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OPTIMIZATION OF THE BIOAGENT *Bacillus subtilis* BIOMASS PRODUCTION AND ANTIBOSIS AGAINST *Acremonium strictum*

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ABSTRACT

All *A. stritum* strains, isolated from grain sorghum plants showing Acremonium wilt disease symptoms, varied in their virulence on both tested grain sorghum genotype with the superiority of strain No. 2.

Among 5 different recommended media, King's medium was the most favorable one for propagation and highest antagonistic activity of *Bacillus subtilis*, previously isolated from rhizosphere of resistant grain sorghum cultivar (Dorado) against *A. strictum*.

The optimum environmental conditions needed for *B. subtilis* to give maximum antagonistic efficiency against *A. strictum* were 48h incubation period, 30-35^{o}C incubation temperature and pH 7.0. in shake-flask submerged culture. Sucrose (9.0 g.l^{-1}) and KNO_3 (20 g.l^{-1}) were the best carbon and nitrogen sources, respectively.

Utilization of agro-industrial wastes for *B. subtilis* propagation in modified King's medium showed that glucose syrup and corn steep liquor as cheap carbon and nitrogen sources, respectively supported the highest antagonistic potential of *B. subtilis* using shake-flask submerged culture.

Keywords: *Bacillus subtilis,* Biological control, Acremonium wilt, *Acremonium strictum*, Agro-industrial wastes, Antifungal activity, Optimization, Propagation.

INTRODUCTION

Grain sorghum (*Sorghum bicolor* L. Moench) occupies a unique position among cereal crops in Egypt and all over the world. It is used mostly as food and feed in addition to its importance in several fermentation industries such as beverages and biofuels (Ratnadass *et al.*, 2003). Grain sorghum is vulnerable to different fungal diseases of which Acremonium wilt, caused by the soil-borne fungus *Acremonium strictum* W. Gams (*Cephalosporium acremonium* corda), which is responsible for crop yield losses of up to 50% (Ibrahim and Zein El-Abedeen, 2000 and Osman, 2004).

Biological control of plant pathogens, based on the management of a natural source to develop the antagonistic activity against harmful organisms, common component of ecosystems, is currently accepted as a key practice in sustainable griculture and as potential alternative to the intensive use of chemical pesticides which are harmful to the environment. Several investigators reported the use of biological control against soil-borne diseases (El-Kazzaz *et al.,* 2000 and Chincholkar and Mukerji, 2007).

Bcillus subtilis has been used as bicontrol agent for many soil-borne diseases such as late wilt of maize (Ibrahim, 1990), sesamum root-rot

(Dinakaran *et al.,* 1995) stem-rot of ground nut (Kulkarni *et al.,* 1996), Fusarium wilt in tomato (Sarhan *et al.,* 2001), Acremonium wilt of grain sorghum (Ali *et al.,* 2005) and sugar beet damping-off (Abo-Elnaga, 2006).

In the literature several media were used for propagation of *B. subtilis* with enormous variation among obtained results with respect to growth and antagonistic potential (Ezzat *et al.,* 2001 and Abd-Alla *et al.,* 2003).

Nutritional and environmental parameters influence markedly microbial metabolism and production of antifungal substances. Certainly, this is the case with *B. subtilis.* Thus, optimization of these parameters may led to strain improvement which may enhance antagonistic activity and open the possibilities of efficient disease control.

The present work aimed to optimize cultural conditions for propagation and supporting antagonistic potential of *B. subtilis* against *A. strictum* the incitant of grain sorghum Acremonium wilt. Here, incorporation of some agro-industrial wastes in the propagation medium was investigated.

MATERIALS AND METHODS

Bacterial candidate

Bacillus subtilis, a promising antagonistic strain against grain sorghum Acremonium wilt disease (Osman, 2004) was used in this study. It was maintained refregerated on nutrient agar medium (Jacobs and Gerstein, 1960).

Sorghum grains

Grains of two susceptible grain sorghum genotypes (Giza 113 and Giza 15) obtained from Sorghum Res. Dept., Field Crops Res. Inst., ARC, Giza, Egypt, were used for carrying out the pathogenicity test under greenhouse conditions.

Agro-industrial wastes and by-products

Sugar-cane molasses, obtained from Sugar and Integrated Industries Company, El-Hawamedia, Giza, and sugar beet molasses, obtained from Delta Sugar Company, Kafer El-Shikh, were clarified according to Pyke (1958) and kept refrigerated until use. Glucose syrup, corn steep liquor and glutovin were kindly provided by Egyptian Company of Starch and Glucose, Torah, Cairo while soybean cake was obtained from Food Processing Unit, ARC, Giza. Corn steep liquor was clarified according to Dokhan (2005) before use while glutovin and soybean cake were subjected to acid hydrolysis according to Ahmed *et al.* (1992) and the hydrolyzates were kept refrigerated until use.

Isolation and identification of the phytopathogen

Plant samples of grain sorghum showing acremonium wilt disease symptoms were collected from different governorates in middle and upper Egypt. The lower internodes (3^{rd}-5^{th} above soil level) of the rotted stalks of the wilted plants were washed with running water and left to dry, surface sterilized by ethanol (70%), flamed and peeled under aseptic conditions. Small pieces of the internal tissues were cut out and plated on PDA medium (Booth, 1971) containing streptomycin (200 ppm). Plates were incubated at 30^{o}C for 3-7 days and examined daily for the occurance of fungal growth. The

growing fungi were examined miroscopically and purified using single spore technique.

The obtained fungal isolates were identified by morphological characteristics and microscopic examination according to Barnett (1960) and the specifications of Sabet *et al.* (1966a) and confirmed by comparing these isolates with the culture collection of the Maize, Sugar and Forage Crops Res. Dept., Plant Pathol. Res. Inst., ARC, Giza, Egypt. The isolates were maintained on PDA slants under mineral oil in a refrigerator for further studies.

Virulence of *Acremoium strictum* strains

The virulence of 12 *A. strictum* strains was carried out under greenhouse conditions using soil infestation technique. Two susceptible grain sorghum cultivars (Giza 113 and Giza 15) were used. The phytopathogenic inoculants were prepared by growing the obtained *A. strictum* strains separately in sterilized glass bottles of 500 ml capacity, each containing 100 g sorghum grains moistened with 50 ml H_2O for 15 days at 30°C. Bottles were shaken every 2 days for homogenicity.

Soil infestation was carried out by adding the previously prepared inoculant of each strain at the rate of 100 g. pot^{-1} to sterilized clay pots (25 cm diameter) filled with autoclaved Nile silt soil prior to sowing as described by Sabet *et al.* (1966b). Untreated soil was used as control. Sorghum grains were surface sterilized using sodium hypochlorite solution (5%) for 2 minutes. Five seeds were sown in each pot and 4 replicates were allocated. Superphosphate (15.5% P_2O_2) was added at the rate of 3.0g. pot^{-1} before sowing. Plants were fertilized 21 days after sowing at the rate of 3.0 g urea (46.5% N) and 3.0 g potassium sulphate (48% K_2O) per pot. Irrigation was done when necessary using tap water. Disease monitoring was recorded after 90 days of sowing as percentage of wilted plants.

Re-isolation of the pathogenic fungal isolates from diseased plants was carried out to meet Koch's postulates.

Estimation of *B. Subtilis* growth

Bacillus subtilis growth was determined turbidimetrically as follows: a loopful of 48 h-old culture was transferred into 50 ml sterilized nutrient broth in 250 ml – conical flask and incubated on a rotary shaker incubator at 150 rpm and 25°C for 48 h. After incubation period, bacterial cells were counted as $cfu.ml^{-1}$ broth culture by plate count technique (using nutrient agar medium, 25°C for 48 h). Optical density (OD) of each broth culture dilution, with known bacterial count, at 620 nm was recorded using Perkin Elemer 55 E spectrophotometer. Bacterial growth (cfu. ml^{-1}) was determined by multiplication of sample OD at 620 nm by $slope^{-1}$ of relation line plotted between bacterial count ($cfu.ml^{-1}$) and OD at 620 nm.

In vitro* estimation of *B. subtilis* antagonistic potential against *A. strictum

Antagonistic potential of *B. subtilis* culture filtrate against *A. strictum* was determined using well cut-diffusion technique (Brock, 1973). After incubation period, cultures were centrifuged at 3000rpm for 20 min. The supernatants were then filter sterilized using sterile syringe filter of 25 mm in diameter and 0.2 μm pore size. Wells (5 mm in diameter) were made

equidistantly from each other in 9 cm – Petri plates containing PDA supplemented with 0.5% peptone medium seeded with *A. strictum* ($3x10^4$ spores. ml^{-1}) using sterile cork borer. Then, 0.2 ml of each culture filtrate was transferred separately into each well using sterile micropipette. The plates were then incubated for 5 days at 30°C before measuring the inhibition zone diameters. Absolute unit of inhibition zones (AU) were calculated as follows: $AU = Y^2/X^2$ where: Y is the radius of inhibition zone diameter and X is the radius of the well.

Optimization of cultivation conditions for magnifying of *B. subtilis* growth and antagonistic potential.

In order to optimize nutritional and environmental conditions to support *B. subtilis* growth and antagonistic potential against *A. strictum,* 8 sets of experiments were conducted using shake-flask submerged culture: a conical flasks of 250 ml capacity containing 50ml sterilized medium were inoculated with a loopful of *B. subtilis,* 48 h-old culture. The inoculated flasks were incubated on shaker incubator at 150 rpm and 30°C for 72 h. At the end of incubation period, the bacterial growth and antagonistic potential were determined as previously mentioned.

To select for the most favorable basal medium, 5 different recommended media [Czapek-Dox (Thom and Raper, 1945), King (King *et al.,* 1945) potato-dextrose (Booth, 1971), nutrient broth (Stanisich and Holloway, 1972) and Richard (Richard, 1954)] were tested. Time course of cell growth and antagonistic potential were examined up to 84h incubation period. Optimum pH and incubation temperature were also studied using King's medium with initial pH values of 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 and different incubation temperatures of 25, 30, 35, 40 and 45°C. The carbon (glucose, mannose, sucrose, maltose, lactose and corn starch) and nitrogen [$(NH_4)_2 SO_4$, NH_4Cl, $NaNO_3$, KNO_3, urea, peptone and yeast extract] sources were examined. The most favorable concentrations of carbon (3,6,9, 12 and 15 $g.l^{-1}$ sucrose) and nitrogen (5, 10, 15, 20, 25 and 30 $g.l^{-1}$ KNO_3) sources were also investigated.

Utilization of some agro-industrial by-products as carbon and nitrogen sources

Some agro-industrial by-products, sugar cane molasses, sugar beet molasses and glucose syrup (as carbon sources) and corn steep liquor, glutovin and soybean cake (as nitrogen sources) replaced sucrose and KNO_3, respectively, in order to obtain an economic medium for propagation of *B. subtilis.*

Statistical analysis

Statistical analysis was computed using analysis of variance procedure described by Sendecor and Cochran (1980), the significant mean differences between treatment means were separated by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Isolation and identification of the phytopathogen

The most prevalent fungi (12 isolates) were isolated from samples of grain sorghum plants, showing Acremonium wilt disease symptoms, collected

from seven governorates in middle and upper Egypt. All isolates exhibited the same identical characteristics. Their growth on PDA supplemented with 0.2% yeast extract at 28°Cwas moderately rapid covering the plate surface in about 15 days. They produced a dense mycelial mat with a distinctly raised smooth margin. The hyphae were hyaline, septate, slender and simple varying in length. Conidia were hyaline, single-celled, oval, straight or slightly curved produced at the tip of the conidiophore and collected to form spore heads embedded in a slime matrix. When compared with those reported by Barnett (1960) and Sabet *et al.* (1966a) for *Acremonium strictum,* they were in conformity. Therefore, the isolates were all identified as *A. strictum*, the well known causal pathogen of Acremonium wilt of grain sorghum. The isolated fungus is very similar to *Cephalosporium acremonium* which was isolated from wilted grain sorghum plants (El-Shafey *et al.,* 1979 and Ali *et al.,* 2004) and was the causal pathogen of Acremonium wilt disease for American sorghum cultivars (Natural *et al.,* 1982) and Chinese grain sorghum ones (Xu *et al.,* 1995).

Virulence of *Acremonium strictum*

Results illustrated in Figure (1) show that the tested strains varied in their virulence and the infection was a cultivar dependent. Genotype Giza 113 was less susceptible to *A. strictum* strains (30-93.5% infection) with a mean infection percentage of 45.29 whereas Giza 15 was more susceptible (50-97.5%) with a mean infection percentage of 60.63. Strain No 2 isolated from Giza governorate (Ayat) was the most aggressive, causing the highest infection percentage (97.5 and 93.5) on Giza 15 and Giza 113, respectively with a mean infection percentage of 95.5. Differences in virulence between *A. strictum* strains indicate that different pathotypes of this pathogen may exist (William and Asher, 1996 and Osman, 2004).

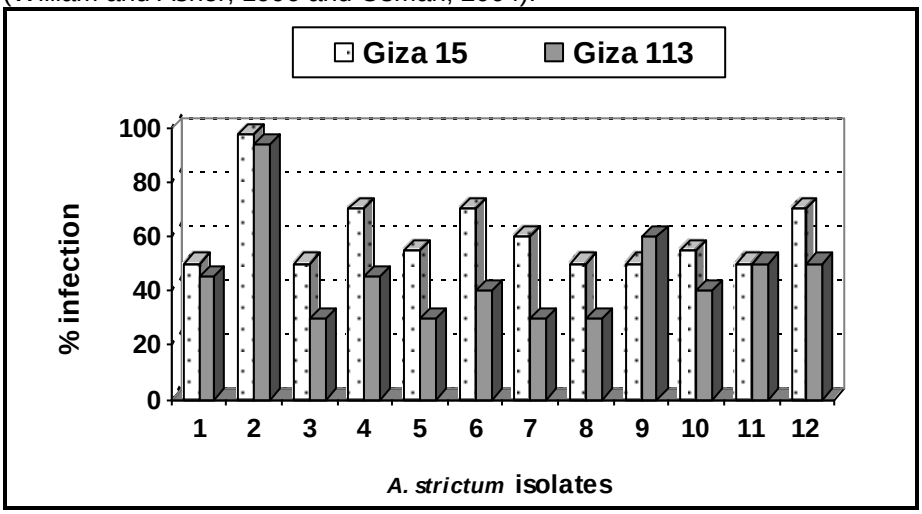

Figure 1:Virulence of *A. strictum* strains against two susceptible [grain sorghum genotypes under greenhouse conditions.

Similarly, El-Assiuty (1982) found that some strains of *C. acremonium* causing stalk rot disease of grain sorghum were more virulent than others. On the other hand, sorghum and maize varieties showed a wide susceptibility to *C. acremonium* strains and could be classified into 6 major categories according to their degree of infection (Mansour *et al.,* 1986). Also, El-Shafey *et al.* (1999) recorded highly significant variation in virulence of large number of *A. strictum* strains when their pathogenicity was tested on four grain sorghum cultivars under greenhouse conditions. On the other hand, Giza 15 was the most susceptible genotype.

For the superiority of strain No 2 among all tested *A. strictum* strains in respect to its infection of both susceptible genotypes, it was selected for further studies.

Optimization of cultivation conditions for enhancement of *B. subtilis* growth and antagonistic potential against *A. strictum*

A comparative study was carried out to gain some more antagonistic potential of the promising antaognsitic *B. subtilis* strain (Osman, 2004) against *A. strictum.*

1. Selection of suitable medium

Cultivation of *B. subtilis* was undertaken in five different recommended media using shake-flask submerged culture. Data illustrated in Figure (2) show that nutrient broth is the most suitable medium for growth of *B. subtilis* ($30x10^7$ cfu. ml^{-1}) followed by King's and Richard's media ($28x10^7$ and $27x10^7$, respectively), while King's broth is the most suitable for *B. subtilis* antagonistic potential against *A. strictum* expressed as absolute unit of inhibition zone (29.16 AU), followed by nutrient broth and potato dextrose media (23.04 and 21.16 AU, respectively). However, Czapek-Dox's broth was the least medium for both growth and antagonistic potential. These findings are in line with those obtained by Ezzat *et al.* (2001). In contradiction, Ibrahim (1990) reported that Czapek-Dox's broth was the best cultural medium for maximum antagonism by two different *B. subtilis* strains against *Cephalosporium maydis.* On the other hand, Abd-Alla *et al.* (2003) stated that Richard's medium was suitable for maximum antagonistic potential of *B. subtilis* against *Sclerotium rolfsii*. Such contradiction might be due to difference in bacterial strain, pathogen and/or other cultural conditions (Abo-Elnaga, 2006). Therefore, King's medium was selected for further studies.

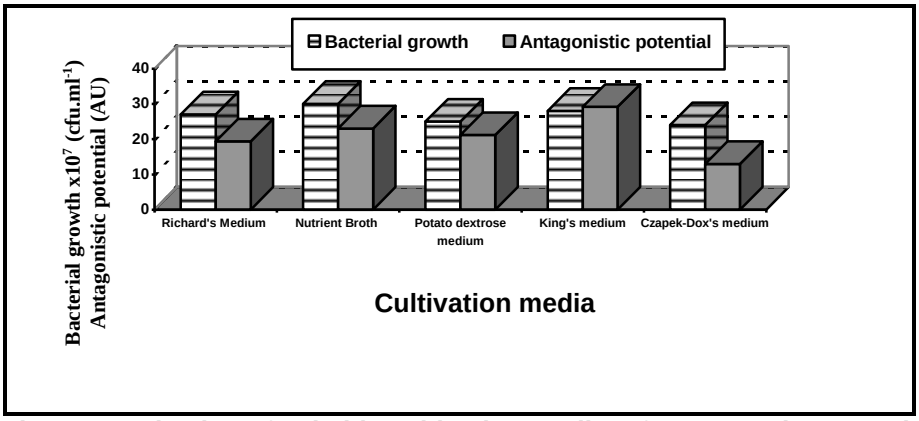

Figure 2: Selection of suitable cultivation medium for supporting growth and antagonistic potential expressed as absolute unit of inhibition zone (AU) of *B. subtilis* against *A. strictum.*

2. Time course

Time course of *B. subtilis* growth and antagonistic potential aganist *A. strictum* was investigated. Results illustrated in Figure (3) show that there was close association between bacterial growth and antifungal substance (s) production (expressed as AU). Bacterial growth increased markedly with time to reach its maximal ($30x10^7$ cfu.ml^{-1}) after 60 h, then slightly decreased. In parallel, antagonistic potential of *B. subtilis* increased to reach its maximal (30.47 AU) after 48 h, then also slightly decreased. The obtained results were similar to those reported by Ibrahim (1990) and Ezzat *et al.* (2001) who stated that the highest antifungal production by *B. subtilis* was two days of incubation in buffered medium at pH 7.0. Abd-Alla *et al.* (2003) mentioned that maximum antagonistic potential of *B. subtilis* against *S. rolfsii* was achieved at 72 h incubation period. Such difference might be attributed to variations in bacterial strain, pathogenic fungi and/or other cultural variations.

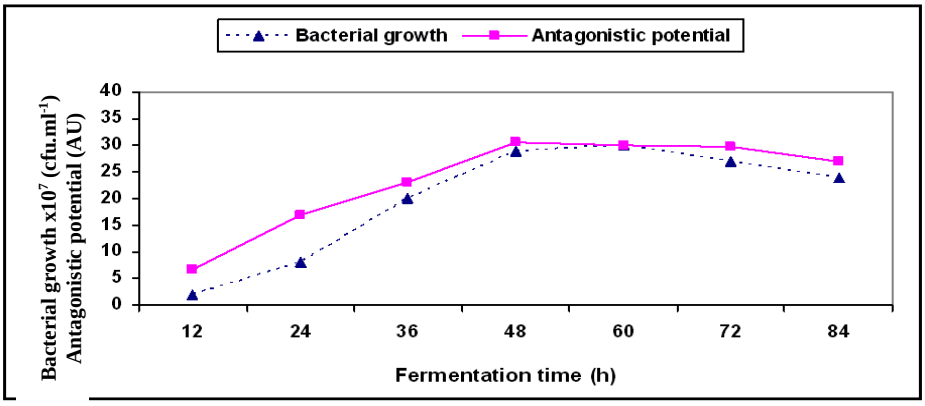

Figure 3: Time course of *B. subtilis* growth and antagonistic potential against *A. strictum* expressed as absolute unit of inhibition zone (AU).

3. Effect of fermentation pH

Growth medium pH is considered to be one of the most important factors affecting the microbial growth and production of biologically active materials including anti-fungal substances (McKeen *et al., 1986)*. Therefore, cultivation of *B. subtilis* was conducted in King's medium with six initial pH levels. Data illustrated in Figure (4) clearly show that antagonistic potential of *B. subtilis* gradually increased by increasing initial pH of the growth medium, reaching its maximal activity (31.36 AU) at pH 7. then the antagonistic potential markedly decreased by increasing initial pH levels. These results are in consistence with those reported by Ezzat *et al.* (2001) and are generally in agreement with Ibrahim (1990) who stated that maximal antagonistic activity of two strains of *B. subtilis,* grown in Czapek-Dox's medium, against *C. maydis* was achieved at initial pH of 7.5. Abd-Alla *et al.* (2003) reported that maximum antagonistic activity of *B. subtilis,* grown in Richard's medium, against *S. rolfsii* was attained at initial pH of 6.0. These variations are more likely attributed to certain variations the in bacterial strain, pathogenic fungus and/or other environmental and nutritional factors.

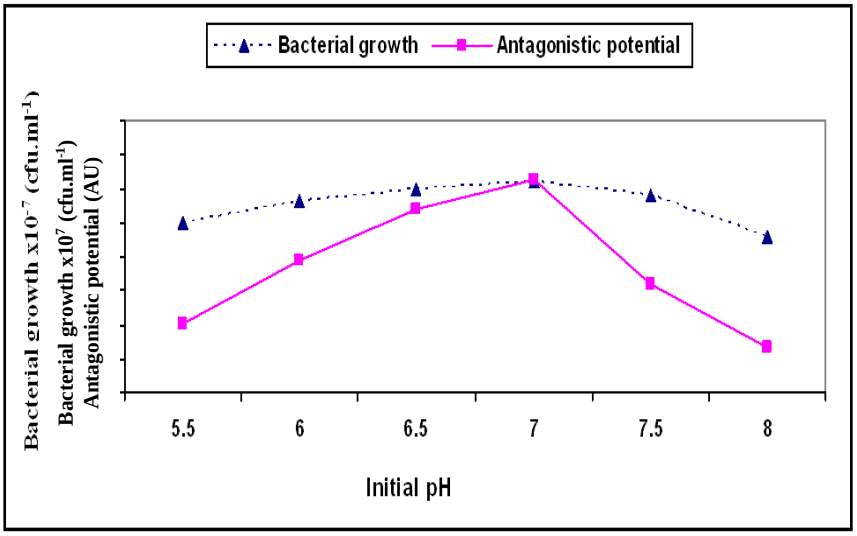

Figure 4: Effect of initial pH levels of the cultivation medium on growth and antagonistic potential of *B. subtilis,* expressed as absolute unit of inhibition zone (AU) against *A. strictum*.

4. Effect of fermentation temperature

Results in Figure (5) show the effect of fermentation temperature on growth and antagonistic potential of *B. subtilis* against *A. strictum.* Temperature ranging between 25 and 35°C supported cell growth and enhanced antagonistic activity. Higher temperature had a negative effect on both variables. The maximal antagonistic potentials of 33.64 and 32.49 AU were attained in cultivations carried out at 30 and 35°C, respectively. The obtained results are in a parallel line with Sellam *et al.* (1978) who stated that production of antifungal substance by *B. subtilis* against *C. maydis* was

stimulated by growing the bacteria at 30-35ºC in Czapek-Dox's medium. Abd Alla *et al.* (2003) achieved the highest antagonistic activity against *S. rolfsii* by *B. subtilis* at 35ºC in Richard's medium.

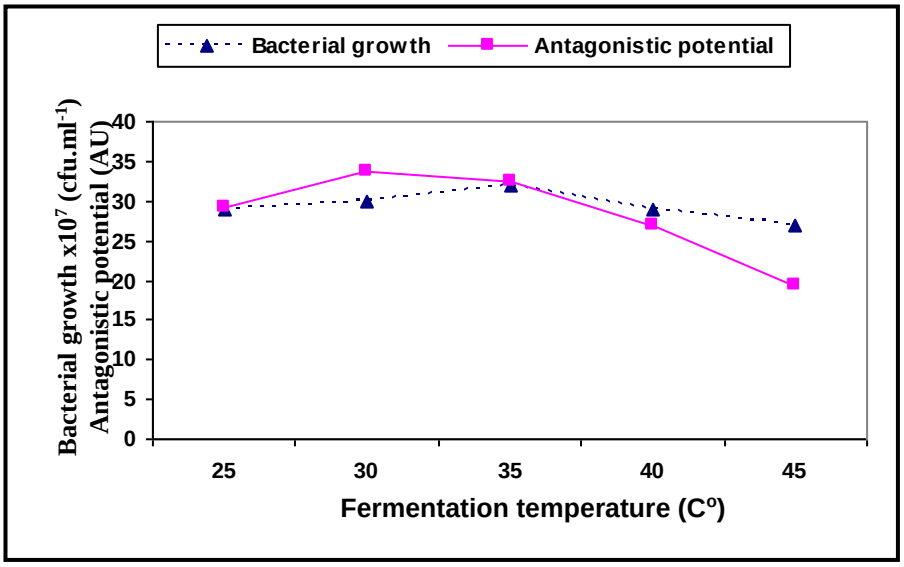

Figure 5: Effect of fermentation temperature on growth and antagonistic potential of *B. subtilis* against *A. strictum* expressed as absolute unit of inhibition zone (AU).

5. Effect of carbon sources

Seven different carbohydrates were tested as carbon sources to achieve maximal growth and antagonistic activity of *B. subtilis* against *A. strictum.* Table (1) reveals that sucrose supported the best performance of *B.subtilis* against *A. stritcum* (33.64 AU) followed by glucose and glycerol (32.49and 31.36 AU, respectively). Maximal bacterial growth ($32x10^7$ cfu.ml^{-1}) was attained using glucose while, lactose supported the lowest bacterial growth and antagonistic activity ($20x10^7$ cfu. ml^{-1} and 1.44 AU, respectively). In agreement with the obtained results Abd-Alla *et al.* (2003) advocated sucrose to glucose for supporting *B. subtilis* antagonistic potential against *S. rolfsii*.

Table 1:Effect of different carbohydrates as carbon sources on growth and antagonistic potential of *Bacillus subtilis* against *A. strictum*.

Carbon source[(a)]	Final pH	Bacterial growth X 10^7 ($cfu.ml^{-1}$)	Antagonistic potential of *B. subtilis* culture filtrate against *A. strictum*		
			Y (mm)	Y^2 (mm^2)	$AU = Y^2/X^2$
Corn starch	6.75	28	7.50	56.25	9.00
Glucose	6.85	32	14.25	203.06	32.49
Mannose	6.80	25	10.00	100.00	16.00
Sucrose	6.80	30	14.50	210.25	33.64
Maltose	6.40	28	9.00	81.00	12.96
Lactose	6.10	20	5.00	25.00	4.00
Glycerol (control)	7.00	29	14.00	203.06	31.36

Y, radius of inhibition zone **X, radius of the well= 2.5 mm**
Au, absolute unit of inhibition zone **(a), at the rate of 3.9 g carbon. l^{-1}**

6. Effect of initial concentration of sucrose

As shown in Figure (6), antagonistic potential of *B. subtilis* against *A. strictum* increased by increasing initial sucrose concentration reaching maximum value (33.64 AU) with maximal bacterial growth ($30x10^7$ cfu. ml^{-1}) with sucrose concentration of 9.0 $g.l^{-1}$. Higher concentration of sucrose led to a decrease in both variables. Results reveal that the antagonistic activity correlated positively with the increase of bacterial growth. The obtained results thus confirm those of earlier reports (Sellam *et al.,* 1978 and Abd-Alla *et al.,* 2003).

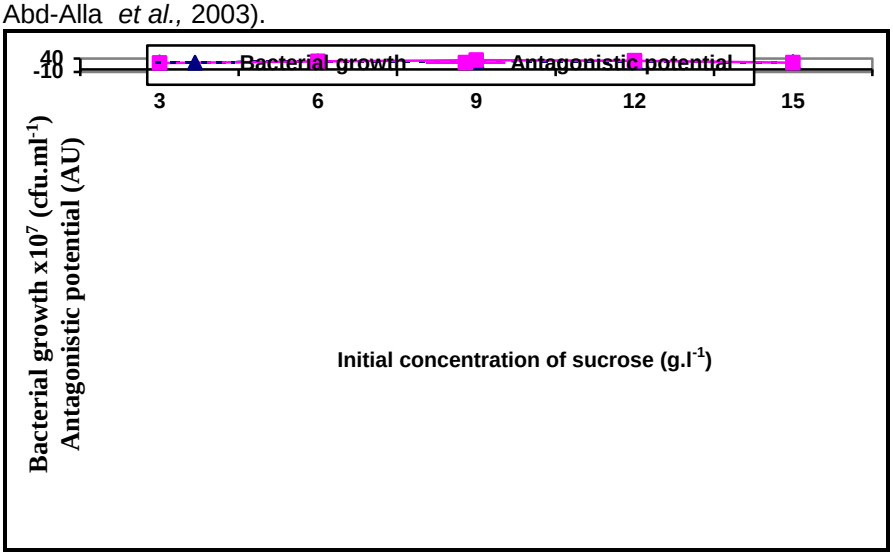

Figure 6: Effect of initial concentrations of sucrose on growth and antagonistic potential of *B. subtilis* against *A*. strictum expressed as absolute unit of inhibition zones (AU).

7. Effect of nitrogen sources

Among the seven tested nitrogen sources (Table 2), KNO_3 was the best which maximized the antagonistic potential against *A. strictum* (34.81

AU) followed by peptone and yeast extract (33.64 and 31.36 AU, respectively). Whereas, yeast extract stimulated bacterial growth followed by peptone and urea. Addition of NH_4Cl or $(NH_4)_2SO_4$ resulted in lowest antagonistic activity. Abd-Alla *et al.* (2003) achieved the highest antagonistic potential of *B. subtilis* against *S. rolfsii* with KNO_3 as nitrogen source.

Table 2: Effect of nitrogen sources on growth and antagonistic potential of *B. subtilis* against *A. stricum*.

Carbon source[(a)]	Final pH	Bacterial growth X 10^7 (cfu.ml^{-1})	Antagonistic potential of *B. subtilis* culture filtrate against *A. strictum*		
			Y (mm)	Y^2 (mm^2)	$AU = Y^2/X^2$
Ammonium sulphate	6.95	27	8.00	64.00	10.24
Ammonium chloride	7.00	25	8.00	64.00	10.24
Sodium nitrate	6.85	30	12.00	144.00	23.04
Potassium nitrate	6.85	30	14.75	217.56	34.81
Peptone (control)	6.80	31	14.50	210.25	33.64
Urea	7.1	31	12.50	156.25	25.00
Yeast extract	6.85	32	14.00	196.00	31.36

Y, radius of inhibition zone **X, radius of the well= 2.5 mm**
Au, absolute unit of inhibition zone **(a), at the rate of 3.9 g carbon. l $^{-1}$**

8. Effect of initial concentration of KNO_3

As illustrated in Figure (7), the stimulatory effect of varying KNO_3 concentrations on antagonistic potential is interesting. While optimal growth of *B. subtilis* requires higher concentrations, only 20 g.l^{-1} of KNO_3 were needed for maximum antagonism (36.0 AU). When initial KNO_3 concentration was reduced to 5.0 g.l^{-1}, a considerable decrease in antagonistic activity was observed. The obtained results are in agreement with those reported by Abd-Alla *et al.* (2003) with respect to the antagonism of *B. subtilis* against *S. rolfsii.*

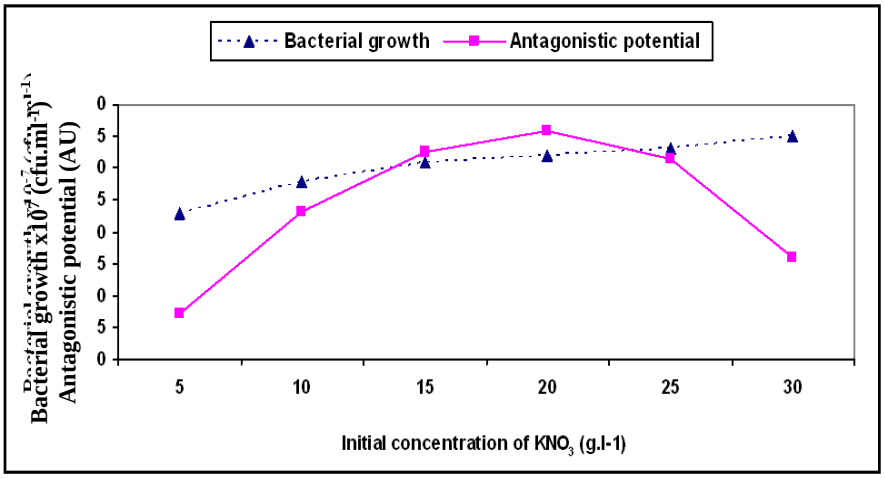

Figure 7: Effect of initial concentration of KNO_3 on growth and antagonistic potential of *B. subtilis* against *A. strictum* expressed as absolute unit of inhibition zones (AU).

Agro-industrial wastes as carbon and nitrogen sources

The feasibility of economic production of *B. subtilis* for application and use as biocontrol agent requires low production costs and high yield of the antifungal substance(s). These requirements could not be met upon using mineral synthetic media (El-Gamal and Hamed, 2003 and Dokhan, 2005). Therefore, sucrose in modified King's medium was replaced by pre-determined quantities of each of three different cheap carbon sources. Figure (8) shows that *B. subtilis* exhibited the lowest bacterial growth and the poorest performance in sugar beet and sugar cane molasses (2.56 and 4.0 AU, respectively). The lowest bacterial growth and antagonistic activity of *B. subtilis* against *A. strictum* most probably due to presence of a number of inhibitory substances such as phenolic compounds in molasses. The inhibitory effect of these substances on microbial metabolism is well documented (Castro *et al.,* 1994 and Dokhan, 2005). Results also reveal that glucose syrup supported simultaneously the highest antagonistic potential (36.0 AU) and bacterial growth. These improvements in both variables might be attributed to presence of certain minerals and/or organic growth factors that could be presented naturally in this by-product (Taherzadeh *et al.,* 2003).

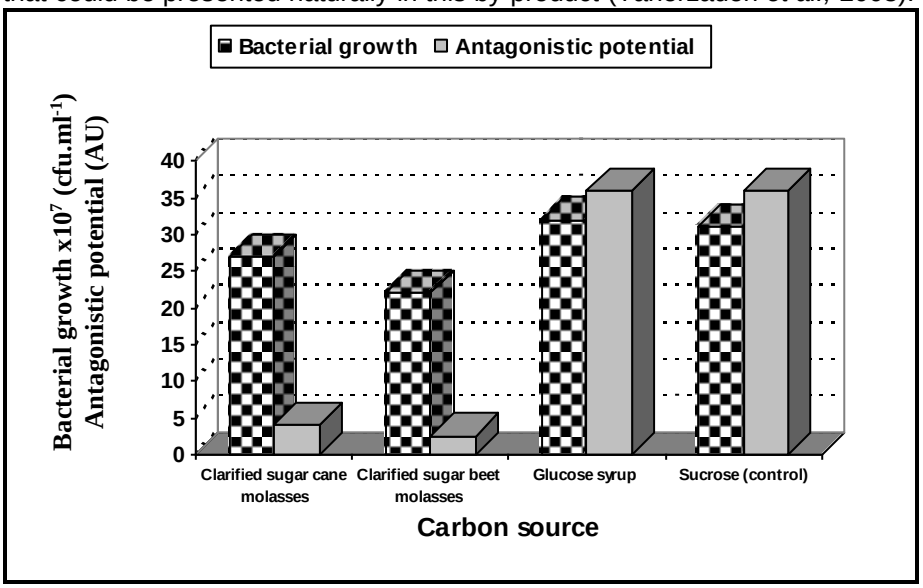

Figure 8:Utilization of agro-industrial wastes as carbon sources for enhancing growth and antagonistic potential of *B. subtilis* against *A. strictum* expressed as absolute units of inhibition zones (AU).

On the other hand, KNO_3 in modified King's medium was also replaced by predetermined quantities of each of three cheap organic wastes as nitrogen source. Generally, all tested pretreated wastes induced growth and antagonistic activity of *B. subtilis* against *A. strictum* with different efficiencies (Figure 9). With corn steep liquor, *B. subtilis* exhibited the highest growth and antagonistic potential ($35x10^7$ cfu. ml^{-1} and 36.48 AU,

respectively). This represented the highest achievement propagation conducted. These results are in line with those reported by El-Gamal and Hamed (2003).

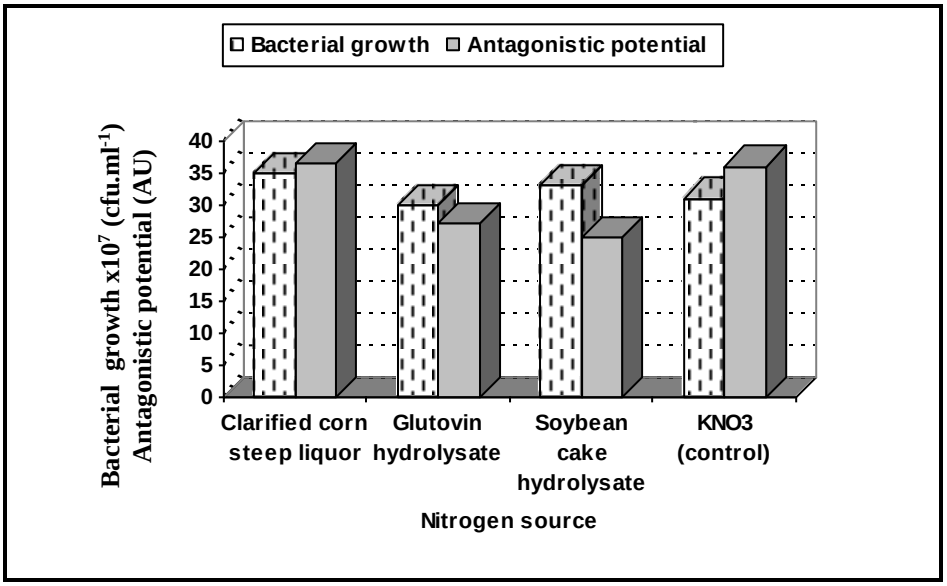

Figure 9: Utilization of agro-industrial wastes as nitrogen sources for enhancing growth and antagonistic potential of *B. subtilis* against *A. strictum* expressed as absolute units of inhibition zones (AU).

According to the aforementioned results, glucose syrup, and corn steep liquor were selected as most appropriate and cheap carbon and nitrogen sources, respectively for propagation of *B. subtilis.*

Work is in progress concerning extraction, purification and characterization of antifungal substance(s) of *B. subtilis* in addition to formulation of this promising bacterial strain as biocontrol agent against *A. strictum*.

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تحديد الظروف المثلى لتنمية وإكثار بكتيريا *Bacillus subtilis* وتعظيم قدرتها على مقاومة فطر *Acremonium strictum* المسبب لمرض الذبول الأكريمونيومي في الذرة الرفيعة

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يعتبر فطر *Acremonium strictum* المسبب لمرض الذبول الأكريمونيومي في الذرة الرفيعة من أهم مسببات الأمراض الكامنة في التربة. وقد تم عزل 12 عزله من هذا الفطر من نباتات ذرة رفيعة تبدو عليها أعراض الإصابة بالمرض وتم تعريفها واختبار قدرتها على إصابة صنفين من الذرة الرفيعة بالمرض (جيزة 113 وجيزة 15). وقد أظهرت جميع العزلات قدرتها على إصابة كلا الصنفين ولكن بدرجات متفاوتة وجاءت العزلة رقم (2) التي تم عزلها من محافظة الجيزة أشد هذه العزلات قدرة على إحداث المرض حيث بلغت نسبة الإصابة 93.5% و 97.5% على الترتيب.

عند تنمية بكتيريا *Bacillus subtilis* التي سبق عزلها من ريزوسفير نباتات ذرة رفيعة مقاومة للمرض على خمس بيئات مختلفة كانت بيئة King الأكثر ملائمة لنمو البكتيريا وإعطاء أعلى نشاط مضاد لأشد عزلات الفطر *A. strictum* ضراوة. وبدراسة الظروف البيئية المثلى لنمو هذه البكتيريا وتعظيم قدرتها على مقاومة الفطر الممرض وجد أن تنمية *B. subtilis* في بيئة King السائلة لمدة 48 ساعة في دوارق مهتزة عند درجة حرارة 30- 35°م ورقم هيدروجيني 7 يساعد على نمو البكتيريا ويزيد من قدرتها على تضاد الفطر بدرجة ملموسة. وجاء السكروز كأنسب مصدر كربوني من بين سبع مصادر تم اختبارها وبتركيز أمثل 9جم/لتر كما كانت نترات البوتاسيوم أفضل مصدر نيتروجيني من بين 7 مصادر تم اختبارها وبتركيز أمثل 20جم/لتر.

وعند استخدام بعض المخلفات الزراعية الصناعية في بيئة King المعدله كمصادر كربون ونيتروجين رخيصة الثمن بدلا من السكروز ونترات البوتاسيوم، أظهر كل من شراب الجلوكوز (كمصدر كربون) وسائل نقع الذرة (كمصدر نيتروجين) أعلى كفاءة في دعم نمو وإكثار بكتيريا *B. subtilis* وتعظيم قدرتها على مقاومة فطر *A. strictum*.