and 3:2 air-to-medium ratios were obtained. The medium was adjusted to pH 7.0 and then sterilized by autoclaving. Flasks were inoculated with the same amount of inoculum (two loopfuls of *B. thuringiensis* culture grown on nutrient agar for 24 hours). Flasks were placed on a rotary shaker at 200 rpm for 36-48 hours. Two flasks were used for each experiment.

Method of addition of glucose

The medium described by Avignone Rossa *et al*. (1990) was used as a basal medium. The medium was distributed in 500-ml Erlenmeyer flasks in 50 ml aliquots and autoclaved. Glucose, to make 0.8% (w/v) in the final medium, was autoclaved separately and added aseptically to bring the final volume of the culture in each tube to 10.0 ml. The sugar was added aseptically either batch-wise (all at once) or fed-batch at a rate of 0.3% at zero time, 0.25% after 24 hours and 0.25% after 48 hours. The media were inoculated and incubated at 37$\pm$2ºC on a rotary shaker at 200 rpm. The pH measurements were taken daily every 24 hours for 72 hours. Total viable cells and spores counts were determined at the end of incubation periods.

Total viable cells count (TVCC)

Total viable cells count per ml of *B. thuringiensis* isolate suspension was carried out by pour plate method. One hundred micro-liters of each dilution was mixed thoroughly with melted plate count agar (40–50^{0}C) and allowed to set. Plates were incubated at 32$\pm$2^{0}C for16–18 hours. Colony forming units (CFU/ml) were determined.

Spore count (SC)

Ten ml of *B. thuringiensis* isolates cultures were warmed in a water bath at 80^{0}C for 15 minutes, serially diluted, mixed with melted plate count agar (40–50^{0}C) and allowed to set. Plates were incubated at 32 $\pm$2^{0}C for 21–36 hours for spore count. Colony forming units (CFU/ml) were determined. Some of the results were subjected to statistical analysis.

RESULTS AND DISCUSSION

Figure 1 shows the effect of different aeration levels on the process of sporulation of different tested *Bacillus thuringiensis* isolates. The rate of sporulation of isolates BR12 and EA3 increased as the rate of aeration increased. Isolate BR12 produced high spore count at 19:1 and low count at 3:1 (v/v) air-to-medium ratios. Isolate EA3 was found to sporulate at 19:1 and 9:1 at a relatively high rate when compared to sporulation at 4:1 and 3:1 ratios. This finding indicated that, for the two isolates, oxygen supply is a very fundamental element in sporulation. The same result was obtained by Avignone Rossa *et al.* (1992) who found that spore count and viable cell count of *B. thuringiensis* subspecies *israelensis* and *galleriae* decreased when they were grown in cultures with low aeration rate and the counts remarkably increased when grown in culture with high aeration rate. Sug-Joon *et al.* (2009) came to the same conclusion**.** However, isolate N10 was found to sporulate best at 4:1 ratio, while an increase in aeration rate to 9:1 and 19:1 resulted in a decrease of sporulation. Similar findings were reported by Abdel-Hameed *et al*. (1991) who mentioned that an increase in aeration to a certain limit gave higher toxin production and any further increase in aeration resulted in a decrease of toxin production by a strain of *B. thuringiensis* isolated in Egypt.

Figure 2 shows that addition of glucose sugar all at once (at zero time) resulted in a decrease in pH values of the culture medium of *B. thuringiensis* isolates during the first day of incubation. This is mainly because of the fact that the bacterium metabolizes glucose by Embden-Meyerhof-Parnas pathway and produces acetic acid in the medium (Nickerson and Bulla, 1974). However, the pH value started to increase for

all isolates. This is probably due to production of alkaline material from nitrogenous nutrients that could neutralize the acidic component produced from sugar metabolism (Dulmage *et al.*, 1990).

Addition of the sugar in a fed-batch method resulted in an increase in pH values (Figure 3). Different patterns of pH changes were observed when the final pH values of the culture media due to both methods of glucose sugar addition were compared. Similar results were obtained by Foda *et al.* (1985).

In the present study, it was found that batch addition of the sugar resulted in low pH values of the culture medium and as a result of that many cells were killed. On the other hand, fed-batch addition caused a rise in pH level which resulted in high spore and viable cells counts (Figure 4). This finding goes in line with the results shown by Foda *et al.* (1985) who found that incremental addition of sugar(fed-batch) resulted in survival and sporulation of large numbers of cells of *B. thuringiensis* subspecies *entomocidus* that would have been killed upon batch (all at once) addition. The two

methods of glucose addition resulted in statistically significant differences in both total viable cells counts and spores counts of the three isolates EA3, N10 and BR12 (Table 1).

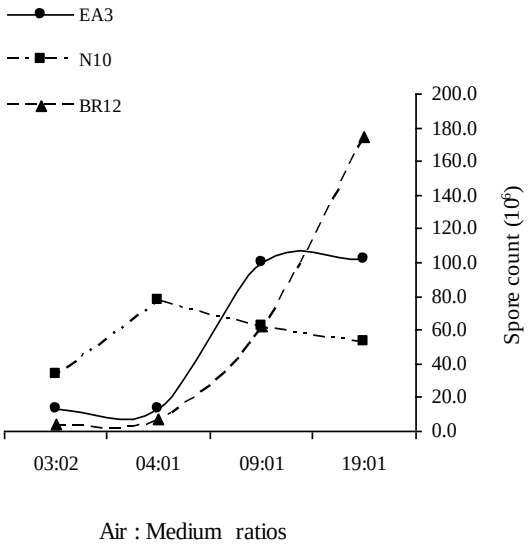

Fig. 1. Effect of different aeration levels on sporulation of different *B.thuringiensis* isolates after 24 hrs of shaking .

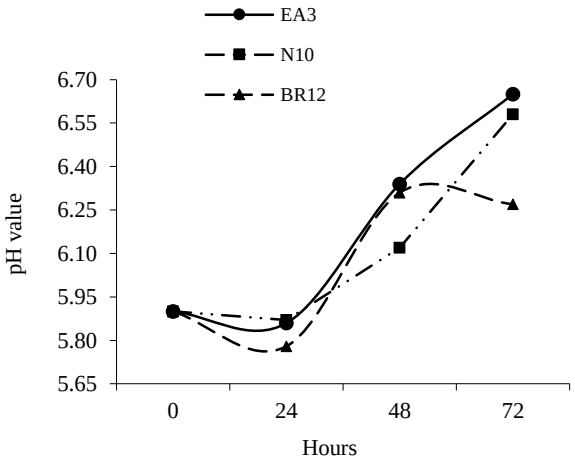

Fig. 2. Effect of addition of glucose sugar all at once (at zero time) on pH change during the growth of selected *B. thuringiensis* isolates.

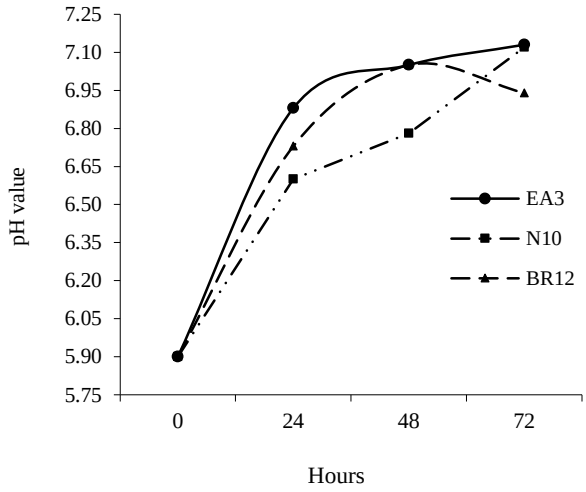

Fig. 3 .Effect of fed-batch addition of glucose sugar on pH changes during the growth of selected *B. thuringiensis* isolates.

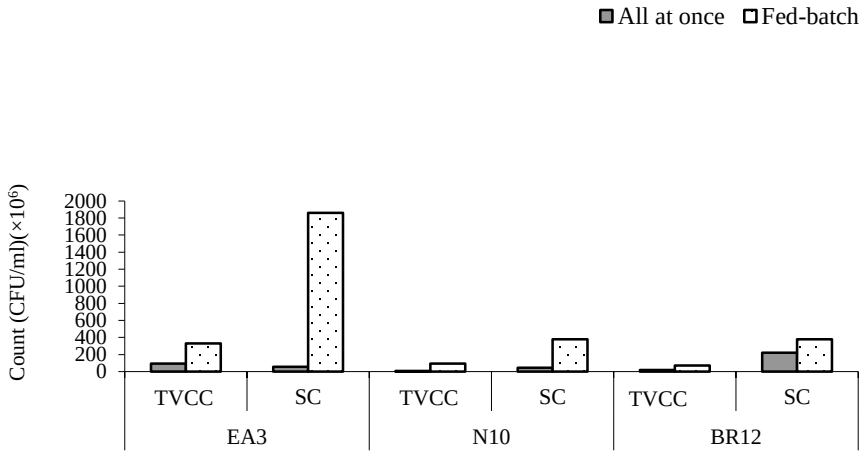

Fig. 4. Effect of method of addition of glucose on growth and sporulation of *B. thuringiensis* isolates.

Table 1. Effect of method of addition of glucose on growth and sporulation of selected *B. thuringiensis* isolates

Method of glucose addition	TVCC	SC
All at once	8.023 b	7.910 b
Fed-batch	8.113 a	8.807 a
Mean	8.068	8.358
Significance level	**	***
C.V	0.32	o.56
SE(±)	0.0106	0.0192

TVCC =Total Viable Cell Count, SC= Spore Count.
** denotes significance at P=0.01, *** denotes significance at P=0.001.
Means within a column followed by different letters are significantly different according to t- test.

CONCLUSION

From the present study, it could be concluded that different isolates of *Bacillus thuringiensis* need different aeration rates of oxygen supply for attaining good growth. However, high aeration levels *viz.* 9:1 and 19:1 provide good oxygen demand for the growth of two of the tested *Bacillus thuringiensis* isolates (BR12 and EA3). Moreover, for better growth of the bacterium, fed-batch addition of glucose sugar to the culture medium is recommended.

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تأثير التهوية ونمط إضافة سكر الجلوكوز إلي وسط النمو علي نمو وتجرثم بعض العزلات الباكتيرية من نوع *Bacillus thuringiensis* والتي تم عزلها من الترب السودانية

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الخلاصة

أجريت تجربة بمعمل الأحياء الدقيقة بكلية الزراعة، جامعة الخرطوم، في العام 2003م لدراسة تأثير التهوية ونمط إضافة السكر علي نمو وتجرثم بعض سلالات البكتيريا من نوع *Bacillus thuringiensis* السودانية تم عزلها من الترب السودانية تم . استجابت العزلات للتهوية بمعدلات مختلفة، حيث وجد أن معدل BR12 ، N10 ، EA3 اختيار ثلاث عزلات لهذه الدراسة هي وأعلي معدل تجرثم للعزلتين حدث عند معدل تهوية 19:1 (هواء : EA3 ، BR12 التجرثم ازداد بزيادة التهوية للعزلات فقد كان أعلي معدل تجرثم عند معدل التهوية 4:1 ووجد أن أي [N10) بينما أقل معدل عند 3:1. أما العزلة v/v وسط نمو زيادة في التهوية أدي إلي نقصان التجرثم. وفي الجانب الآخر فقد وجد أن نمط إضافة السكر إلي وسط النمو يؤثر علي نسق الأس الهيدروجيني بوسط النمو ومعدل التجرثم للعزلات المختارة. أدي إضافة السكر علي جرعات إلي نجاة الكثير من الخلايا الباكتيرية والتي كانت ستموت في حالة إضافة السكر جرعة واحدة مما حسن معدل التجرثم. إحصائيا وجد أن هناك فرق معنوي لنمط إضافة السكر عند احتمال 1% .