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by I Zam Syukria

The effect of nitrogen sources on anti-phytopathogenic activities fermented filtrate of *Bacillus subtilis* AAF2

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Abstract. The filtrate from *Bacillus subtilis* AAF2 fermentation was reported to have high anti-phytopathogenic activity in the corn steep liquor modified medium with glucose as its carbon source. Besides media and carbon sources, the nitrogen source also affects these activities. This study aims to obtain the best nitrogen source on anti-phytopathogenic activities fermented filtrate of *B. subtilis* AAF2. The nitrogen sources used were yeast extract (15 G L^{-1}), peptone (15 G L^{-1}), NH_4NO_3 (1.71 G L^{-1}), and $(NH_4)_2SO_4$ (2.83 G L^{-1}), with phytopathogen test were *Fusarium oxysporum* and *Sclerotium rolfsii*. The parameters measured were pH fluctuations, the number of *B. subtilis* AAF2 cells, and the efficacy of phytopathogen growth inhibition (measured every 24 hours for 3 x 24 hours). The results showed pH ranged from 5.7 to 7.1; the number of cells ranges from $2.1X10^6$ - $1.00X10^{15}$ CFU ml^{-1}; the efficacy against *F. oxysporum* ranged from 7.10 - 71.40% and against *S. rolfsii* ranged from 17.70 - 71.80%. The best nitrogen source in the fermentation of anti-phytopathogenic compounds by *B. subtilis* AAF2 was peptone.

1. Introduction

B. subtilis AAF2 strain is an endophytic bacterial isolate obtained from the leaves of *Artocarpus altilis* (Parkinson) Fosberg. The filtrate produced by this bacterium has high anti-phytopathogenic activities against *Fusarium oxysporum* and *Sclerotium rolfsii* [1], so that it is potential to be developed as a producer of anti-phytopathogenic compounds.

The anti-phytopathogenic compound from ethyl acetate extract was identified as L-homocysteine [2]. L-homocysteine is a chemical compound consisting of two L-homocysteine which is connected by disulfide bonds [3]. L-homocysteine is a non-protein amino acid that is homologous with cysteine, as a precursor on methionine synthesis [4].

The filtrate of *B. subtilis* AAF2 fermentation has high anti-phytopathogenic activities against *F. oxysporum* and *S. rolfsii*, if grown on modified corn step liquor medium [5] with glucose as a carbon source [6]. Another thing that needs to be considered in the fermentation of anti-phytopathogenic

compound is the nitrogen source. Based on the investigation conducted, there is no information available on the best nitrogen source in the fermentation of anti-phytopathogenic compound by the *B. subtilis* AAF2 strain, so further research needs to be done. This study aims to find the best nitrogen source on anti-phytopathogen activities fermented filtrate of *B. subtilis* AAF2 against anti-phytopathogenic activity.

2. Material and methods

Method used in this research was experimental. The data obtained were analyzed descriptively.

2.1. Sterilization

Sterilization of heat-resistant tools and materials used an autoclave at 121°C with a pressure of 15 lbs for 15 minutes. Non heat-resistant materials were sterilized by the filtration method used a 0.2 µm sterile filter membrane.

2.2. Selection of Nitrogen Sources

The nitrogen source used were: peptone 15 g L^{-1}, yeast extract 15 g L^{-1}, $(NH_4)_2SO_4$ 2.83 g L^{-1} and NH_4NO_3 1.71 g L^{-1} with initial medium pH 7. Fermentation process is carried out by inoculating 10% (v v^{-1}) of the inoculum source with cell number 10^6 (CFU mL^{-1}) into each Erlenmeyer flask containing a modified corn step liquor medium with a different nitrogen source, then incubated at 27°C with agitation 120 rpm 3 x 24 hours.

2.3. Parameters

The parameters observed were fluctuations of medium pH, bacterial cell counts and efficacy of phytopathogens growth inhibition. Observations on all parameters were carried out every 24 hours for 3 x 24 hours [7]. Fluctuations in medium pH were measured used a pH meter; the number of bacterial cells was measured using the pour plat method [8]; and the efficacy of phytopathogens growth inhibition were carried out used a food poisoning technique [9] by formula:

$$D = (C-T)/C \times 100\%$$

Wherever: D = Efficacy; C = Control colony diameter; and T = Treatment colony diameter.

3. Results and discussion

B. subtilis AAF2 shows almost the same growth pattern in medium with different nitrogen sources. The high cell numbers at 24-hour fermentation time in all nitrogen sources, and subsequently decreased growth at 48 hours and 72 hours, except in medium with NH_4NO_3 as nitrogen sources, the growth continued, although was low (Figure 1).

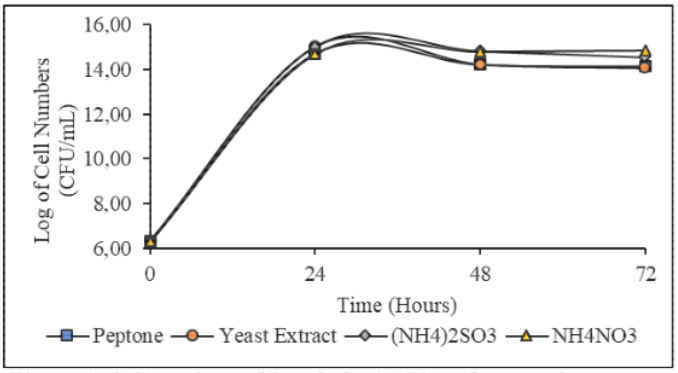


Figure 1. Cell numbers of *B. subtilis* AAF2 on fermentation process

A sharp decrease in pH occurred after 24 hours of fermentation in a medium containing yeast extract, $(NH_4)_2SO_3$, and NH_4NO_3 as a nitrogen source (ranging from 1.2 to 1.3), whereas in media containing peptone there was a decreased in pH, but with a low value, which is 0.6. After 24 hours the pH value then increases for each nitrogen source (Figure 2). These results indicate *B. subtilis* AAF2 will produce acidic metabolites which are high enough if grown on media containing glucose as a source of carbon without nitrogen sources [6], whereas if used nitrogen as a nitrogen source acid only occurs at 24 hours fermentation, and after that this bacteria will produce alkaline metabolites.

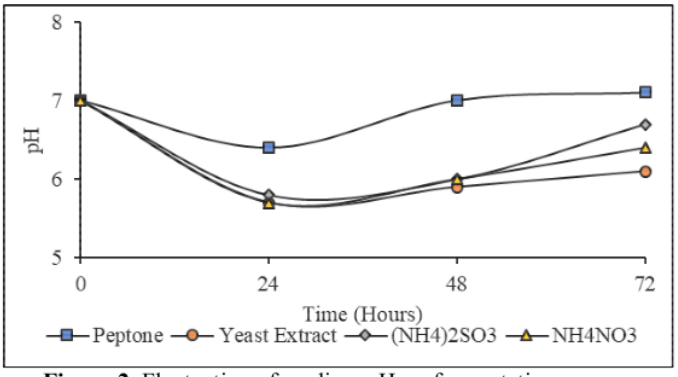
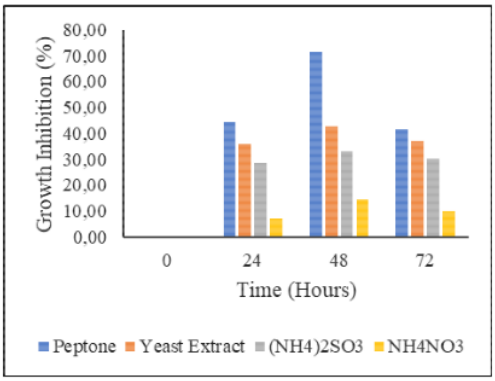


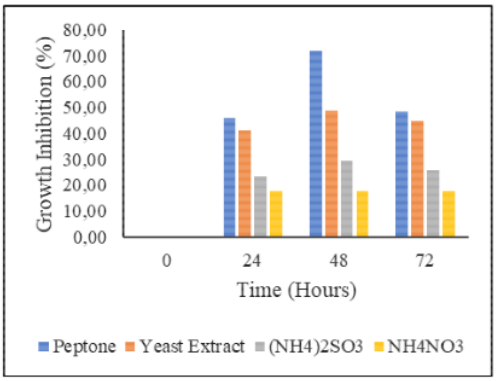
Figure 2. Fluctuation of medium pH on fermentation process

Peptone is the best nitrogen source in this study. The additions of peptone increased the anti-phytopathogenic activities of fungi, namely 71.4% against *F. oxysporum* and 71.8% against *S. rolfsii* at 48 hours fermentation time (Figure 3). In general, organic nitrogen sources increase the production of anti-phytopathogenic compounds and increase their activity (10). Crueger & Crueger (1984) and Stanbury *et al*. (2003) stated that peptone provides nitrogen in the form of peptides and essential amino acids which are easily metabolized by microbes. Amino acids are known to be precursors for the formation of anti-phytopathogenic compounds [11,12].

The selection of carbon sources [13] and nitrogen sources needs to be done to obtain the best of anti-phytopathogenic compounds. Because carbon and nitrogen are involved in metabolism, which may initiate precursor biosynthesis that controls metabolism and is involved in product synthesis [14]. Carbon acts as a major component of protoplasm, whereas nitrogen acts as a major component of amino acids in all parts of the cell, major components of purine and pyrimidine rings, as well as substantial components of murein and complex lipids [15].



a. F. oxysporum



b. S. rolfsii

Figure 3. Growth inhibition of anti-phytopathogenic compounds from fermentation filtrate

4. Conclusion

The best nitrogen source on anti-phytopathogenic activities fermented filtrate of *B. subtilis* AAF2 was peptone (71.4% anti-phytopathogenic activity against *F. oxysporum* and 71.8% against *S. rolfsii*) at 48 hours fermentation time.

Acknowledgment

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