Int.J.Curr.Microbiol.App.Sci **(2014) 3(11) 633-639**

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 3 Number 11 (2014) pp. 633-639**
http://www.ijcmas.com

Original Research Article

Effects of wood ash on the growth of known strains of *Bacillus subtilis*

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A B S T R A C T

Keywords

Wood ash, nutrient agar, nutrient broth, Gram positive, *Parkia biglobosa*, *Annacadium occidentalis*, sun-dried

The effects of varying concentrations of wood ash on the growth of known strains of *Bacillus subtilis* were studied. Different concentrations of ash (0-5% w/v) were prepared in distilled water and filtered. The pH of the filtrates that ranged between 9.6 and 9.8 were titrated, using 0.1M HCl (hydrochloric acid) to pH of 7.2. The ash filtrates were used to prepare nutrient agar and nutrient broth. The growth of five strains of *Bacillus subtilis* (8B, 1A, 2B, 3A, 5A and BC4333) on the ash-incorporated nutrient agar were determined by pour-plate method at 35^{o}C. Also, the growth of the strains of *Bacillus* in ash-incorporated nutrient broth was determined spectrophotometrically at OD_{540nm}. The microbial load of strain 8B decreased from 7.87×10^{7}cfu/ml to 5.27×10^{7} cfu/ml on agar plates, as the concentration of the wood ash increased from 0 to 5%w/v. Similar trend of decrease in growth, with increasing ash concentration was observed in the broth culture. Thus, wood ash did not support the growth of the strains of *Bacillus subtilis* involved in fermentation of African locust beans to produce 'iru'.

Introduction

Bacillus is a genus of Gram positive, rod-shaped, spore-forming bacteria and a member of division Firmicutes. Members of the genus *Bacillus* are generally found in the soil. They have the ability to break down protein in fermentation processes**.** Many species of *Bacillus* are of considerable importance. Some produce antibiotics, bacitracin and polymyxin. *Bacillus subtilis* is an ubiquitous bacterium commonly recovered from water, soil, air, and decomposing plant residue and have the ability to form tough protective endospores allowing the organisms to tolerate extreme environmental condition. *Bacillus subtilis* strains grow optimally in the temperature range of 25^{o}C to 35^{o}C. *Bacillus subtilis* are generally non-pathogenic; though, some strains have been found to cause rots in potatoes. They grow in food that is non-acidic and cause ropiness in spoilt bread and some strains of *Bacillus subtilis* are capable of producing toxins. *Bacillus subtilis* is very useful in the fermentation industries, most especially during the fermentation of African locust beans to a soup condiment called 'iru'.

Ash is the inorganic residue remaining after the organic matter in wood has been burnt (Rose and Henry, 2001). Wood ash contains calcium carbonate, potash, phosphate and trace elements like iron, manganese, zinc, copper and some heavy metals, but it does not contain nitrogen because of the presence of calcium carbonate Pan, (2004). Manganese, a growth factor is responsible for sporulation and also required for proteinase production in *Bacillus subtilis* Stockton and Wyss, (1946). Wood ash is used in the production of 'kuuru', a local softening agent used by the local women in the production of 'iru-pete'. This paper aimed at elucidating the effects of wood ash on the growth of some known strains of *Bacillus subtilis* involved in fermentation of *Parkia biglobosa* cotyledons to produce 'iru-pete'.

Materials and Methods

Collection of isolates: The strains of *Bacillus subtilis* used for the research were collected from the stock cultures in of the Department of Microbiology, Ekiti State University, Ado-Ekiti, Ekiti State.

Preparation of wood ash

A branch of cashew tree (*Annacadium occidentalis*) was cut, sun-dried after which it was burnt completely to ash.

Preparation of media:

Different amounts (0g, 1g, 2g, 3g, 4g and 5g) of wood ash were weighed and dissolved in 100ml of distilled water in a conical flask separately and filtered using Whatman filter paper. The pH of the filtrates was determined using a pH meter, and titrated using 0.1M HCl to pH 7.2. The filtrates were used to prepare the nutrient agar (NA) and broth (NB) according to the manufacturer's instructions, sterilized in the autoclave at 121^{o}C for 15minutes.

Determination of growth in ash-incorporated media:

Nutrient broth cultures of the *Bacillus subtilis* strains (8B, 1A, 2B, 3A, 5A and BC4333) were diluted serially and plated (pour-plate) in the ash-incorporated media. Plates were incubated at 35^{o}C for 24h. One milliliter (1ml) each of the serially diluted cultures were also aseptically inoculated into ash-incorporated broth media and incubated for 24h at 35^{o}C, using shaker incubator (200rpm). Colonies developed on the ash-incorporated agar plates were counted and the microbial load was determined and expressed as cfu/g using the method of Huarrigan and McCance (1976). The microbial growth in the ash-incorporated broth was determined spectrophotometrically at OD_{540nm} at 18h and 24h respectively.

Results and Discussion

Table 1 shows the microbial load of the strains of *Bacillus subtilis* on the ash-incorporated agar media after 24hours of incubation. The microbial load of strain 8B (7.87×10^{7} cfu/g) on NA without ash was higher than that in all the ash-incorporated media (ranged from 5.27×10^{7} to 7.10×10^{7} cfu/g). Similar results were observed for the growth of the other strains 1A, 2B, 3A, 5A and BC4333 on the ash-incorporated NA.

Fig. 1 shows the growth turbidity at (OD_{540nm}) of *Bacillus subtilis* 8B in NB and ash incorporated NB at 18 and 24 hours. At 18 hours of growth, culture medium without ash, had the highest turbidity of (0.39×10^{7}) while the growth in ash-incorporated media decreased (from 0.38×10^{7} to 0.24×10^{7}) as the ash concentration increased. The same trend was observed at 18 hours and 24hours of growth.

Figures 2, 3, 4, 5 and 6 show the growth turbidity at (OD_{540nm}) of the *Bacillus subtilis* strains 1A, 2B, 3A, 5A and BC4333 having 0 to 5%(w/v) of ash incorporated NB medium respectively. Similar trends of reduction in growth (turbidity measurement) as the concentration of ash increased were observed in all the strains both at 18h and 24h incubation periods.

The increase in pH of distilled water after the addition of wood ash was due to the alkaline nature of ash. The alkaline nature of ash has been reported by Haimi, (2000) and Fritz, (2000), that when soil was treated with wood ash, the soil was de-acidified, thereby leading to a rise in pH of the soil.

The relatively higher microbial load of all the strains (8B, 1A, 2B, 3A, 5A, BC4333) of *Bacillus subtilis* on nutrient agar (without ash) could be attributed to the pronounced decreased in the pH of the medium. The decrease in the microbial load with increasing concentration of ash in the ash-incorporated medium (from 1%w/v to 5%w/v) could be attributed to the increase in pH of the ash-incorporated medium. It was also as a result of the absence of nitrogen source in the ash-incorporated medium which is a form of macro nutrients needed for growth by the microorganism. Similar result was reported by Pan, (2000), that nitrogen in wood ash is normally insignificant due to the conversion of wood nitrogen to NH_3, NO_2 and N_2 during the combustion of wood. Similar result was observed from the growth measurement on the ash-incorporated broth.

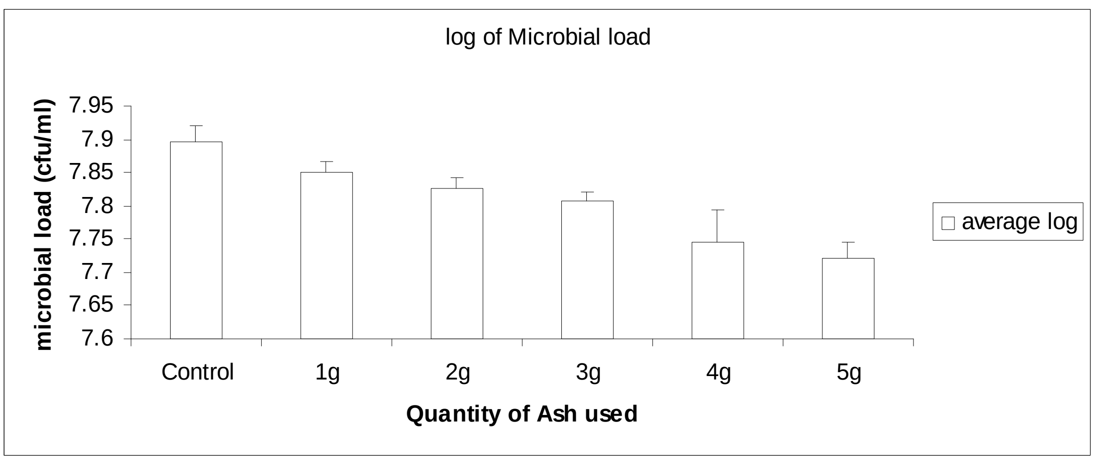

Fig.1 Microbial load for *Bacillus subtilis* strain 8B

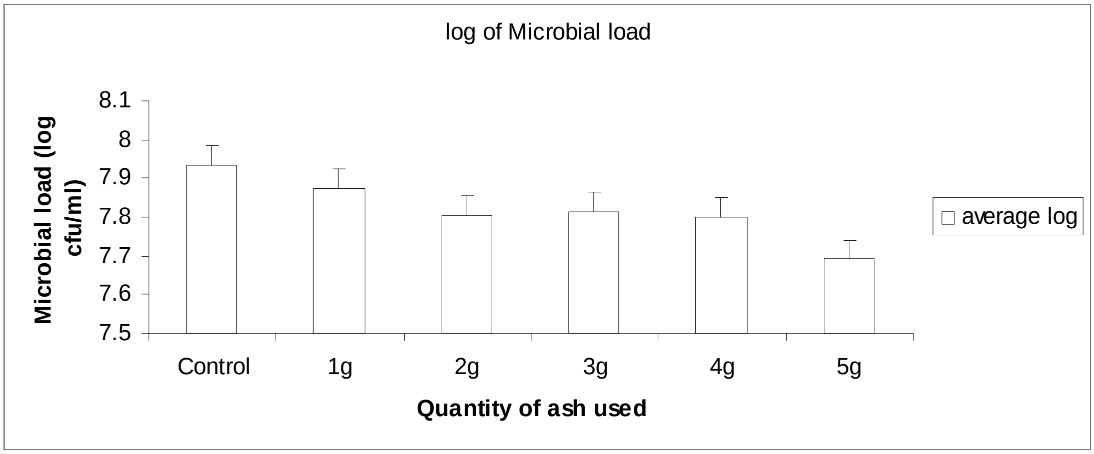

Fig.2 Microbial load for *Bacillus subtilis* strain 1A

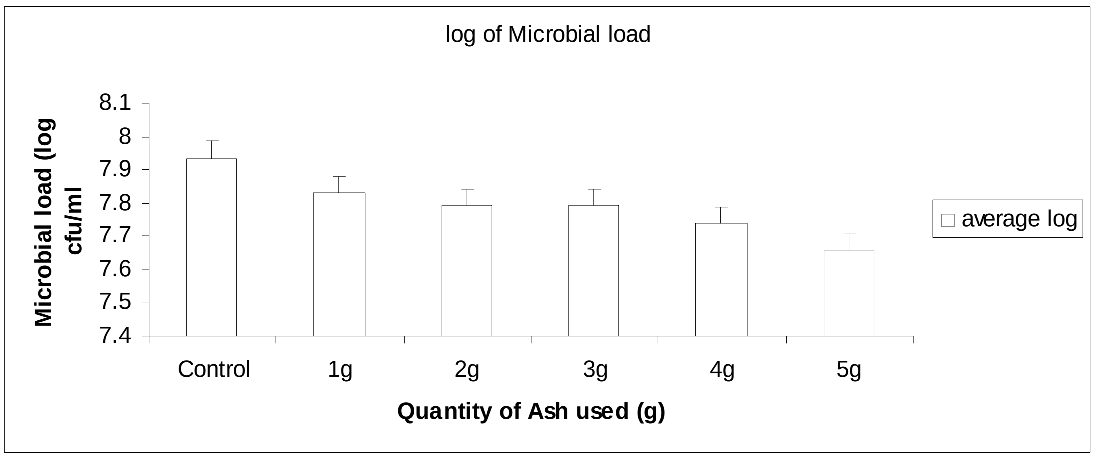

Fig.3 Microbial load for *Bacillus subtilis* strain 2B

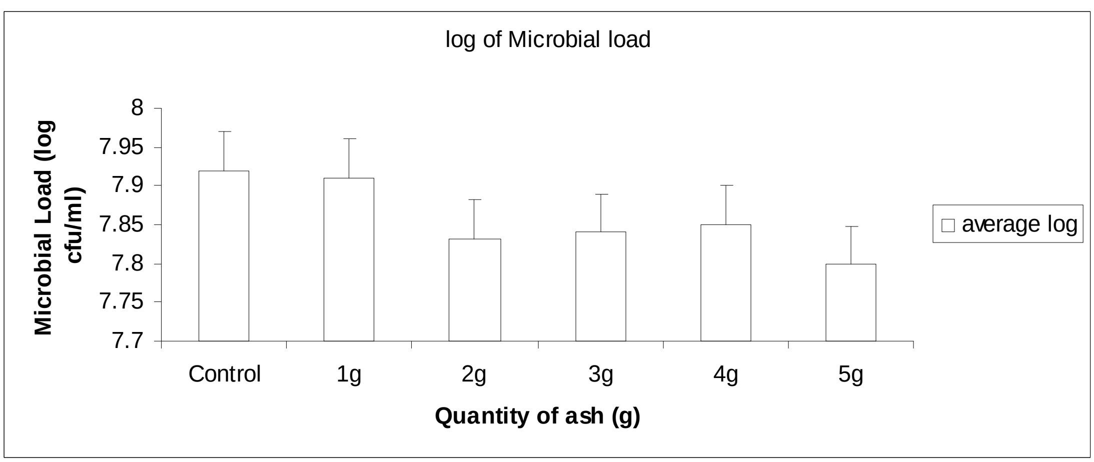

Fig.4 Microbial load for *Bacillus subtilis* strain 3A

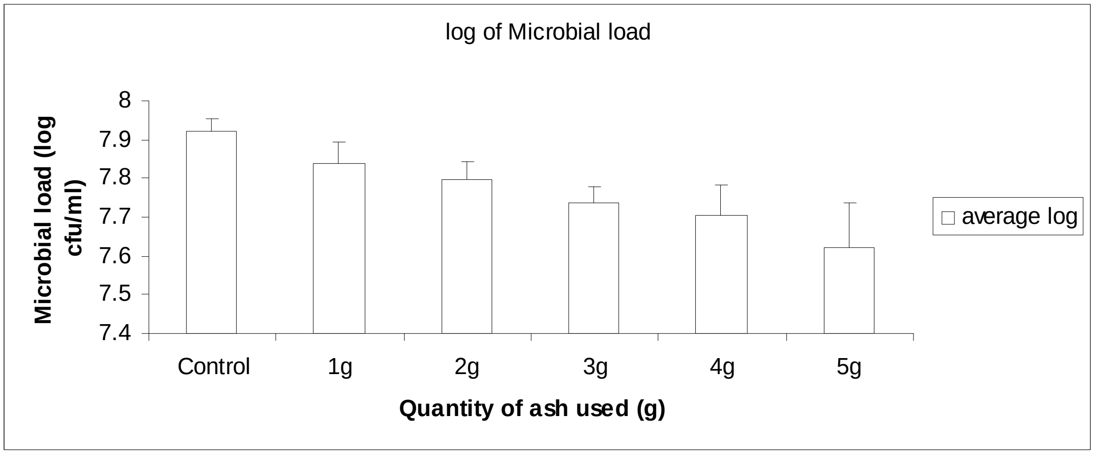

Fig.5 Microbial load for *Bacillus subtilis* strain 5A

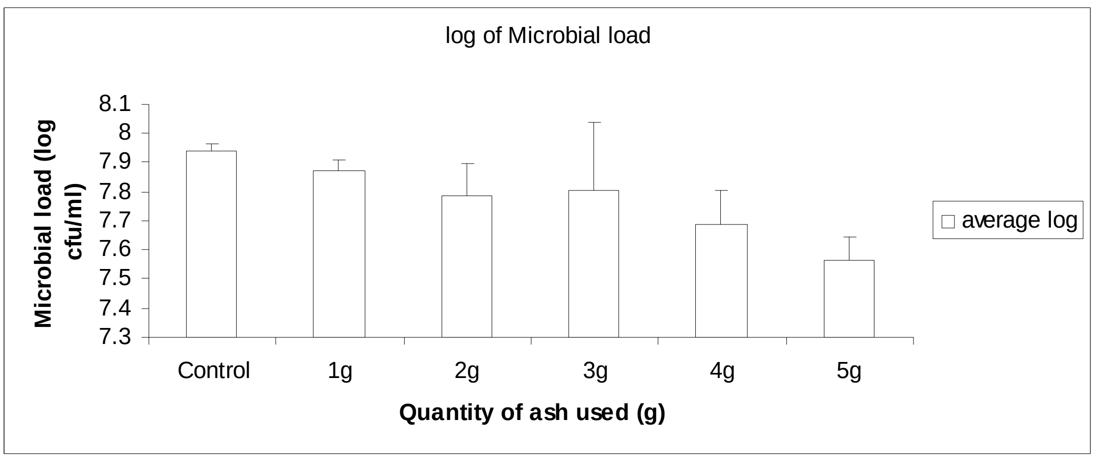

Fig.6 Microbial load for *Bacillus subtilis* strain BC4333

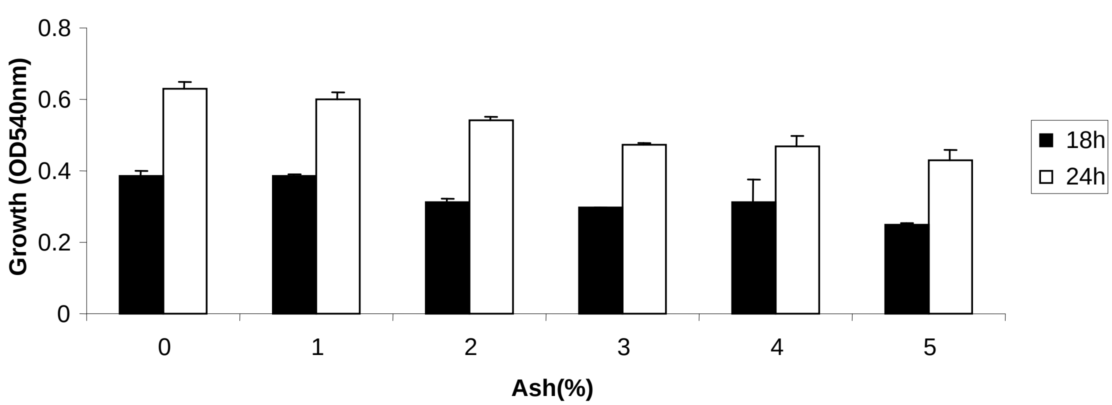

Fig.7 Growth (OD_{540nm}) of *Bacillus subtilis* strain 8B in the NB media (0-5% w/v ash)

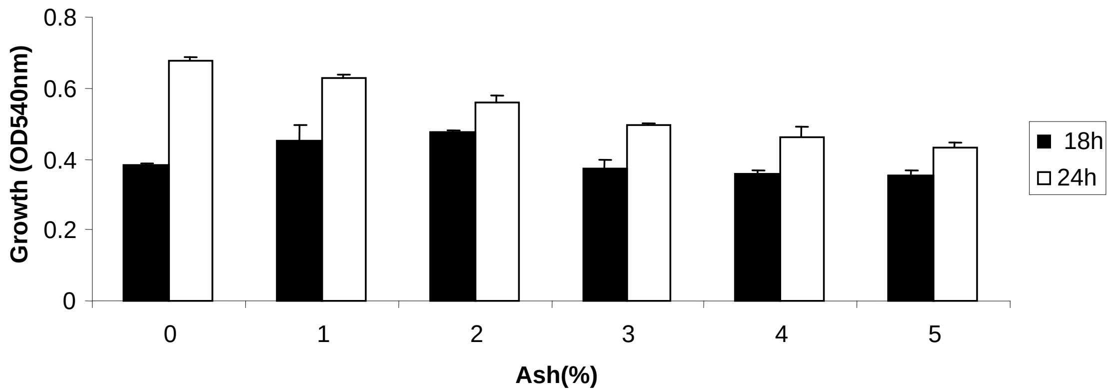

Fig.8 Growth (OD_{540nm}) of *Bacillus subtilis* strain 1A in the NB media (0-5% w/v ash)

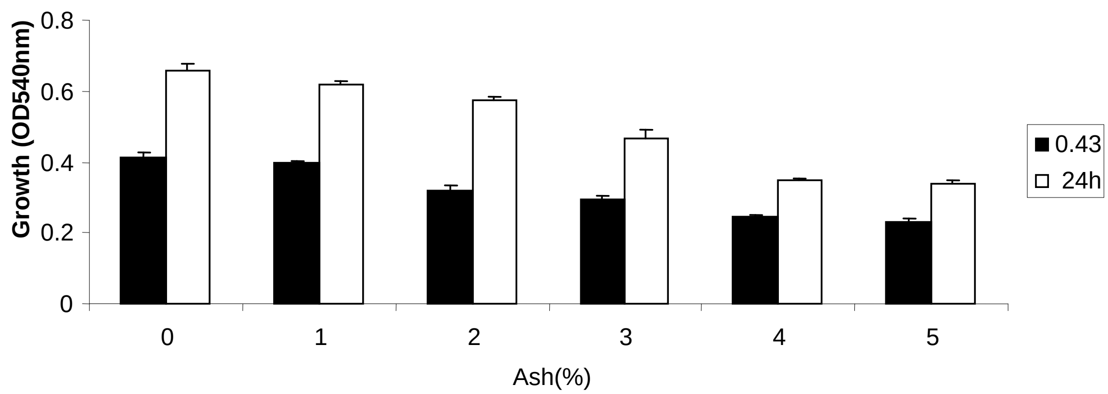

Fig.9 Growth (OD_{540nm}) of *Bacillus subtilis* strain 2B in the NB media (0-5% w/v ash)

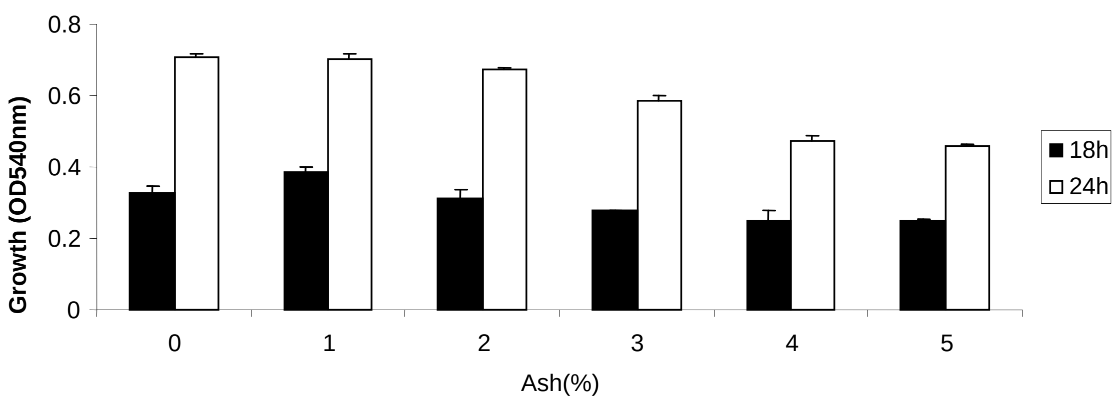

Fig.10 Growth (OD_{540nm}) of *Bacillus subtilis* strain 3A in the NB media (0-5% w/v ash)

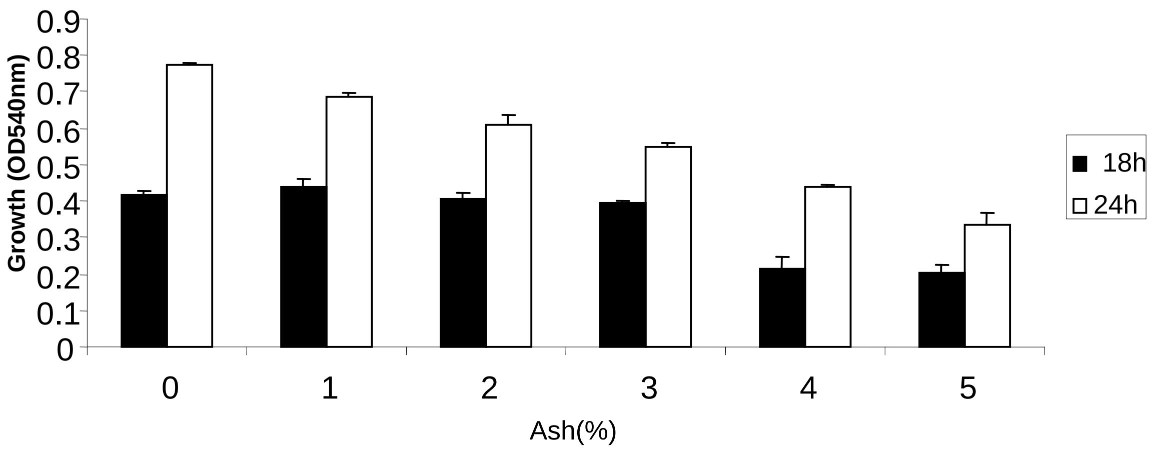

Fig.11 Growth (OD_{540nm}) of *Bacillus subtilis* strain 5A in the NB media (0-5% w/v ash)

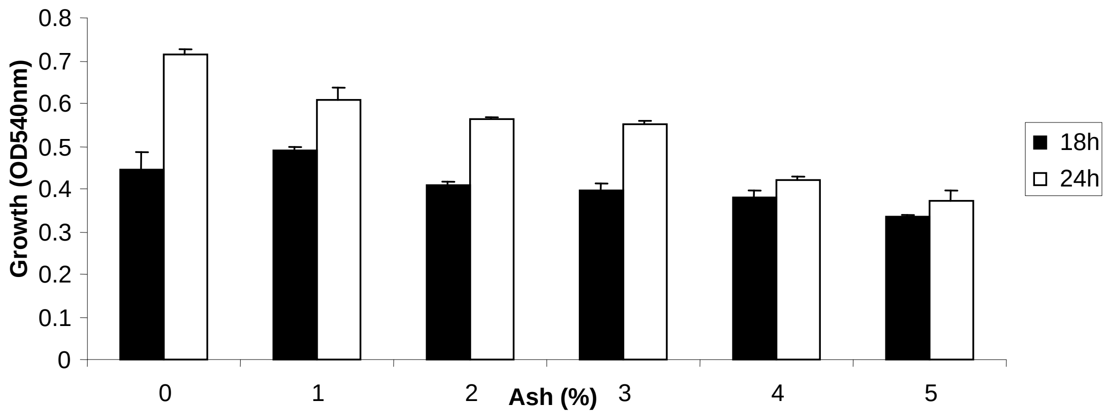

Fig.12 Growth (OD_{540nm}) of *Bacillus subtilis* strain BC4333 in the NB media (0-5% w/v ash)

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