Detection of the optimal conditions for inulinase productivity and activity by *Acinetobacter baumannii* isolated from agricultural rhizosphere soil

Sahira Nsayef Muslim and Alaa N. Mahammed Ali, Istabreq Muhammed Ali Salman, Israa M.S. AL_Kadmy, Sraa N. Muslim

***Abstract*—**Inulinase is an enzyme catalyzing the hydrolysis of inulin, a plant reserve polysaccharide, into fructoses and fructo-oligosaccharides which are widely used in pharmaceutical and food industry. Although several substrates for the production of inulinase have been reported as being economically effective such as utilization of inulin-rich substrate rather than pure inulin, however, there is still need to develop the substrate to make the entire process much cheaper and more effective. Here we reported inulinase production from *Acinetobacter* as there is not found any report on inulinase production from *Acinetobacter*. Eight isolates of *Acinetobacter baumannii* were isolated from different agricultural rhizosphere soil samples and screened for higher inulinase production, *Acinetobacter baumannii*Rs5 gave higher inhibition zone around the colony in comparison with other isolates. The enzyme activity was increased to 3.97 U/ml when this isolate was cultivated under the optimal conditions which consisted of using basal medium containing 4% (w/v) inulin extract from *dahlia* tubercles and 2% (w/v) treptone at pH7.5 at 28°C for 22 hour. The enzyme revealed maximum activity(4.32 U/ml) in the presence of dahlia tubercles (2% w/v) followed by garlic with activity of 4.09U/ml as substrates. Consequently, dahlia has a potential as an effective and much cheaper (economical) substrate for inulinase production and inulinase activity in comparison with traditionally used substrates like pure inulin and other saccharides.

***Keywords*—** Inulinase, *Acineto bacterbaumannii*, rhizosphere.

I. INTRODUCTION

The rhizosphere, considered to be a hot spot of bacterial diversity , harbours bacterial flora whose diversity is mainly expressed in terms of functions adapted to the root presence, and in particular to favour plant growth. This is in turn beneficial to the whole rhizosphere microbiota through the highly nutritive and energetically rhizodepositions[1]. Rhizosphere is a rich source of various microorganisms that can benefit plant growth and survival. Among those *Bacillus, Enterobacter, Acinetobacter, Alcalogenes, Arthrobacter, Flavobacterium, Rhizobium, Erwinia, Azospirillum, Burkholderia, Enterobacter, Pseudomonas, Rhizobium* and *Serratia* [2]. They promote growth of plant *via* acidolytic solubilization of minerals, chelation of iron, production of enzymes to mineralize N, S or P from organic compounds to provide plants corresponding inorganic ions, nitrogen fixation, phytohormone production and exoploymer synthesis [1].

Acinetobacter baumannii causes opportunistic infections because of limited number of virulence factors and are thus considered as low grade pathogen[3]. *Acinetobacter baumannii* are able to survive on moist and dry surfaces, It has been isolated from soil and water samples , also it isolated in low numbers from fresh fruit and vegetables and from health human skin so that the environment soil, water and animals are the natural habitats for it [4]. Non specific nutritional requirements, resistance to desiccation and the ability to form biofilm permit *Acinetobacter baumannii* to grow in various environment, and therefore initial contact with the pathogen preceding infection can be made in a variety of ways [4]. Most of the *Actinetobacter baumanii* infections resulted in pneumonia , urinary tract , blood stream and surgical wound infections[3].

Inulin is present as a reserve carbohydrate in the roots and tubers of composite plants such as Jerusalem artichoke (*Helianthus tuberosus* L.), Chicory (*Cichorium intybus* L.), Sunflower (*Helianthus annuus* L.) and Dahlia (*Dahlia pinnata* Cav.)[5]. Onions, leeks and garlic are also rich in fructan and are the most common sources of this polysaccharide in the Australian diet. Not only these plants are rich in fructans but about 15% of flowering plant species have fructans as their main storage carbohydrate[6]. The yields of the roots and tubers are very high[7]. Inulin consists of linear chains of β-2,1-linked D- fructofuranose molecules terminated by a glucose residue through a sucrose-type linkage at the reducing end[6,7].

Many inulin sources have recently received attention as a renewable raw material in the production of inulinase i.e. ethanol, acetone, butanol, pullulan, gluconic acid, sorbitol, inulooligosaccharides and ultra-high-fructose syrup in pharmaceutical industries[8]. Inulin is degraded by inulinase, which cleaves glycoside bonds to form largely (95%) D-fructose by a single-step process[9]. Inulinase (2,1-b-D-fructano -hydrolases EC 3.2.1.7) hydrolyses the inulin in to inulooligosaccharides and pure fructose. The inulooligosaccharides have wide applications in various types of foods like confectionery, fruit preparations, milk desserts, yogurt and fresh cheese, baked goods, chocolate, ice cream, and sauces [10]. The fructose is being an excellent alternative for the production of fructose syrups[11]. This sugar is used by the food and beverage industries, besides sweeteners and shows several advantages in comparison to sucrose, being less cariogenic, highly soluble and hygroscopic and therefore, less prone to form crystals, has low calorie content and does not cause arterioscelerosis. Furthermore, fructose may be used by

diabetic patients and mask the bitter taste of saccharm[12]. The inulooligosacchrides acting as growth factors for improvements of the intestinal microbial flora, thus they are considered a prebiotic agents[9], relief of constipation, decrease of total cholesterol and lipid in the serum and promotion of animal growth[13].

Microorganisms are the best source of inulinases for commercial production. Microbial inulinases are an important class of industrial enzymes, which are usually inducible and extracellular[9].Earlier studies focused on inulinase production using various microorganisms such as yeasts, fungi and bacteria[9,11].Among the bacteria, *Bacillus* spp., *Pseudomonas* spp*.,* and *Streptomyces* sp. have been reported as high-yielding inulinase strains[14]. The bacteria *Acinetobacter baumannii* has been rarely used for this purpose. To our knowledge there is not any report about inulinase production by this bacteria. Therefore, the aim of this study was to investigate inulinase production by *Acinetobacter baumannii* besides to optimize medium conditions for increasing the activity of inulinase and detection of substrate specificity.

Materials and Methods

Samples collection

Forty five agricultural rhizosphere soil samples were collected from different locations by using pre-sterilized sample bottles and sterile spatula from Army channel in Baghdad city . One gram of each of the samples was suspended in 10 ml of sterile distilled water and shake vigorously for 10 min. later, 0.1 of the resulting liquid was spread on the surface of blood agar and MacConkeys agar, then incubated at 30℃ for 18-24 hour using L- shaped glass rod[15].

Bacteriological analysis

The bacterial isolates were observed for morphological characters and identified by using the tests guided by Berge's Manual of Systemic Bacteriology. Suspicious isolates of *Acinetobacter* spp. were identified by characteristic colonies (non Lactose-fermenting, glistening, small mucoid colonies), Gram staining pattern and standard biochemical reaction like catalase test(+),oxidase test(-), indole production(-), citrate utilization test(+), haemolysis test (-), histamine assimilation test (-), glucose oxidation test, gelatin liquefaction test (-), urease activity(-), unability to motile coccobacilli and ability to produce acid from glucose, xylose, galactose, rhamnoseand lactose[16,17].Further, the *Acinetobacter baumannii* isolate was confirmed by using API 20NE biochemical kit and the Vitek 2 system by using Vitek GNI card (bio Mérieux, France) according to the manufacturer's instructions.

Primary screening for inulinase activity:

All the bacterial isolates were inoculated in to the inuline agar plates containing 2g/L of inuline, 10g/L of yeast extract , 20 g/L of $MgSo_4.7H_2O$, 2g/L of KCl, 10% of NaCl, 20g/L of agar. Inulin was used as the sole source of carbon in this medium, thus, bacterial growth after 24 hour of incubation at 30℃ shows the presence of inulinase activity, then the diameters of clear zones around the colonies were measured(18).

Preparation of substrate and crude inulin extract from garlic, onion and leek

Garlic, onion and leek were obtained from local market and washed thoroughly with cold water, sliced and then dried at 100 °C. The dried slices were then milled to a fine powder with mill and used as substrate. The inulin extract was prepared with modification the method that described by[6] by suspending the powder in distilled water (3% w/v) then boiled for 10 min. After that it was filtered through muslin cloth, centrifuged at 8000 rpm for 10 min and used as a sole carbon source.

Preparation of substrate and crude inulin extract from artichoke and dahlia

Jerusalem artichoke's root tubers and dahlia tubercles were washed in running water, sliced, dried and ground into powder and used as substrates. The inulin extract was prepared as described by[19,20]by suspending of each powder in distilled water (3% w/v) then boiled for 10 min. After that it was filtered through muslin cloth, centrifuged at 8000 rpm for 10 min and used as a sole carbon source.

Preparation of substrate and crude inulin extract from chicory roots

Chicory roots was washed with cold water, grounded to a fine powder with mill and used as substrate ,then blended by a mixer with of 0.2 M potassium phosphate buffer(pH 7.0)(3% w/v) as described by[5].The whole blend of chicory extract was used as a sole carbon source throughout the study.

Studying of growth parameters for inulinase production

1- Effect of different carbon sources on inulinase production

Mixtures of basal medium ($(NH_4)_2SO_4$ 2.1 g, $MgSO_4.7H_2O$: 0.3 g, $CaCl_2.2H_2O$: 0.3 g, $FeSO_4.7H_2O$: 0.5 g, KH_2PO_4: 10.0 g) supplemented with 2% (w/v) yeast extract as nitrogen source were mixed with 25 ml of 3% garlic, onion, leek ,artichoke, chicory and dahlia extracts(pH7.0) besides to 5%pure inulin (chicory root, Sigma), sucrose, fructose, dextrose, lactose, mannose and glucose were used as carbon sources for inulinase production. The selected isolate was inoculated to these media and incubated at 30℃for 24 hour. The fermented broth was centrifuged at 3500 rpm for 10 minutes and the cell free supernatant obtained was collected and used as crude inulinase enzyme, then the inulinase activity was determined.

2-Effect of nitrogen sources on inulinase production

Effect of different nitrogen sources including peptone, treptone, beef extract, yeast extract, and urea (organic nitrogen sources) and ammonium chloride, sodium nitrate, potassium nitrate and ammonium sulfate a(inorganic nitrogen sources) was studied by incorporating 2% (w/v) of each nitrogen source in dahlia extract medium. The selected isolate was inoculated to these media and incubated at 30℃ for 24 hour, then the inulinase activity was determined.

3-Effect of different concentrations of dahlia extract on inulinase production.

Mixtures of basal medium with dahlia extract of different concentrations (1,2,3,4,5, and 6%) were prepared. The pH was adjusted at 7.0, inoculated with selected isolate and incubated at 30°Cfor 24 hour, then the inulinase activity was determined.

4- Effect of pH on inulinase production

Dahlia extract medium was prepared at different pH values (5-9).This medium was inoculated and incubated at 30ºC for 24hours. The inulinase activity was determined.

5-Effect of incubation temperature on inulinase production.

Dahlia extract medium was incubated with selected isolate at different temperatures (20,25,28,30, 35,37,40,45 ºC) for 24 hours. The inulinase activity was measured .

6-Effect of different incubation periods on inulinase production

Dahlia extract medium was inoculated with selected isolate and incubated at 28℃ for different periods (12,18, 24,26, ,30,36 and 48 hours). The inulinase activity was determined.

Substrate specificity

A study of substrate specificity for inulinase was made by using garlic, onion, leek, Jerusalem artichoke's root tubers, dahlia tubercles and chicory roots as powders. Inulinase assay was done by using these plant powders at concentration 2% instead of pure inulin.

Assay of inulinase

Endoinulinase activity was assayed by incubating 2 ml enzyme solution with 2% (w/v) inulin prepared in 10 mM citrate-phosphate buffer pH 7.0 at 35℃ for 60 min. After incubation, the reaction tubes were kept in a boiling water bath for 10 min. to stop the enzyme reaction and then cooled to room temperature. The reaction mixture was assayed for reducing sugar as fructose by DNS method as described by[18] by reading the absorbance at 575 nm. The calibration curve was prepared with fructose solutions of known concentration and blanks were run simultaneously with enzyme and substrate solutions. One unit of inulinase activity was defined as the amount of enzyme, which produced 1µ mole of fructose under the assay conditions.

Isolation of *Acinetobacter baumannii*

In an attempt to determine the distribution of *Acinetobacter* in nature, 45 agricultural rhizosphere soil samples were collected from an area approximately 150 miles long and 60 miles wide. Eight(18%) *Acinetobacter baumannii* isolates were obtained out of twelve(27%) *Acinetobacter* spp. Isolates. The nutritional properties of *Acinetobacter* and their ubiquitous occurrence in soil suggest that these organisms may be very important agents in the aerobic mineralization of organic matter in nature[21]. The large majority of *Acinetobacter* strains normally occurring in water and soil do not require growth factors, and can grow in a mineral medium containing acetate or some other organic sources of carbon and energy [22].

Results and Discussion

Screening for Inulinase-Producing Isolates

Among eight *Acinetobacter baumannii* isolates subjected to rapid screening for extracellular inulinase production by using inulin agar plates, five isolates were found to be positive for inulinase activity by growth on this medium. *Acinetobacter baumannii* Rs5 gave higher clear zone around the colony in comparison with other isolates(figure-1) so that this isolate was selected as the best inulinase producer.

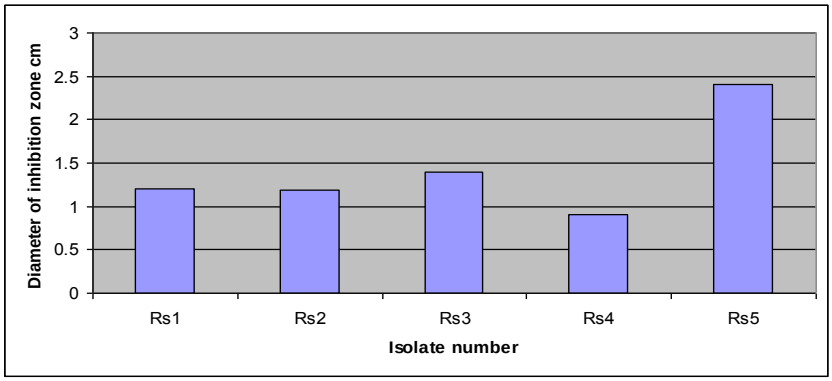

Figure-1:Diameters of inhibition zones of *Acinetobacter baumannii* isolates

Studying of growth parameters for inulinase production

1-Effect on carbon source on inulinase production

To determine the effect of carbon sources on inulinase production *by Acinetobacter baumannii* Rs5, different carbon sources were tested which include 3% garlic, onion, leek, artichoke, chicory and dahlia extracts besides to 5%pure inulin, sucrose, fructose, dextrose, lactose, mannose and glucose.. As shown in figure-2, inulin was the best carbon source for inulinase production yielding the maximum enzyme activities of about 1.23 U/ml with pure inulin and about 1.98 U/ml, 2.05U/ml and 2.56U/ml with the inulin chicory, garlic and dahlia extracts at 3%(w/v),respectively. Dahlia is an interesting alternative, since, it is a relatively cheap and easily available substrate, that can serve as a feedstock for large-scale fermentation, whereas pure inulin is only available in limited quantities and at very high cost.

The inulinase was most probably an inducible enzyme, with the physiological inducer being inulin, and that some repression of the biosynthesis might occur in the presence of fructose. In contrast, inulin hydrolysis produces fructose; thus, the bacterial strain might be able to regulate the inulinase level by a dual mechanism of substrate (inulin) induction and product (fructose) repression[23]. Mahmoud *et al*.[6]reported that among the different carbon sources

tested, sugarcane molasses and sugar beet molasses were found to support inulinase synthesis, whereas glucose, lactose, galactose, arabinose and soluble starch showed a repressive effect on inulinase production in *Aspergillus niger* also demonstrated that the enzyme activity by using extracted inulin as a carbon source was about 1.2-fold higher than activity obtained by using pure inulin as a carbon source. Although many plants such as garlic, onion and leek contain fructan of inulin type but there is distinct variable differences in inulinase activity. The possible differences in fructan synthesis in these species, probable number and concentration of polymers present in addition to their relative molecular weights, all these reasons may be responsible for the variable activities. *Bacillus* is also active producer of extracellular inulinase, 30-42 U/ml inulinase activity were obtained on sucrose as substrate[24].The maximum activity of endo-inulinase from *Pseudomonas* sp. was reached when 50g inulin/L as substrate was used[25]. Inulin was the best carbon source for inulinase production by *Streptomyces* sp., the maximum enzyme activity of about 0.55 U/ml [19].

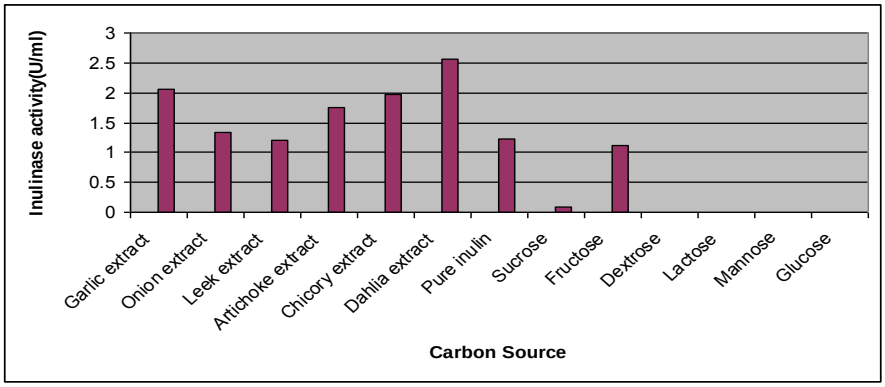

Figure-2:Effect of carbon source on inulinase production by *Acinetobacter baumannii* Rs5

2-Effect of nitrogen source on inulinase production

The supplementation of additional nitrogen sources (either organic or inorganic) such as peptone, treptone, beef extract, yeast extract, urea, ammonium chloride, sodium nitrate, potassium nitrate and ammonium sulfate to the production medium had show a profound impact on the production of inulinase by *Actinetobacter baumanii* Rs5(Figure-3). Among the various nitrogen sources tested , treptone in the dahlia extract medium promoted enhanced growth of microorganism and consequently the inulinase production with activity of 2.72U/ml. peptone was important for the cell mass formation and inulinase synthesis in high concentration in *Marinimicrobium* sp.[26]. In contrast, tryptone was found to be the best nitrogen source for inulinase production by *Streptomyces* sp. and the optimal concentration was obtained at 0.7% (w/v)[19]. The complex nitrogen sources have been reported to be better than inorganic nitrogen sources[14]. Yeast extract was found to be the best nitrogen source to be used in conjunction with dahlia extract for inulinase production followed by beef extract[20]. Ammonium salts usually cause acidic conditions, because acid is liberated in the medium after the utilization of ammonium ions and highly acidic conditions may inhibit the growth and synthesis of inulinase [14].

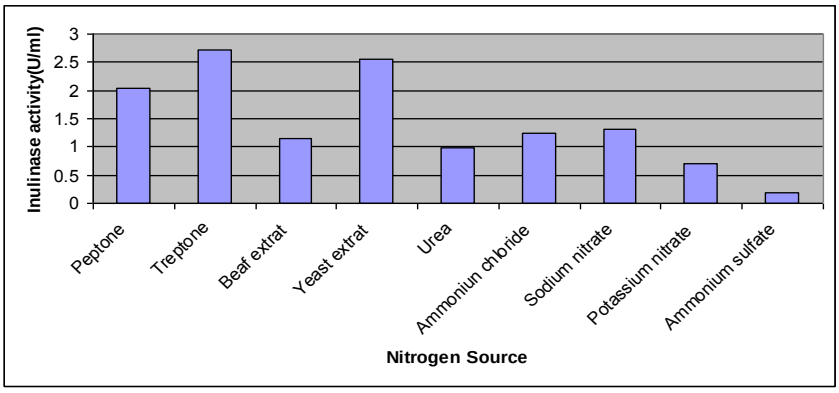

Figure-3:Effect of nitrogen source on inulinase production by *Acinetobacter baumannii* Rs5

3-Effect of different concentrations of dahlia extract on inulinase production

The best concentration of dahlia root extract in media for inulinase production was 4% with 3.11U/ml inulinase activity(figure-4). In this respect, other authors reported different optimum concentrations of composite plants

extracts:3% for Jerusalem artichoke and 5% for sunflower as carbon sources for inulinases production. These different concentrations varied according to the type of plants[5].

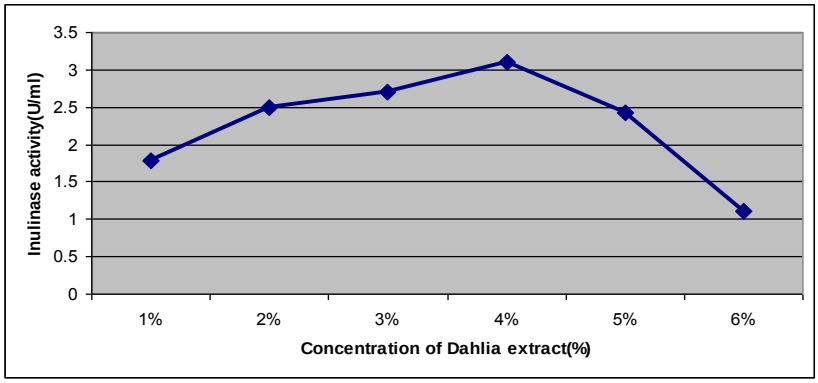

Figure-4:Effect of different concentrations of dahlia extract on inulinase production by *Acinetobacter baumannii* Rs5

4-Effect of initial pH on inulinase production

Experiments were executed to find out the optimum pH in order to maintain the favorable conditions for increasing inulinase production .The fermentation medium pH was adjusted accordingly with 1N HCl /NaOH from 5-9. The significance of pH on the production of inulinase was observed . The maximum inulinase production of 3.58U/ml was obtained at pH 7.5(Figure-5). This may be attributed to the balance of ionic strength of plasma membrane. The pH affects in enzyme production because of its role in the solubility of medium substrates and its effect on the ionization of the substrate and it's availability for the bacterial growth. Moreover ,the pH affects the productivity and enzyme stability[27].*The optimal pH for inulinase production by Marinimicrobium* sp. was7.5[26]. The inulinase from bacterial strains shows pH optima between 4.8 and 7.0[14]. The maximum inulinase production by *Erwinia* was obtained at pH 6[28].

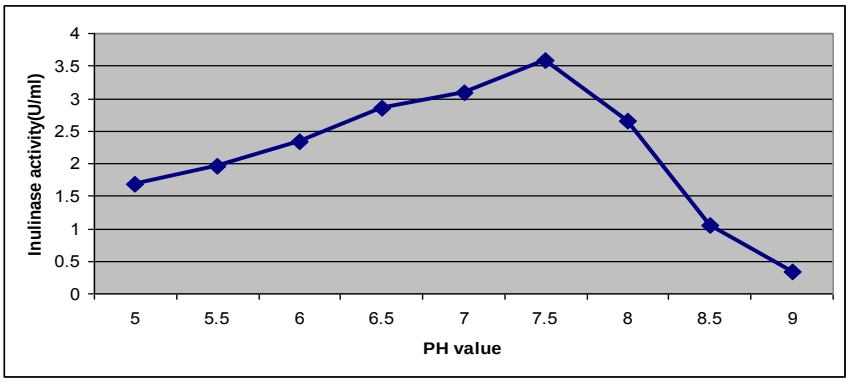

Figure-5:Effect of pH value on inulinase production by *Acinetobacter baumannii* Rs5

5-Effect of temperature on inulinase production

Incubation temperature has a profound effect on enzyme production . So the fermentation was carried out at different temperatures ranging from 20 to 45℃ by *Actinetobacter baumanii* Rs5 under submerged culture conditions . The maximum enzyme activity of 3.89U/ml was obtained at 28℃ (Figure-6) .The enzyme production reduced gradually with further increase in incubation temperature. This may be due to the denaturation of microbial strain at higher temperatures. Lower and higher temperatures decreases the specific activities because of the thermal effects of these temperatures on the microorganism growth and on the enzymatic reaction rate inside the cells which reflects on the vital creation of the enzyme[27]. Fungal and bacterial inulinases show temperature optima in the mesophilic and thermophilic range[14].The maximum inulinase production by *Erwinia* was obtained at 30℃[28].

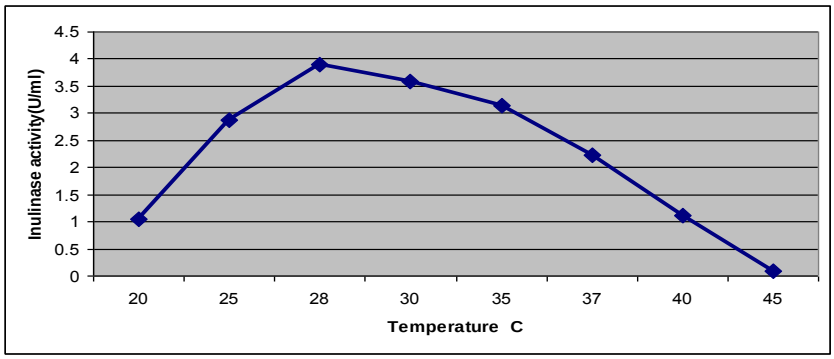

Figure-6:Effect of temperature on inulinase production by *Acinetobacter baumannii* Rs5

6-Effect of incubation period on inulinase production

The optimum incubation period for inulinase production was determined by conducting experiments with different time interval from 12 to 48hour. (Figure-7). It can be concluded that there were variations in enzyme level produced rise from an initial of 1.16 U/ml at 12 hour giving its peak activity of 3.97 U/ml at 22 hour of fermentation .Fermentation beyond 24hour showed a decrease in enzyme production, which could be either due to the inactivation of the enzyme because of the presence of some kind of proteolytic activity or the growth of the organism might have reached a stage from which it could no longer balance its steady growth with the availability of nutrient source. By using the optimized fermentation parameters, the enzymatic activity was at lowest values in the log phase and increasing in the exponential phase, at 22 hours it reached to the maximum values and in the early stationary phase (up to 30h) of the growth cycle the activity was stable and continued in decreasing at late stationary phase[29]. The enzyme production decreases after 24 hour of incubation is due to the production of reducing sugar such as glucose and fructose in culture medium which may lead to repression of inulinase production because these sugars are more readily carbon source than inulin. This decrease in enzyme production occurred as a result of the reduce in nutrients of the medium and as a result of accumulation the catabolic repression of enzyme[30]. Inulinase production by *Pseudomonas aeruginosa*, *Xanthomonas oryzae*, *Lactobacillus casei* and *Achromobacter* sp. was started from 8th hour and the maximum enzyme activity was seen during the late logarithmic phase at 22nd hour of fermentation [31].

Substrate specificity

The crude inulinase from *Actinetobacter baumanii* Rs5 showed maximum activity(4.32U/ml) with dahlia root powder(2% w/v), followed by garlic powder(table-1)but it decreased with all other substrates such as artichoke root tubers and chicory roots powders in addition to onion and leek powders. This indicating that dahlia root powder might be the most suitable substrate for maximum inulinase activity. It is interesting to note that although plants considered in this study all contain fructan but there is distinct variable differences in inulinase activity. The possible differences in fructan synthesis in these species, probable number and concentration of polymers present in addition to their relative molecular weights, all these reasons may be responsible for the variable activities[6].

Table(1):Effect of substrates on activity of inulinase from *Actinetobacter baumanii* Rs5

Substrate	Enzyme activity(IU/ml)
Artichoke	3.89
Dahlia	4.32
chicory	3.87
Garlic	4.09
Onion	3.26
Leek	3.15
Pure inulin	3.97

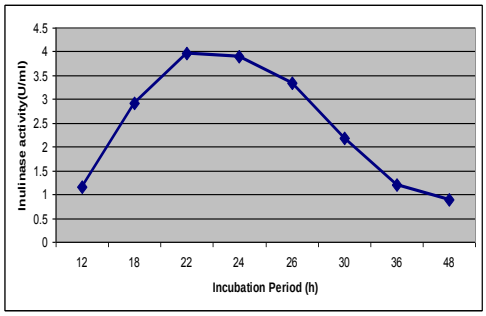

Figure-7:Effect of incubation period on inulinase production by *Acinetobacter baumannii* Rs5

REFERENCES

[1] Rawat, S.; Izhari A. and Khan, A.(2011).Bacterial Diversity in Wheat Rhizosphere and their Characterization. Advan. Appli. Scien. Res.2 (2): 351-356.

[2] Sahin, F., R. Cakmakci and F. Kantar.(2004). Sugar beet and barley yields in relation to inoculation with N2-fixing and phosphate solubilizing bacteria. *Plant and Soil*.265: 123-129. http://dx.doi.org/10.1007/s11104-005-0334-8

[3] 3-Patricia R.; Kathy A.; Michael C.; Linda G.; Marie K.and Stephen A.(2010).Guide to the Elimination of Multidrug-resistant *Acinetobacter baumannii* Transmission in Healthcare Settings. APIC.pp:1-54.

[4] 4-Espinal, P.; Martí, S.and Vila, J.(2012). Effect of biofilm formation on the survival of *Acinetobacter baumannii* on dry surfaces. J. Hospit. Inf. 80 (1):56–60.

[5] 5-Fawzi, E.M.(2011).Comparative Study Of Two Purified Inulinases From thermophile *Thielavia Terrestris* Nrrl 8126 and mesophile *Aspergillus Foetidus* Nrrl 337 Grown On *Cichorium Intybus* L. Brazilian J. Microbiol. 42: 633-649.

[6] 6-Mahmoud, D.A.R.; Mahdy, E.M.E.; Shousha, W.G.; Refaat, H.W. and Abdel-Fattah, A.F.(2011). Raw Garlic as a New Substrate for Inulinase Production in Comparison to Dry Garlic. Australian J. Basic Appli. Scien. 5(10): 453-462,

[7] 7-Yun, J.W.; Kim,D.H.; and Song, S.K.(1997). Production of inulo-oligosaccharides from inulin by immobilized endoinulinase from *Pseudomonas* sp. J.ferment. & bioengin. 84(4): 369-371.

[8] 8-Erdal,S.; Canli,O. and Algur, O.F.(2011). Inulinase production by *Geotrichum candidum* using Jerusalem artichoke as sole carbon source. Romanian Biotechnol. Lett.16(4):6378-6383.

[9] 9- Nascimento, D.; Jiunio,V.; Fernades,P.; Ribero, G.; Danyo, M.; and Sandra, A.(2012). Production, characterization and application of inulinase from fungal endophyte CCMB 328. Ann. Brazilian Acade. Scien.84(2):443-453.

[10] 10-Gao,L.; Chi, Z.; Sheng, J.; Wang, L.; Li ,J. and Gong, F.(2007). Inulinase producing Marine Yeasts: Evaluation of their Diversity and Inulin Hydrolysis by Their Crude Enzymes. Microbial Ecology. 54:722–729.

[11] 11-Dilipkumar, D.; Rajasimman, D.; and Rajamohan, N.(2011).Responce surface methodology for the optimization of inulinase production by K. *Marxianus var. marixianus*. J. Appl. Scien. Environ. Sanit. 6 (1): 85-95.

[12] 12-Nandagopal, S. and Kumari, B.D. (2006). Enhancment of inulinase production from Chicory and Fenugreek rhizosphere soil. American-Eurasian J. Agrric. Environ.Sci., 1(3):225-228.

[13] 13-Bharathi, S.; Saravanan, D.; Radhakrishnan, M.; Balagurunathan, R.(2011). Bioprospecting of Marine Yeast with special reference to Inulinase production. International J. Chem.Tech. Research. 3(3):1514-1519.

[14] 14-Singh, R.S. and Singh, R.P.(2010). Production of Fructo-oligosaccharides from Inulin by Endoinulinases and Their Prebiotic Potential. Food Technol. Biotechnol., *48* (4):435–450.

[15] 15- Kamble, K. D.; Bidwe, P. R.; Muley, V. Y.; Kamble, L. H.; Bhadange, D. G. and Musaddiq, M.(2012). Characterization of l-asparaginase producing bacteria from water, farm and saline soil. Bioscience Discovery, 3(1):116-119.

[16] 16-Constantiniu, S.; Romaniuc, A.; Smaranda, L.; Filimon,R. and Taraşi, I.(2004). cultural and biochemical characteristics of *Acinetobacter* spp. strains isolated from hospital units. the journal of preventive medicine. 12 (3-4): 35-42.

[17] 17- Holt, J.G.(1994). Bergey's manual of determinative bacteriology. 2nd ed. Vol.2, Williams and Wilkins. Baltimore.

[18] 18- Jenny, S.; Kavitha1, C.; Vidyadharani1, G. ; Priya, R. and Dhandapanil, R.(2012).Isolation of inulinase producing bacteria from sugarcane soil. Internat. J. appli. biol. pharmaceut. technol.3(4):320-326.

[19] 19-Laowklom, N.; Chantanaphan, R.; and Pinphanichakarn,P.(2012).Production, Purification and Characterization of Inulinase from a Newly Isolated *Streptomyces* sp. CP01. Natural Resources. 3:137-144.

[20] 20-Jain,S.; Jain, P. and Kango, N.(2012).Production of inulinase from *Kluyvermyces marxianus* using dahlia tubers extract. Brazilian J.of Microbiol.: 62-69.

[21] 21-Baumann, P.(1996). Isolation of *Acinetobacter* from Soil and Water. J. Bacteriol.96(1):39-42.

[22] 22-Warskow, A. and Juni, E.(1999).Nutritional Requirements of *Acinetobacter* Strains Isolated from Soil, Water, and Sewage. J. Bacteriol.112(2):1014-1016.

[23] 23-Allais, J.J.; Lopez, G.H.; Kammoun, S. and Baratti, J.C.(1987). Isolation and Characterization of Thermophilic Bacterial Strains with Inulinase Activity. Appl. Environ. Microbiol. 53(5):942-945.

[24] 24-Camelia, N.; and Gabriela, B.(2011). Inulinase-a veasatile tool for biotechnology. Innovative Romanian Food Biotechnol. 9(2):1-11.

[25] 25-Kim, D.; Choi, Y.; Song, K. and Yun, W.(1997). Production of inulo-oligosaccharides using endo-inulinase from a *Pseudomonas* sp. Biotechnol. Lett..19(4):369-371.

[26] 26-Li, A.X.; Guo, L.Z. and Lu, W.D.(2012).Alkaline inulinase production by a newly isolated bacterium *Marinimicrobium* sp. LS–A18 and inulin hydrolysis by the enzyme. World J. Microbiol. Biotechnol. 28(1):81-89.

[27] 27-Moat,A.G.; Foster, J.W. and Spector, M.P.(2002).Microbial Physiology. 4th ed. Wiley-Liss, Inc., New York. 1:1-28. http://dx.doi.org/10.1002/0471223867

[28] 28-Amith, A. and Jayachandran, K.(2007).Fermentative Production of the Extracellular Exo-acting Inulinase from a Novel Strain of *Erwinia* sp. Res. J. Biotechnol. 2(2).

[29] 29-Shah, A.J. ; Karadi , R.V. and Parekh, H.(2010). Isolation, optimization and production of l-asparaginase from coliform bacteria , Asian J. Biotechnol. 2(3):169-177.

[30] 30-Kheng, P. P. and Omar, I. O.(2004). Inulinase production by a local fungal isolate, *Aspergillus niger* USM AI 1 via solid state fermentation using palm kernel cake (PKC) as substrate. Songklanakarin J. Sci. Technol. 27(2): 326-336.

[31] 31-Angel;,S.J.;Kavitha,C.; Vidyadharani, G.; Roy,P. and Dhandapani,R.(2012).Isolation Of Inulinase Producing Bacteria From Sugarcane Soil Internat. J. Appli. Biol. Pharmaceut. Technol.3(4):320-326.