Parâmetros de crescimento para otimização do cultivo de isolados de *Bacillus thuringiensis* (Berliner, 1911) no controle de *Aedes aegypti* (Linnaeus, 1762)

Growth parameters for optimizing the cultivation of *Bacillus thuringiensis* (Berliner, 1911) isolates to control *Aedes aegypti* (Linnaeus, 1762)

Parámetros de crecimiento para optimizar el cultivo de aislados de *Bacillus thuringiensis* (Berliner, 1911) para el control de *Aedes aegypti* (Linnaeus, 1762)

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RESUMO

Objetivo: Obter os parâmetros de crescimento de linhagens de *Bacillus thuringiensis* ativas a *Aedes aegypti*. **Métodos:** Dezoito combinações de pH e temperaturas foram testadas para obtenção das melhores condições de crescimento para três isolados do BBENMA, BtMA-690, BtMA-750 e BtMA-1114. Para comprovação das melhores condições de crescimento foi realizada a quantificação do inóculo obtendo a concentração de esporos/mL e massa celular (g/L). As CL_{50} dos isolados cultivados nas condições extremas de temperatura e das duas melhores condições de crescimento foram testadas em bioensaios com de *A. aegypti* para confirmação de toxicidade. **Resultados:** Os isolados BtMA-690 e BtMA-750 demostraram melhores condições de crescimento nas combinações de 34°C com pH 8,0 para concentração de esporos/mL e 30°C com pH 6,0 para massa celular, enquanto BtMA-1114 apresentou melhor crescimento nas condições de 28°C com pH 7,0 para concentração de esporos/mL e 34°C com pH 6,0 para massa celular. Após 72 horas, BtMA-690 apresentou mortalidade de 35%, na combinação 26°C com pH 6,0, BtMA-750 acima de 90% para nove das condições testadas e BtMA-1114 com 14%, para 36°C com pH 7,0. **Conclusão:** A otimização do crescimento das linhagens de Bt em laboratório possibilitará melhor adequação dos parâmetros de crescimento utilizados no cultivo dos isolados.

Palavras-chave: Bactéria entomopatogênica, Controle biológico, Vetor.

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ABSTRACT

Objective: To obtain the growth parameters of *Bacillus thuringiensis* (Bt) strains active against *Aedes aegypti*. **Methods**: Eighteen combinations of pH and temperatures were tested to determine the best growth conditions for three isolates from the Maranhão Entomopathogenic Bacillus Bank: BtMA-690, BtMA-750, and BtMA-1114. To confirm the best growth conditions, the inoculum was quantified by measuring the spore concentration (spores/mL) and cell mass (g/L). The LC_{50} of the isolates grown under extreme temperature conditions and the two best growth conditions were tested in bioassays with *A. aegypti* larvae to confirm toxicity. **Results**: Isolates BtMA-690 and BtMA-750 showed the best growth conditions at 34°C with pH 8.0 for spore concentration and 30°C with pH 6.0 for cell mass. In contrast, BtMA-1114 exhibited better growth at 28°C with pH 7.0 for spore concentration and 34°C with pH 6.0 for cell mass. After 72 hours, BtMA-690 showed a mortality of 35%, in the combination 26°C with pH 6.0, BtMA-750 above 90% for nine of the conditions tested and BtMA-1114 with 14%, for 36°C with pH 7.0. **Conclusion**: Optimizing the growth of Bt strains in the laboratory will enable better adjustment of growth parameters for the cultivation of these isolates.

Keywords: Entomopathogenic bacteria, Biological control, Vector.

RESUMEN

Objetivo: Obtener os parámetros de crecimiento de *Bacillus thuringiensis* ativas a *Aedes aegypti*. **Métodos:** Dezoito combinações de pH y temperaturas foram testadas para obtener das melhores condições de crescimento para três isolados do BBENMA, BtMA-690, BtMA-750 e BtMA-1114. Para comprobar las mejores condiciones de crecimiento al realizar la cuantificación del inóculo se obtiene una concentración de esporos/mL y masa celular (g/L). As CL50 dos aislados cultivados nas condiciones extremas de temperatura y das dos mejores condiciones de crecimiento foram testadas em bioensaios com de *A. aegypti* para confirmación de toxicidade. **Resultados:** Los aislados BtMA-690 y BtMA-750 demuestran mejores condiciones de crecimiento en combinaciones de 34°C con pH 8,0 para concentración de esporos/mL y 30°C con pH 6,0 para masa celular, mientras BtMA-1114 Presenta un mejor crecimiento en condiciones de 28°C con pH 7,0 para concentración de esporos/mL y 34°C con pH 6,0 para masa celular. Después de 72 horas, BtMA-690 presentó una mortalidad de 35%, en combinación 26°C con pH 6,0, BtMA-750 acima de 90% para nuevas condiciones probadas y BtMA-1114 con 14%, para 36°C con pH 7,0. **Conclusión:** La optimización del crecimiento de Bt en el laboratorio possibilitará mejor según los parámetros de crecimiento utilizados en el cultivo.

Palabras clave: Bacterias entomopatógenas, Control biológico, Vector.

INTRODUCTION

Controlling mosquitoes such as *Aedes aegypti* (Linnaeus, 1762), *Anopheles darlingi* (Root, 1926), and *Culex quinquefasciatus* (Say, 1823), which are vectors of dengue, malaria, and lymphatic filariasis respectively, is a significant challenge for national public health (BRASIL, 2023; FERREIRA MU and CASTRO MC, 2016; LIMA-CAMARA TN, 2016).

New alternatives for controlling these species are being discussed, as numerous studies have documented the resistance of these mosquitoes populations to various classes of chemical insecticides (VARGAS LDL, et al., 2022; MOYES CL, et al., 2017; FLORES-SUAREZ AE, et al., 2016). Therefore, there is a need to replace the use of chemical insecticides, especially biological insecticides, which are considered ecologically safe (BRÜHL CA, et al., 2020; GUO S, et al., 2015; SCOPEL W and ROZA-GOMES MF, 2011).

The bacterium *Bacillus thuringiensi*s (Berliner, 1911) is one of the main active ingredients used in the manufacture of biolarvicides for biological control, due to its insecticidal properties, as it presents protein crystals that are formed by δ-endotoxins, Cry and/or Cyt proteins, which are toxic to a wide range of insects,

but in a specific way for each group, presenting multiple advantages, especially when compared to chemical control, which cause serious problems for human health and the environment (PEÑA LC, et al., 2022; HECKEL DG, 2020; BEN-DOV E, 2014; BRAVO A, et al., 2011; BRAVO A, et al., 2007; POLANCZYK R e ALVES S, 2003; ALVES SB, 1998).

This bacterium has significant potential for controlling mosquito vectors, and several strains have already been isolated with specific activity for mosquitoes in different parts of the world, mainly in studies with *A. aegypti* larvae (VIEIRA-NETA MRA, et al., 2020; LOBO KS, et al., 2018; SOARES-DA-SILVA J, et al., 2017; EL-KERSH TA, et al., 2016; CAMPANINI EB, et al., 2012).

Due to the good effectiveness of *B. thuringiensis* in vector control, several studies were conducted to explore alternative methods to produce this bacterium in laboratory conditions (VIEIRA-NETA MRA, et al., 2020; LOBO KS, et al., 2018; MOURÃO AHC, 2017; PEREIRA EL and MARTINS BA, 2016). Optimizing the growth conditions of each strain and choosing the appropriate cultivation medium are extremely important for the successful production of the bacteria. The ideal growth of bacterial strains must provide maximum production of toxic crystals, with minimum cost of laboratory inputs (MOURÃO AHC, 2017; COUCH TL, 2000).

Currently, Maranhão Entomopathogenic Bacillus Bank (BBENMA) is one of the largest in the Northeast region of Brazil, featuring strains with high toxicity in larvae of disease vectors, which were isolated from different biomes (VIEIRA-NETA MRA, et al., 2020; VIANA JL, et al., 2020; LOBO KS, et al., 2018; SOARES-DA-SILVA J, et al., 2017).

Because these strains are isolated from different biomes, it is necessary to understand the nutritional requirements, to optimize the growth of the bacteria in the laboratory, in order to increase the obtainment of insecticidal toxins, and subsidize the future production of low-cost bioproduct, for the control of mosquito vectors. With this, the objective was to obtain physical and chemical growth parameters in the laboratory for strains of *Bacillus thuringiensis* (Bt) active in disease vector mosquitoes.

METHODS

Selection of *Bacillus thuringiensis* isolates

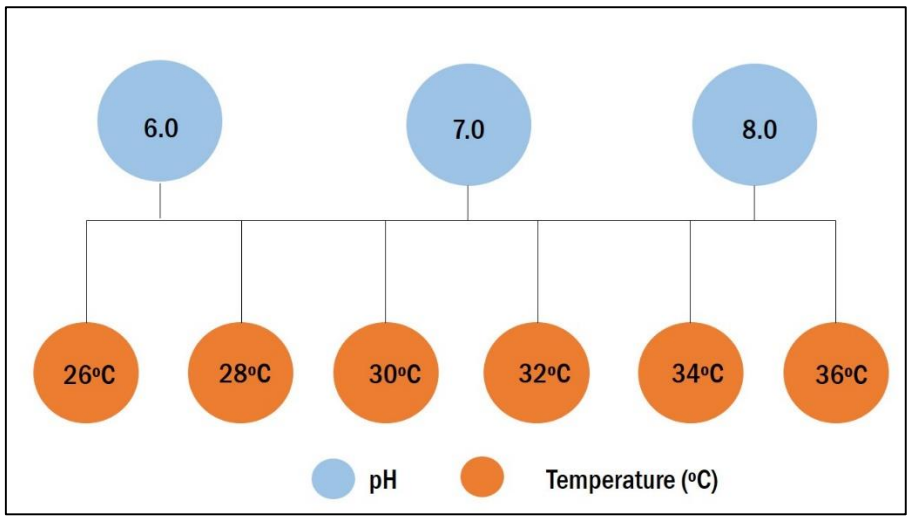
The *B. thuringiensis* strains were selected from BBENMA maintained at the Medical Entomology Laboratory (LABEM) of the State University of Maranhão (UEMA) Caxias Campus. We currently have around 1.500 isolates of *B. thuringiensis* from the Amazon, Cerrado and Caatinga biomes, the Mangrove and Restinga ecosystem in the state of Maranhão, and the Caatinga biome in the state of Piauí, isolated samples from soil, water and dead insects.

In the evaluation of growth factors and parameters, three strains were selected, BtMA-690, BtMA-750 and BtMA-1114, which were isolated from soil and insects from the Cerrado and Amazon biomes, that showed greater toxicity in *A. aegypti* larvae with significant values of LC_{50}(IC 95%) 0.004 mg/L (0.003-0.006), 0.041mg/L (0.030-0.063) and 0.025 mg/L (0.019-0.030), respectively, and also the presence of insecticidal toxins with different gene profiles, including combinations of mosquitocidal genes (VIEIRA-NETA MRA, et. al., 2020; VIANA JL, et al., 2020; SOARES-DA-SILVA J, et al., 2017).

pH and temperature for evaluation of *Bacillus thuringiensis* growth factors

To verify the best growth conditions for each of the three selected *B. thuringiensis* isolates, after growth on eight plates containing Nutrient Agar medium for 24 hours in a bacteriological oven, the entire content of each isolate was scraped using a platinum loop and inoculated in 200 mL of Nutrient Broth medium enriched with salt solution and subsequently incubated in a shaker at 200 rpm, for around five days, until complete sporulation was verified. Each strain was subjected to eighteen different combinations of pH and temperature, three of which were tested for pH (6.0, 7.0 and 8.0), combined with six different temperatures (26°C, 28°C, 30°C, 32°C, 34°C and 36°C), with the three pH values tested at six different temperatures (**Figure 1**). To balance the pH of the culture medium, a 0.5 M sodium hydroxide solution was used.

Figure 1 - Different combinations of pH and temperatures to prove the best growth conditions for *Bacillus thuringiensis*.



Source: Lobo KS, et al., 2024.

To confirm the best growth conditions for each isolate tested, the inoculum was quantified to obtain the concentration of spores/mL, in addition to determining the cell mass. Spore counting was performed according to the methodology described by Alves SB and Moraes SA (1998), which consists of preparing serial dilutions from the original culture and plating 0.1 mL of each dilution on plates containing nutrient agar using a *Drigalsky* loop in three replicates. Then, the plates were incubated in a bacteriological oven for approximately 24 hours. Colonies were subsequently counted to determine viable spores. For the calculation, plates containing 30 to 300 colonies were selected, this number being multiplied by the dilution that the plate represents.

To analyse the cell mass, 200 mL of the fermented product (culture medium + bacterial cells) were centrifuged and after centrifugation at 5.000 rpm for 20 minutes of the total bacterial content, followed by three washes with sterile water, the supernatant was discarded, the pellet frozen, freeze-dried for about eight hours in an Enterprise freeze-drying device (Terroni) at the Food Laboratory of the Federal Institute of Piauí (IFPI), Campus Teresina Zona Sul and subsequently the freeze-dried was used to calculate cell mass, which was expressed in g/L.

Toxicity confirmation bioassays

From the freeze-dried isolates obtained from different growth combinations (temperature and pH), two solutions were prepared, 5000 mg/L (solution I) and 50 mg/L (solution II), from which were prepared the LC_{50} of the isolates, BtMA-690, BtMA-750 and BtMA-1114, of 0.004mg/L, 0.041mg/L and 0.025mg/L, respectively, used in bioassays.

The LC_{50} tested were obtained from isolates cultivated under combined conditions of three pHs (6.0, 7.0 and 8.0), with minimum and maximum temperatures, 26°C and 36°C, and also from conditions considered ideal for each of the isolates, according to spore concentration and cell mass (BtMA-690 and BtMA-750: pH 6.0 at 30ºC and pH 8.0 at 34ºC; BtMA-1114: pH 6.0 at 34ºC and pH 7.0 at 28ºC), which were used in bioassays to confirm toxicity.

The bioassays with minimum and maximum temperature conditions and three pH conditions consisted of replicas of five plastic cups with a final volume of 150 mL of water, 20 third-stage *A. aegypti* larvae, obtained from the insectary at the Federal University of Maranhão (UFMA) Campus Codó, and LC_{50} of each isolate, of

each of the selected combinations. For each bioassay, a negative control was prepared, which consisted of a replicate without bacterial inoculation.

The bioassays with isolates cultivated under the best growth conditions followed the same methodology as the previous one, however, two bioassays were carried out for each combination, in different environments and populations of *A. aegypti*. The first repetition was carried out at the Biology Laboratory at UFMA/Codó, with *A. aegypti* larvae obtained from breeding maintained in the laboratory. The second repetition was carried out at LABEM at UEMA/Caxias, with *A. aegypti* larvae obtained from breeding maintained by the laboratory.

Larvae mortality readings were taken at intervals of 24, 48 and 72 hours after application of the bacterial solution. Larvae that exhibited no response to tactile stimuli with sterile toothpicks were considered dead, and all living and dead individuals were counted, obtaining the larvae mortality percentage (DULMAGE HT, et al., 1990). The tests were carried out under controlled humidity (85%) and temperature (28 ± 2 ºC) conditions and a 12-hour.

RESULTS

Evaluation of *Bacillus thuringiensis* growth factors

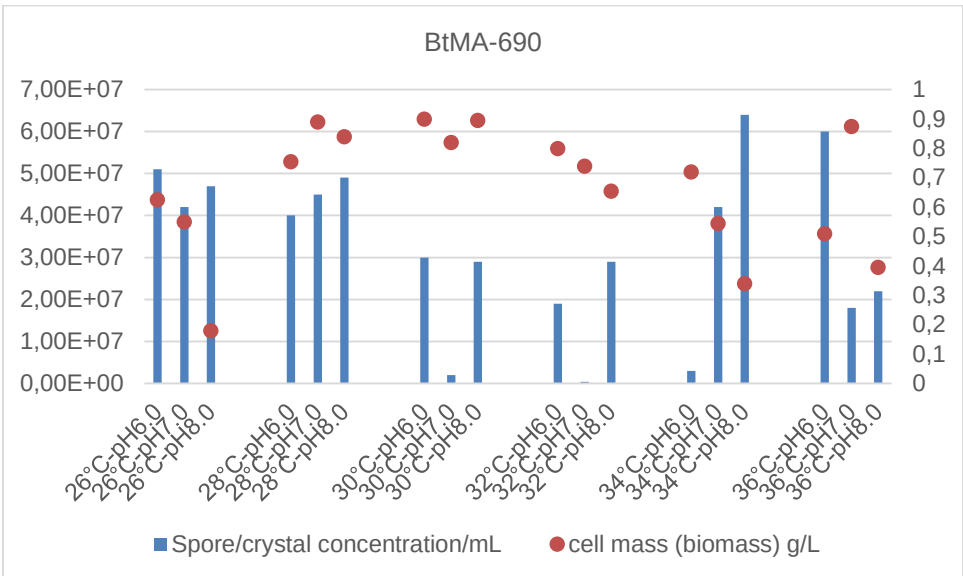
An evaluation of the chemical and physical growth factors of the three selected isolates, BtMA-690, BtMA-750, was carried out. By subjecting these isolates to 18 different combinations of pH and temperature, the best growth condition for each of them was observed.

The isolate BtMA-690 showed better growth conditions in the combination of factors pH 8.0 with a temperature of 34°C, with a concentration of 6.40 x 10^7 spores/mL and in the combination of pH 6.0 with a temperature of 30°C with 0.9 g/L of cell mass (**Figure 2**).

BtMA-750 in the combination pH 8.0 with temperature 34°C showed more satisfactory growth, presenting a concentration of 6.70 x 10^7 spores/mL and in the combination pH 6.0 with temperature 30°C it provided 1.065 g/L of biomass (**Figure 3**).

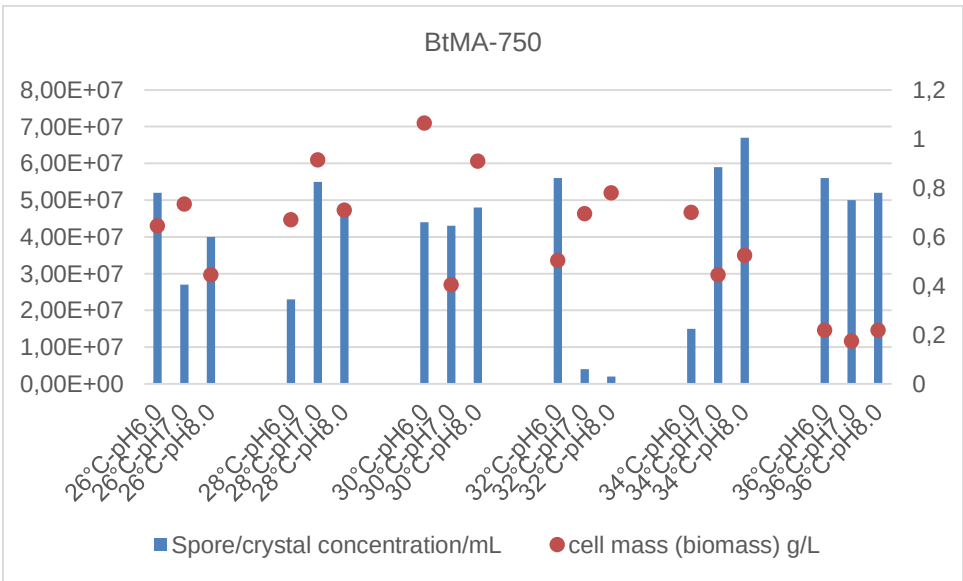
BtMA-1114 had better bacterial growth with the combined factors of pH 7.0 with temperature 28°C, showing a concentration of 2.40 x 10^7 spores/mL and with factors pH 6.0 with temperature 34°C showing 0.99 g/L of cell mass (**Figure 4**).

Figure 2 - Spore/crystal concentration and cell mass (biomass) of isolate BtMA-690 in different combinations of pH and temperature.



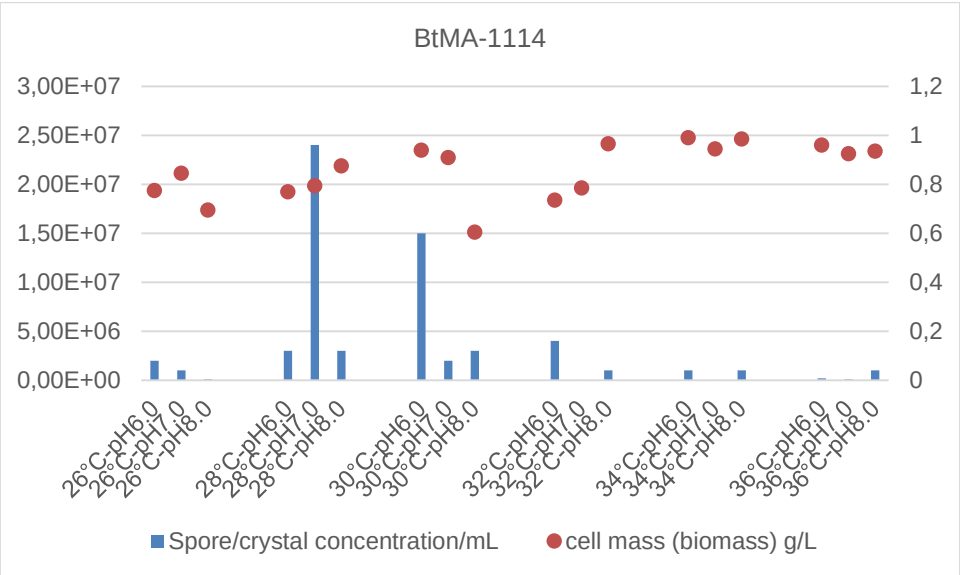
BtMA – *Bacillus thuringiensis* do Maranhão.
Source: Lobo KS, et al., 2024.

Figure 3 - Spore/crystal concentration and cell mass (biomass) of the BtMA-750 isolate in different combinations of pH and temperature.



BtMA – *Bacillus thuringiensis* do Maranhão.
Source: Lobo KS, et al., 2024.

Figure 4 - Spore/crystal concentration and cell mass (biomass) of isolate BtMA-1114 in different combinations of pH and temperature.



BtMA – *Bacillus thuringiensis* do Maranhão.
Source: Lobo KS, et al., 2024.

Confirmation of toxicity of isolates

Bioassays with BtMA-690 showed mortality rates below 50% at temperature and pH combinations of 26°C and 36°C with pH 6.0, 7.0, and 8.0. Specifically, mortality rates were 35% at 26°C with pH 6.0, and 30% at 36°C with pH 8.0. For all other combinations, mortality was below 20% (**Table** 1). Even under the optimal temperature and pH conditions for this bacterium, for both cell mass and spore concentration, larval mortality remained below 20% after 72 hours. (**Table 2**).

For BtMA-750, at a temperature of 26ºC, mortality rates exceeded 50% after 72 hours under pH conditions of 6.0 and 7.0, with mortality rates of 93% and 99%, respectively. However, at pH 8.0 combined with the same temperature, mortality was 36% after 72 hours. At a temperature of 36ºC, mortality was 100% for pH combinations of 6.0 and 8.0, and 99% for pH 7.0 after 72 hours (**Table** 1). Under the two conditions considered ideal for cell mass and spore concentration, larval mortality reached 96% at the end of bioassay I and 100% in bioassay II (**Table 2**).

On the other hand, of the six combinations of temperatures (26°C and 36°C) and pH levels (6.0, 7.0, and 8.0) for the BtMA-1114 isolate, five showed low larvicidal activity, with the highest mortality being 14% after 72 hours, achieved only for the combination of 36°C and pH 7.0. For the other combinations, mortality was below 5%. It is worth noting that these mortality rates were well below the expected LC_{50} of 50% (**Table** 1). Under the ideal conditions for this isolate, mortality was slightly higher but still below 50%, reaching 39% at a temperature of 34°C and pH 6.0 after 72 hours (**Table 2**).

Table 1. Percentage of mortality of *Aedes aegypti* larvae in bioassays with the LC_{50} of *Bacillus thuringiensis* isolates cultivated under conditions of minimum and maximum temperatures combined with three pH.

			Mortality (%)		
ISOLATED	**Temperature (ºC)**	**pH**	**24h**	**48h**	**72h**
BtMA-690	26°	6,0	2%	11%	35%
		7,0	1%	1%	1%
		8,0	3%	7%	14%
	36°	6,0	1%	18%	19%
		7,0	0%	0%	0%
		8,0	6%	22%	30%
BtMA-750	26°	6,0	35%	77%	93%
		7,0	99%	99%	99%
		8,0	31%	33%	36%
	36°	6,0	99%	100%	-
		7,0	96%	97%	99%
		8,0	100%	-	-
BtMA-1114	26°	6,0	0%	0%	1%
		7,0	0%	1%	2%
		8,0	0%	0%	0%
	36°	6,0	1%	1%	1%
		7,0	7%	14%	14%
		8,0	1%	1%	1%

BtMA – *Bacillus thuringiensis* do Maranhão.
Source: Lobo KS, et al., 2024.

Table 2. Percentage of mortality of *Aedes aegypti* larvae in bioassays with the LC_{50} of *Bacillus thuringiensis* isolates cultivated under conditions considered ideal of temperature and pH.

				Mortality (%)		
ISOLATED	**Bioassays**	**Temperature (ºC)**	**pH**	**24h**	**48h**	**72h**
BtMA-690	I	30°	6,0	1%	1%	1%
		34°	8,0	4%	8%	16%
	II	30°	6,0	1%	2%	9%
		34°	8,0	7%	15%	18%
BtMA-750	I	30°	6,0	96%	96%	96%
		34°	8,0	86%	90%	96%
	II	30°	6,0	99%	100%	-
		34°	8,0	99%	100%	-
BtMA-1114	I	28°	7,0	1%	3%	7%
		34°	6,0	17%	21%	22%
	II	28°	7,0	1%	2%	2%
		34°	6,0	31%	37%	39%

BtMA – *Bacillus thuringiensis* do Maranhão.
Source: Lobo KS, et al., 2024.

DISCUSSION

Parameters such as pH and temperature are of fundamental importance for the cultivation and production of endotoxins from *B. thuringiensis*. These conditions differ according to the variety and strain under study and, therefore, must be suitable for each strain, being controlled according to the needs of the microorganism (MALDONADO-BLANCO MG, et al., 2003).

In the present study, it was found that the optimal growth conditions for native strains of *B. thuringiensis* were similar, as for strains BtMA-690 and BtMA-750, isolated from Cerrado and Amazonian soil, respectively, were obtained the best conditions of growth in combinations of 34°C with pH 8.0, in which there was a higher concentration of spores/mL and 30°C with pH 6.0 with better cell mass yield. While for BtMA-1114, isolated from a dead insect, which is another type of substrate used for isolation, the best growth conditions were at 28°C with pH 7.0 and 34°C with pH 6.0, with greater production of spores/mL and better cell mass, respectively.

The temperature considered optimal for the growth and production of *B. thuringiensis* Cry proteins is around 30°C. Temperatures above this value may decrease production yield due to suppression of Cry protein formation and denaturation of cellular material. Low temperatures can slow down the cell multiplication cycle and metabolic reactions, which can lead to an increase in growth time and higher production costs (COUCH TL, 2000).

Fermentation temperature is a critical factor for the metabolic rate and bioactivity of Bt, the growth rate of bacteria is gradually higher with increasing fermentation temperature, and according to Pan X, et al. (2021), in research evaluating and monitoring the fermentation temperature to optimize the growth of this bacteria, the ideal temperature conditions for bacterial growth would be between 30°C and 35°C. Previous studies have also indicated that this bacterium exhibits premature aging at high temperatures, and the virulence of Bt decreases. On the contrary, low temperature leads to slow growth of bacteria, prolongs the fermentation period and decreases bacterial virulence (PEARSON D and WARD OP, 1988). Therefore, adequate temperature is essential for the growth of Bt.

In work on the influence of temperature on cell growth, endotoxin production and sporulation of *B. thuringiensis,* Panarotto C (2006) demonstrated that the best temperatures were 27°C and 30°C, with a temperature of 27°C being considered the most appropriate, also reporting that the optimal values are between 25°C and 35°C, corroborating the results obtained in the present study, where the best growth of the isolates was observed at temperatures of 28°C, 30°C and 34°C.

The pH values around neutrality (7-7.5) are the most suitable for the absorption of nutrients and development of most bacteria, which also occurs with *B. thuringiensis* (MOURÃO AHC, 2017), although, the bacteria can develop in a wide pH range between 5.5 and 8.5 (PANAROTTO C, 2006).

Some studies suggest that the pH during the fermentation process is maintained by adding acid or base when necessary or by adding a buffer solution at the beginning of fermentation. Içgen Y, et al. (2002), reported that the ideal range for growth during the production of *B. thuringiensis* is 5.5 to 6.5 and that this pH range did not alter protein production. Smith DB, et al. (1982) reported that buffering the culture medium to maintain a pH between 5.7 and 8.1 did not interfere with protein production and that varying the pH between 6.5 and 8.0 did not significantly affect protein production. However, in the present study it was observed that pH in the range of 6.0 to 8.0, even considering the same temperature, there was a change in the concentration of bacterial spores, and possibly, in the production and expression of proteins.

The pH of the culture is an important parameter, as it can affect the cell surface charge, and the growth and metabolism of microorganisms are directly related to the pH value (KONG K, et al., 2020). Pan X, et al. (2021), in work carried out on the effect of *B. thuringiensis* biomass and insecticidal activity by cultivation with plant residues, showed that the bacterial concentration was lower in the medium with pH 5.0 and 9.0, implying that both acidic and alkalies would inhibit bacterial growth, and the ideal starting pH range was 7.0 to 7.5 for bacterial growth.

Panarotto C (2006), in cultures testing the influence of pH in relation to cell growth, sporulation and endotoxin production of *B. thuringiensis*, found better results with pH controlled at 5.5, 6.2, 7.0 or with variation between 5.5 and 7.0, indicating that the pH should be close to neutrality. It was also found that cell growth was negatively affected in cultures in which pH was not controlled, also pointing out that low pH values affect sporulation and bacterial growth. In the present study, the best growth conditions, considering the concentration of bacterial spores, were pH 7.0 and 8.0. And it is noteworthy that in relation to cell mass, for all isolates, the best growth conditions were pH 6.0.

When analyzing the concentration of spores/mL for BtMA-690, it was observed that in conditions commonly used for the growth of *B. thuringiensis* (30°C and pH 7.0), the isolate presented the second lowest concentration (2.0×10^6) among the eighteen combinations tested. On the other hand, in the best growth condition (34°C and pH 8.0), a quantity of spores/mL (6.40×10^7) was 32 times greater than in the standard growth condition for *B. thuringiensis*. In relation to cell mass, the best temperature remained 30°C, as well as the standard growth temperature, but with pH 6.0, which showed no difference in yield.

When verifying the same conditions for BtMA-750, it is noted that both for spore concentration and cell mass, the different combinations showed considerable changes in the growth of the isolate, obtaining the best growth conditions (34°C and pH 8.0 for spore concentration; 30°C and pH 6.0 for biomass) approximately 1.56 times (6.70×10^7) more spores/mL and 2.63 times (1.065 g/L) more mass yield cell than in the standard condition (30°C and pH 7.0).

The BtMA-1114 showed a difference in relation to the concentration of spores/mL when compared to the standard condition (30°C and pH 7.0) with the best growth condition (28°C and pH 7.0). In the best growth condition, it reached a value 12 times higher (2.40×10^7) of spores/mL. Regarding cell mass, for both conditions the cell yield was similar, both in the routine growth factors (30°C and pH 7.0) and in the condition considered more satisfactory (34°C and pH 6.0).

Through bioassays carried out with *B. thuringiensis* isolates grown under different conditions, it was found that changing the temperature and pH in the cultivation of the bacteria changed the insecticidal activity of the strains tested against *A. aegypti* larvae, as it was found that when the LC_{50} of the isolates was used, the mortality rates were well below or well above the expected percentages, which may be due to changes in the expression of insecticidal proteins.

In work on nutritional and culture parameters of Bt that influence the synthesis of endotoxins of *B. thuringiensis,* Özkan M, et al. (2003) determined that the synthesis of the Cry4Ba toxin was ideal when the organism was cultivated at 25°C, while the synthesis of Cry11Aa was ideal at 30°C, that is, the study shows that temperature alters the expression of insecticidal proteins.

In bioassays carried out with the best growth conditions for the isolates, it was possible to observe for two strains that of the conditions analyzed, the one with the highest yield of spores/mL obtained higher mortality rates than those with the highest cell mass. Therefore, spores are possibly more important, from the point of view of biological activity, than cell mass.

Studies indicate that, in addition to the toxins, the spores of *B. thuringiensis* also contribute to its toxicity, as they can germinate and grow vegetatively inside the target insect, causing septicemia or enhancing the effect of the toxins in a synergistic action (GALZER ECW e AZEVEDO-FILHO WS, 2016; RAYMOND B, et al., 2008; GLARE TR e O'CALLAGHAN M, 2000). There are several lines of evidence that suggest that vegetative growth of *B. thuringiensis* in the intestine may be important in lethal infections. The importance of live spores in the synergy of mortality caused by endotoxins indicates that the vegetative growth of *B. thuringiensis* contributes to the lethality of the toxins (RAYMOND B, et al., 2008).

CONCLUSION

These results are important to maximize the production of the bacteria in laboratory conditions, by obtaining the growth parameters of each of the Bt strains in a specific way, making it possible to standardize the ideal conditions for the growth and sporulation of each of them, as well as, improve the control of larvae of the *A. aegypti* vector, through understanding the best growth conditions for the production of low-cost biological insecticides.

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