

Influence of Antibiotics and Surfactants Addition on Growth and Methionine Productivity by *Bacillus cereus**

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ABSTRACT

The effects of antibiotics and surfactant addition on methionine accumulation in the broth culture of three strains of methionine yielding *Bacillus cereus* (*B. cereus* DS-13, *B. cereus* AS-9 and *B. cereus* RS-16) recovered from different soil ecovars in Owerri Nigeria were investigated. Ampicillin and erythromycin neither stimulated growth nor enhanced methionine production in *Bacillus cereus* DS-13, while tetracycline and chloramphenicol stimulated growth and enhanced methionine yield in *Bacillus cereus* DS-13 and *Bacillus cereus* AS-9. Ampicillin had the highest stimulatory effect on growth and methionine production in *Bacillus cereus* RS 16. Tween 80 and palmitic acid stimulated growth and improved methionine accumulation in the broth culture of all *Bacillus* strains while stearic acid did not improve yield in all *Bacillus* strains.

Keywords: Methionine; *Bacillus cereus*; Fermentation; Antibiotics; Surfactants

1. Introduction

Methionine is an essential, economically important amino acid used as food and feed supplement. It is a precursor for the other sulfur amino acids, cysteine, taurine and glutathione, and plays an important role in human body by assisting in the breakdown of fats and preventing fat build up in the liver and arteries [1,2]. Methionine is essential for the absorption, transportation and availability of selenium and zinc in cellular functions. It helps in the excretion of cadmium and mercury from the body. In humans, methionine is used to produce creatinine required by the brain. People with AIDS have a low level of methionine. Some researchers suggest that this low level methionine in AIDS patients may explain some aspects causing symptoms including dementia [3]. Also, preliminary trials have suggested that methionine may help to treat some symptoms of Parkinson's disease [3].

Chemical, enzymatic and fermentation processes have been used to synthesize amino acids, and the advantage of microbial methods is that the amino acids are purely optically active [4]. Research on possible utilization of the wild strain revealed that many microorganisms such as bacteria, yeasts and filamentous fungi accumulates amino acids in culture fluid, but only bacteria have sufficient

productivity to warrant the commercial production [5].

Most natural strains cannot produce industrially significant amounts of L-methionine in the culture broth due to various metabolic regulation mechanisms. However, alteration of these mechanisms can lead to L-methionine accumulation [6,7]. The influence of antibiotics and surfactants on amino acid production by bacterial organism has been reported [8-11] although the exact role played by these antibiotics and surfactants are not clearly understood. In our previous work, we reported the production of L-methionine by three methionine yielding strains of *Bacillus cereus* isolated from different soil ecovars in Owerri, Nigeria [12,13]. The present study, therefore, seeks to investigate the effects of antibiotics and surfactant on methionine accumulation in the culture broth of the three newly isolated *Bacillus cereus* strains.

2. Materials and Methods

2.1. Microorganisms

Bacillus cereus DS 13, *Bacillus cereus* AS 9, *Bacillus cereus* RS 16 were previously isolated from different soil ecovars in Owerri, Nigeria. It was maintained on nutrient agar (Oxoid) slants at 4°C. The taxonomic identification was done by the method recommended by [14,15]. Molecular characterization by 16SrRNA conducted at Macro-gen Incorporated Europe confirmed isolates as different strains of *Bacillus cereus*.

*Competing interests: Authors have declared that no competing interest exists.

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2.2. Growth and Cultivation

The medium for seed culture consist of (g/L): peptone, 10.0; yeast extract, 10.0; NaCl, 5.0; water, 1 litre, pH was adjusted to 7.2 with 1N NaOH. The medium was sterilized at 121°C for 15 minutes. Two loopfuls of a 24 h slant of the culture was used to inoculate a 250 ml flask containing 50 ml of the seed medium. The flasks were incubated for 16 - 18 h on a rotary shaker at 120 rpm and 30°C.

3. Determination of Growth of the Isolate

Growth of each isolate was determined turbidimetrically using the culture broth in spectronic 21 spectrophotometer (Camspec, England).

4. Quantitative Determination of Methionine

Quantitative determination of L-methionine in the culture broth without purification was carried out by the modified calorimetric method of [16]. A 5 ml volume of the culture broth of each isolate were centrifuged at 5000 ×g for 20 minutes and the cell free supernatant was assayed for L-methionine. 1 ml of 5N NaOH was added to a test tube followed by the addition of 0.1 ml of 10% sodium nitroprusside solution with through mixing. The mixture was allowed to stand for 10 min. Then two milliliters of 3% aqueous solution of glycine was added to the reaction mixture with frequent shaking over a period of 10 min. After an additional 10 min interval, 2 ml of concentrated *ortho*-phosphoric acid was added drop wise to the mixture with shaking. Colour development was allowed to proceed for 5 min and the colour intensity measured at 540 nm in a spectrometer. A blank containing distilled water and all other reagent served as the 100% transference standard. Results obtained with the test samples were interpolated on a standard methionine curve.

5. Effect of Antibiotics

The effects of various concentrations (0.05 - 1.0 µg/ml) of these antibiotics; tetracycline, ampicillin, erythromycin and chloramphenicol on growth and methionine production was studied. A basal medium consisting of: KH₂PO₄, 0.5 g; K₂HPO₄, 0.5 g; MgSO₄·7H₂O, 0.001 g; MnSO₄·4H₂O, 0.001 g; FeSO₄·7H₂O, 0.001 g CaCO₃, 20.0 g; glucose, 80.0 g; NH₄Cl₂, 20.0 g for *Bacillus cereus* DS13, and *Bacillus cereus* RS 16, glucose, 100.0 g; (NH₄)₂SO₄, 20.0 g for *Bacillus cereus* AS9; distilled water, 1 litre; pH 7.5 was used. Each antibiotic solution was added to the sterilized fermentation medium and fermentation process carried out for 72 h and 30°C on a rotary shaker at 170 rpm. Growth and methionine production was determined as previously described.

6. Effect of Surfactant

The effects of various concentrations (0.1 - 1.0 v/v) of tween 80, oleic acid, linoleic acid, and (0.05 - 1.0 µg/ml) of palmitic and stearic acids on growth and methionine production were examined. A basal medium consisting of; KH₂PO₄, 0.5 g; K₂HPO₄, 0.5 g; MgSO₄·7H₂O, 0.001 g; MnSO₄·4H₂O, 0.001 g; FeSO₄·7H₂O, 0.001 g CaCO₃, 20.0 g; glucose, 80.0 g; NH₄Cl₂, 20.0 g for *Bacillus cereus* DS13, and *Bacillus cereus* RS 16, glucose, 100.0 g; (NH₄)₂SO₄, 20.0 g for *Bacillus cereus* AS9; distilled water, 1 litre; pH 7.5 was used. Fermentation was carried out for 72 h at 30°C on a rotary shaker at 170 rpm. Growth and methionine concentration was determined at the end of fermentation period. All experiments were conducted in triplicates with uninoculated flasks as control.

7. Results and Discussion

Studies on the effect of antibiotics on growth and methionine productivity shown in **Figures 1-3**, reveals that tetracycline and chloramphenicol at 0.05 and 0.1 µg/ml enhanced methionine productivity but did not stimulate

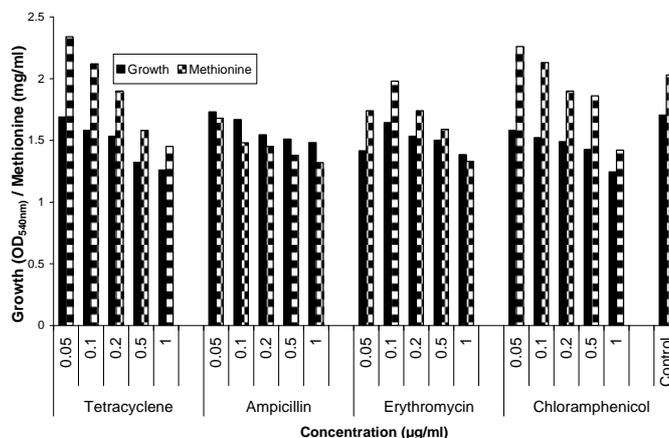


Figure 1. Effect of different concentrations of antibiotics on growth and methionine production by *Bacillus cereus* DS13.

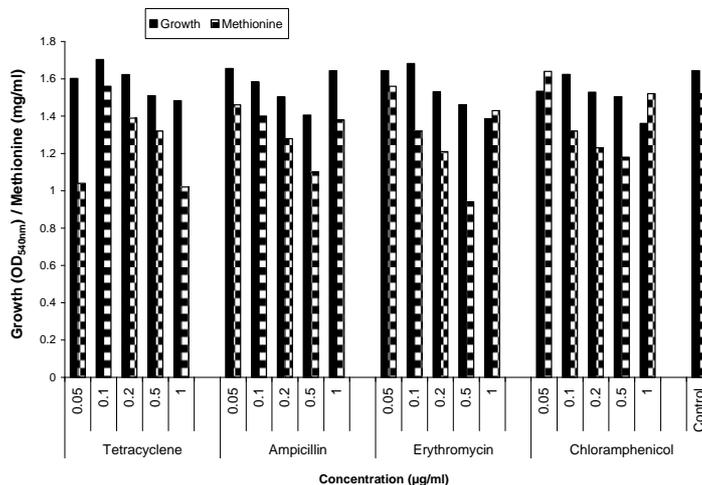


Figure 2. Effect of different concentration of antibiotics on growth and methionine production by *Bacillus cereus* AS9.

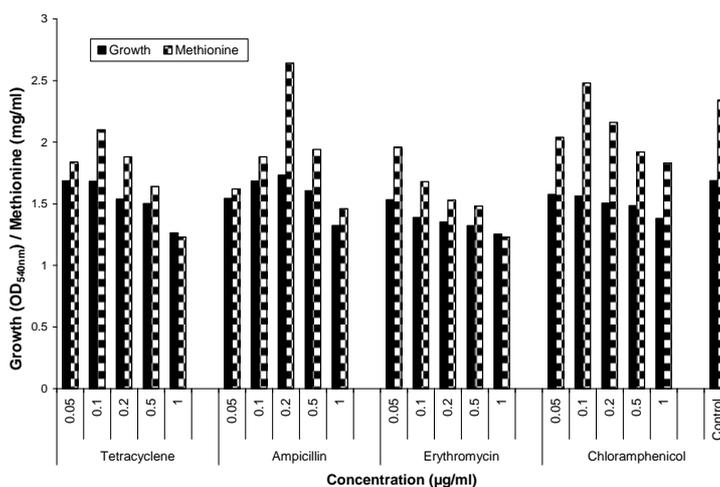


Figure 3. Effect of different concentration of antibiotics on growth and methionine by *Bacillus cereus* RS16.

growth in broth culture of *Bacillus cereus* DS 13. Ampicillin and erythromycin at all concentrations did not enhance methionine yield. In the result shown in **Figure 2**, tetracycline at 0.10 µg/ml and chloramphenicol at 0.05 µg/ml improved methionine yield in broth culture of *Bacillus cereus* AS 9, while ampicillin at 0.20 µg/ml and chloramphenicol at 0.1 µg/ml stimulated methionine yield in broth culture of *Bacillus cereus* RS16 (**Figure 3**). There are no reports on the effect of antibiotics on methionine production. [9,17] have reported increased amino acid production in the presence of small quantities of several kinds of antibiotics, although the exact role played by these antibiotics is not clearly understood. [18] suggested that a change in permeability of the cell wall caused by antibiotics may be responsible for improved yield. This change in permeability affects the intracellular accumulation of amino acids resulting in the inability of the amino acid to regulate its own synthesis by feedback control, thereby releasing high levels of amino acid

into the medium. The stimulatory effect of tetracycline reported in this work is supported by the work of [19]. They reported an increase in lysine yield by *Micrococcus glutamicus* when the antibiotic was added to the fermentation culture.

Surfactants are well known surface agents that are generally used to improve the surface area for microbial action and availability of nutrients to the microorganisms. Results (**Figures 4-6**) indicates that tween 80 (at 0.5 and 1.0 µg/ml) and palmitic acid at all concentration except 0.05 µg/ml enhanced methionine accumulation in broth culture of *B. cereus* DS13 while oleic acid, linoleic acid and stearic acid did not stimulate methionine production. Growth of *B. cereus* DS13 was enhanced with the addition of stearic acid at all concentration. This agrees with the work of [20] who reported a stimulatory effect of tween 80 on lysine production by *Bacillus megaterium*. [8,11] observed a stimulatory effect on lysine production by *Corynebacterium glutamicum* with definite concentrations

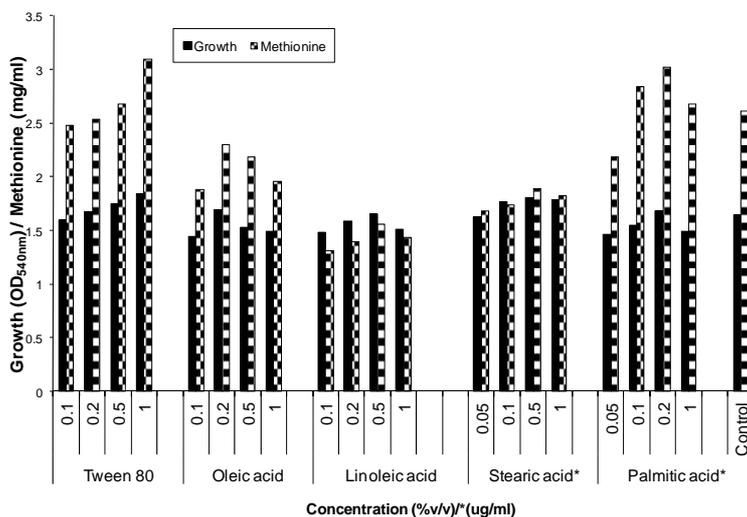


Figure 4. Effect of different concentration of surfactants on growth and methionine production by *Bacillus cereus* DS13.

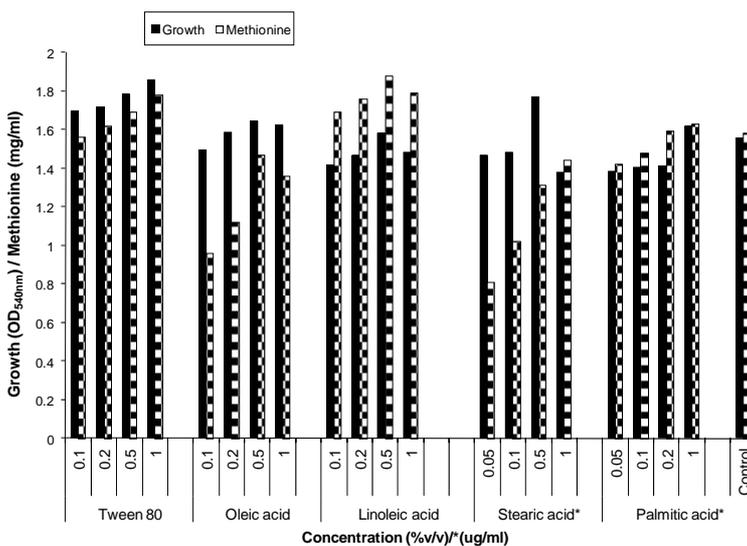


Figure 5. Effect of different concentration of surfactants on growth and methionine production by *Bacillus cereus* AS9.

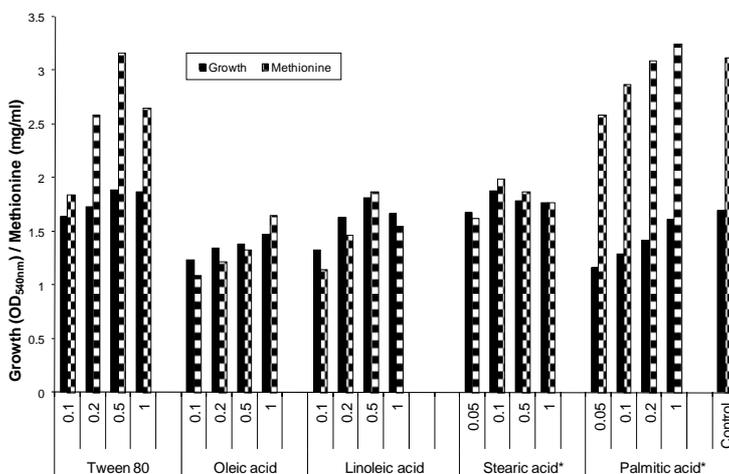


Figure 6. Effect of different concentration of surfactants on growth and methionine production by *Bacillus cereus* RS16.

of liquid tween 80. According to [11,21,22] surfactants stimulate biodegradation of nutrients by increasing the solubility and dispersion of the compounds thus enhancing the release of metabolites into the medium by cell rupturing after its production. These results however, contrasts the work of [23] who reported a non stimulatory effect of tween 80 on production of methionine and glutamic acid by *Serratia marcescens var kiliensis*. Unsaturated fatty acids; linoleic acid enhanced methionine accumulation in the broth culture of *Bacillus cereus* AS 9 while oleic acid stimulated growth and improved methionine production in broth culture of *Bacillus cereus* DS13. There are no reports on the effect of fatty acids on the production of methionine. [20] however reported a stimulatory effect of linoleic acid on lysine production by *Bacillus megaterium*. [23] reported the growth promoting effect of unsaturated fatty acids in *Lactobacillus casei*. They observed that lowering the fatty acid concentration beneath a critical level or prolonging the period of incubation, resulted in growth stimulation. The saturated fatty acid; palmitic acid stimulated growth and improved methionine yield in all *Bacillus* strains. Stearic acid on the other hand enhanced growth in all *Bacillus* strains but did not stimulate methionine yield. The growth promoting effect of the saturated fatty acids reported in this work is in line with the works of [24] who reported a stimulating effect of saturated fatty acids on L-glutamic acid producing bacteria. [25] observed that long chain fatty acids such as lauric acid, stearic acid and oleic acid did not stimulate lysine production in *Brevibacterium lactofermentum*.

[26] have found that inhibitory effects of long chain fatty acid on cell wall digestibility is inversely related to their ability to form insoluble calcium soaps, thus the relative inhibitory effects of the different fatty acids may be partially explained by their selective removal from the medium as insoluble soaps. [27] on their work on L-glutamic acid producing bacteria, noted that C16 to C18 saturated fatty acids-treated in the absence of biotin synthesized insufficient amounts of phospholipids on the cell membrane, thus resulting in enhanced permeability towards L-glutamic acid. It may be possible to suggest that improved methionine accumulation observed in *B. cereus* DS 13 and *B. cereus* RS16 were due to similar permeability process. This experimental study has shown that antibiotics and surfactants stimulated methionine yields in *Bacillus cereus* even though they are strain dependent.

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