Extracellular Metabolites Produced by a Novel Strain *Bacillus alvei* NRC-14: 2. Molasses-Based Growth for High Production of *N*-acetylglucosamine

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Abstract: The pharmaceutically important compound *N*-acetylglucosamine (NAG) is used in various therapeutic formulations, dietary supplements, and in cosmetics. The aim of this study is to evaluate the agroindustrial waste sugarcane molasses as a carbon source for cell growth and production of NAG by the novel strain *B. alvei* NRC-14. Flaked α-chitin was used along with sugarcane molasses as carbon sources for production of NAG. The strain showed the greatest growth and production of NAG in presence of 6 g/L flaked α-chitin along with 4% of sugarcane molasses. Addition of sugarcane molasses to the growth medium actually allowed obvious increase in growth and NAG production by the strain than it did when flaked α-chitin was used alone. The results indicated that molasses does support the bacterial growth and the level of response being dependant on the concentration of molasses. The production of NAG with sugarcane molasses at 2 and 4% did not significantly differ. However, higher concentration of sugarcane molasses as well as the alkaline pH of growth medium decreased the yield of NAG. These results suggest the potential of sugarcane molasses for increasing growth and the effective bioconversion of flaked α- chitin into *N*-acetylglucosamine by the strain.

Key words: Extracellular metabolites, chitin, chinase, degradation, *N*-acetylglucosamine, sugarcane molasses.

INTRODUCTION

Sugarcane molasses, a by-product of sugar refining, is produced during the crystallization of sucrose from the cane juice, and is generally used as an animal feed or a biomass resource for fermentation of ethanol, lactic acid, phenolic compounds, xanthan, and mannitol (Takara et al. 2007; Murugesan *et al.* 2012; Ortiz et al. 2012; Umar *et al.* 2012; Zuko et al. 2012). Molasses contains several phenolic compounds derived from sugarcane (Takara et al. 2007), as well as a high concentration of sugar substances and minerals. Various compounds in sugarcane molasses play important roles in food functionalities and possess anti-oxidative and tyrosinase inhibitory activities (Takara et al. 2007).

Chitin, a homopolymer of ß -1,4 glycosidic bond of *N*-acetylglucosamine, is present in the shells of crustaceans, most fungal cell wall, insect exoskeleton, and parts of invertebrates. Since chitin was first isolated and characterized from mushrooms by French chemist Henri Braconnot in 1811, it has been discovered to be the second most abundant natural biopolymer in the world, amounting in marine biomass alone to approximately 106–107 tons (Park and Kim 2010). Isolated chitin has a highly ordered, crystalline structure and has been found in three polymorphic forms namely: α- , ß– and γ-chitin, which differ in the arrangement of the chains within the crystalline regions. The form α-chitin is the most abundant in shrimp and crab shells and is usually found where extreme hardness is required; it is tightly compacted due to its crystalline structure where the chains are in anti-parallel fashion favoring strong hydrogen bounding. ß–chitin is produced from squid pens and is commercially more expensive. Very few studies have been carried out on the rarer γ-chitin and it has been suggested that γ-chitin is a distorted version of either α- or ß–chitin rather than a true third polymorphic form (Rudall and Kenching (1973). The crystalline form of α-chitin (flaked or powdered chitin) was found to be hardly degraded by most of microorganisms rather than colloidal chitin, which could be easily degraded by most of microorganisms (Khadijeh *et al.* 2011). Chitin is processed in million kilograms quantities in Japan and its degradation products, either in the form of oligomers or monomer (NAG) have received increasing attention because of their broad applications in the fields of agriculture, medicine, food, pharmaceutical, and biotechnology (Arbia *et al.* 2012).

N-acetylglucosamine (NAG), the monomer constituent of chitin degradation, has been widely utilized as a nutritional supplement for therapeutic usage and for treatment of joint damage. Additionally, NGA is used in cosmetics, wound dressing, and as a food additive in milk. Many clinical trials have been performed, using NAG, to treat patients with osteoarthritis, rheumatoid arthritis, and cartilage damadge (Chen et al. 2010). The word "*Osteo*" means bone in Latin, and *arthritis* means joint damage, swelling, loss of movement, and further deterioration. *Osteoarthritis* is a disease that causes damages to the surface of the joints in the body and affects

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the bones. Joints are found in the places of our body where bones meet, and most joints are designed to limit bone movement. A person can, for instance, only bend the arm in one direction, and never move it sideways (Jared 2011). Bone ends are covered by a protective layer of cartilage, that is, the rubbery material which cushions joint. The aminosugar "*D*-glucosamine" is necessary for the formation, maintenance, and repair of cartilage because in osteoarthritis, this cartilage becomes stiff and loses its elasticity (Wong 2007). Recently, NAG is widely used for treatment of osteoarthritis and become more preferable product as a food additive due to its sweet taste and stability (Deng *et al.* 2005; Miro *et al.* 2010). Preparations containing NAG are delivered by parenteral, oral, transmucosal, and topical administration. Results from these deliveries indicate that NAG significantly prevents joint damage (Talent and Garcy 1996; Marcum and Seanor 2007). A large dosage of NAG (20 g) given intravenously to human volunteers results in neither toxicity nor alteration of blood glucose concentrations (Liu *et al.* 2008).

Chemical hydrolysis of chitin to obtain NAG has some defects such as harsh chemical hydrolysis and chemical changes of glucose ring. In addition, the chemically produced NAG has, usually, a bitter taste due to the presence of trace amounts of *O*-acetylated and *di*-acetylated residual substances (Sashiwa *et al.* 2002). However, to avoid the use of such chemical processes, attention is focused on production of NGA using microbial enzymes. Bioconversion of crystalline α-chitin to produce NGA is rarely reported because flaked or crystalline α-chitin could be hardly degraded by most of microorganisms. *B. alvei* NRC-14, a bacterial strain isolated from soil, was proved to be a potent producer of carbohydrate-active enzymes. The present study describes the effective production of NAG by strain *B. alvei* NRC-14 using flaked α-chitin along with sugarcane molasses as carbon sources. To the best of our knowledge, this is the first report deal with the production of NAG using sugarcane molasses by a newly isolated *B. alvei* NRC-14.

MATERIALS AND METHODS

Microorganism and Cultivation:

The bacterial strain, used in this study, was isolated from the Egyptian soil as a potent chitosanase producer using soluble chitosan as both carbon and nitrogen source. This novel strain, identified *B. alvei NRC*-14, was found to produce a variety of extracellular metabolites using minimal growth medium. In the present study, firstly, production of NAG was performed using a minimal growth medium containing (g L-1): 10, flaked α-chitin; 1.5, $(NH_4)_2SO_4$; and 0.5 $MgSO_4.7H_2O$ with a pH adjusted to 6.5. Conical flasks, containing 50ml medium, were inoculated with the strain (4%, v/v, of a 24-hrs pre-culture) in shaken flasks (130 rpm) at 28oC and for 5 days. At intervals, the culture broth was monitored for detection of cell density, pH, reducing sugars, and NAG production. After incubation period, the culture broth was centrifuged at 7000 x g for 20 min at 4oC and the resultant supernatant was tested for determining the enzymes activities as well as estimation of NAG using standard curve with *N*-acetylglucosamine as a standard.

Enzyme Assay:

Chitinase and chitosanase activities were determined using colloidal chitin and soluble chitosan as substrates, respectively. Standard assay conditions, detection of reducing sugars, and the buffers used for detection of enzymes activity were performed as described previously (Abdelaziz *et al.* 2012). For detection of *N*-acetyl-D-glucosaminidase activity, the reaction mixture contained 100 μL *p*-nitrophenyl-*N*-acetyl-*D*-glucosaminide (5mM), 100 μL culture supernatant, and 200 μl of 0.2M citrate-phosphate buffer (pH 5.5). After incubation of the reaction mixture at 37oC for 15 min, 1ml glycine-NaOH (0.2 M, pH 10.6) was added to stop the reaction (Khadijeh 2011). The amount of *p*-nitrophenyl released was determined by measuring the absorbance at 405 nm. One unit of *N*-acetyl-*D*-glucosaminidase activity was defined as the amount of enzyme necessary to liberate 1 μmol *p*-nitrophenyl per minute under standard assay conditions.

Production of N-acetylglucosamine from α-chitin:

After incubation of the strain for 5 days, the culture broth was centrifuged to remove solid residue. The white supernatant obtained after centrifugation was precipitated by absolute ethanol at room temperature (Phakapob *et al*. 2008). White solids obtained were collected and dried to produce the final product, NAG.

Effect of N-acetylglucosamine on Cell Growth:

Effect of NAG as an end product on cell growth of strain NRC-14 was determined. Cells were inoculated into a medium supplemented with molasses (4%) and NAG at different concentrations: 10, 20 and 40 g/L. Cell density (OD600) was determined in samples taken at intervals (Deng *et al*. 2005). Cells were inoculated into the media and cell growth in these cultures was compared to cultures free of NAG (control).

Analyticals:

The IR-spectra of the produced NAG, chemical composition and mineral content of sugarcane molasses, and atomic absorption were detected by the "Central Services Laboratory", National Research Center. The spectrum of the sample was recorded on the spectrophotometer over a wave number range 4000-400 cm-1 using a FT-IR- Raman (Nexus 670, Nicolet-Madison-WIUSA).

Materials:

Sugarcane molasses was a gift from the Chemistry of Natural and Microbial Products Lab. Commercial crab chitin and *N*-acetyl glucosamine were purchased from Sigma Chemical Company. Chromogenic *p*-nitrophenyl-*N*-acetyl-D-glucosaminide was procured from the Microbial Chemistry Lab. All other chemicals were purchased from Sigma Chemical Co., and were of analytical grade. Colloidal chitin was prepared according to the method of Monreal and Reese (1969).

RESULTS AND DISCUSSION

Degradation of α-chitin:

Strain NRC-14 was grown in minimal broth medium using flaked α-chitin. Cell growth, pH, and reducing sugars were measured continuously for 5 days. Cell growth increased after 24 hrs of growth, and slightly decreased after 96 hrs (Fig.1A). The pH value of the culture broth decreased gradually to pH 4.0 (Fig. 1B). Activity of the crude enzyme in culture broth reached, approximately, 4.0 U/ml, after 96 hrs of growth (Fig.1C). These results implied that, *B.alvei* NRC-14 propagated rapidly and that some acidic metabolites were released and accumulated in the culture broth, leading to reduction in the pH value. After 96 hrs growth, the cells decreased due to the acidic conditions of culture broth and entering to the decline phase (Fig. 1A). The concentration of reducing sugars in the culture broth was low within the first 18 hrs, and increased over the tested period, 96 hrs (Fig.1C). The final product, NAG, increased during continuous bacterial growth up to 96 hrs. These results may suggest that, degradation of flaked α-chitin by the effective crude enzymes secreted by the strain may produce some metabolic products that are more accessible as carbon source rather than NAG by which the product did not consumed. Another assumption is that, the amount of reducing sugars formed by enzymatic hydrolysis might be higher than that consumed for metabolic reactions by the strain; the amount of reducing sugar slightly decreased after 96 hrs of growth. The culture supernatant was white after centrifugation with a minor amount of the precipitate, suggesting the potentiality of strain NRC-14 in α-chitin degradation to completion.
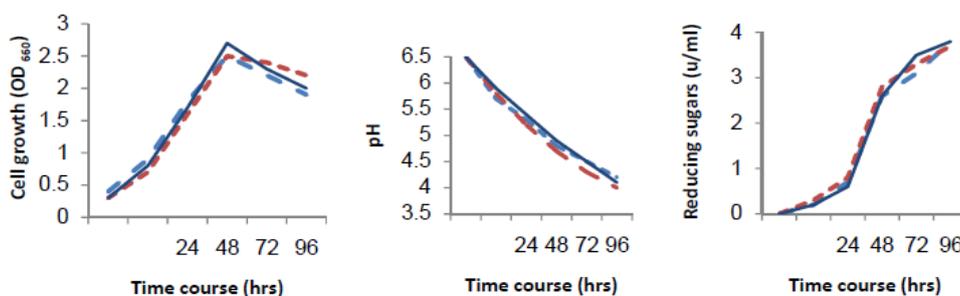

Fig. 1: Parameters of α-chitin degradation, each factor was evaluated more than once (triplicate): cell growth (Fig.1 A), initial pH (F.g.1 B), and reducing sugars released (Fig.1C).

Sugarcane Molasses-Based Medium:

Sugarcane molasses has widely been used for production of ethanol, lactic acid, bacteriocins, phenolic compounds, and polysaccharides such as xanthan. Molasses is a great source of carbohydrates that stimulates the growth of beneficial microorganisms and is a great source of metal ions (Table 1). Cane molasses contains mainly three sugars (glucose, sucrose, and fructose) and may serve as a favorite carbon source for the culture of *B.alvei* NRC-14. In addition to sugars and minerals, molasses contains significant amounts of sulfur, fats, free and bound amino acids, and a variety of micronutrients (Nofziger 1995). A balanced supply of mineral nutrients is essential for beneficial microbes to survive and thrive, especially for the soil microbes, including bacteria that depend on tiny amounts of trace minerals to act as catalysts for enzymes. In the present study, sugarcane molasses was exploited (without any pretreatment) for high production of NAG by the strain *B.alvei* NRC-14. Maximum values for cell growth were obtained when the strain was grown with flaked chitin (6 g/L) along with 4% sugarcane molasses (Fig. 2 and 3). Optimum yield of NAG was obtained when cultures of the strain were incubated at 30oC and a pH value of 5-6 (Fig. 4). Liu *et al* (2012) have reported about the production of lipids

by the algae, *Chlorella protothecoides*. They found that, cane molasses must be pretreated by invertase to release reducing sugars from sucrose so that the organism can utilize it. In our study, no pre-treatment was required for strain NRC-14 because it can utilize sucrose directly for growth (Abdel-Aziz 1999).

Table 1: Chemical composition and mineral content of sugarcane-molasses. Correct NO.

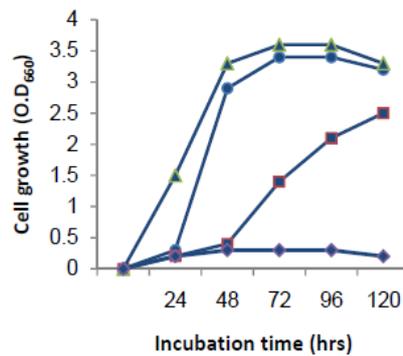| Parameter	Concentration
Chemical composition:	(g/100g)
Moisture	16.45
Total acidity	50.30
Reducing sugars	15.00
Non-reducing sugars (sucrose)	33.80
Ash	9.30
N2	0.47
Minerals and Trace elements:	(mg/100g)
Mn^{2+}	13.10
Fe^{2+}	27.80
Zn^{2+}	13.70
Cu^{2+}	18.40
Ca^{2+}	158.00
Mg^{2+}	106.90
Na^+	787.80
K^+	979.00

Fig. 2: Cell growth as a function of time following growth of *B. alvei* NRC-14 in 6 g/L of flaked chitin along with different concentrations: (●) 2; (▲) 4; (■) 6; or (♦) 8% of sugarcane molasses.
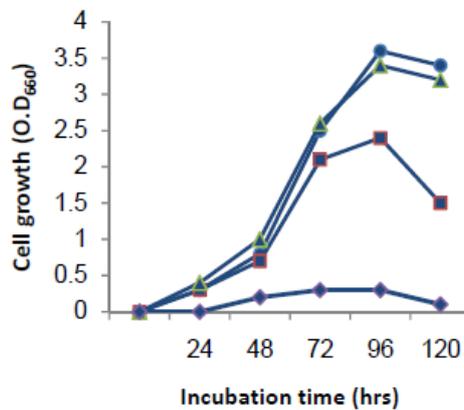

Fig. 3: Cell growth as a function of time following growth of *B. alvei* NRC-14 in 4% of sugarcane molasses along with different concentrations: (●) 6; (▲) 8; (■) 10; or (♦) 12g/L of flaked chitin.
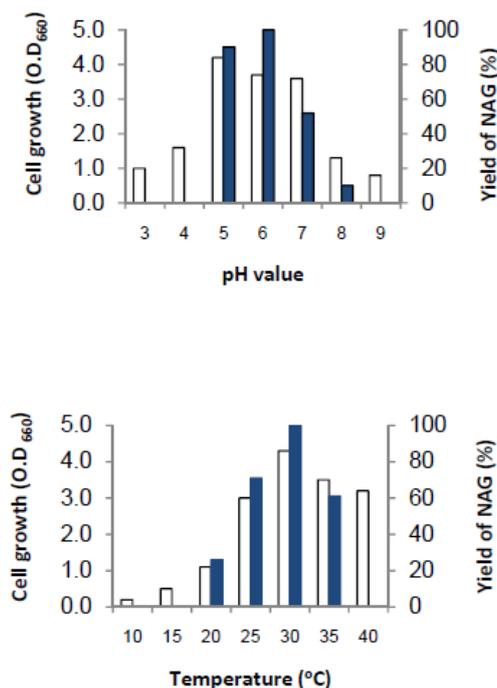
Fig. 4: Yield of *N*-acetylglucosamine produced by *B. alvei* NRC-14 during growth at different pH values and temperatures. The growth medium contained flaked chitin (6 g/L) and sugarcane molasses (4%).

Effect Of Molasses Concentrations:

Previously, *B. alvei* was found to be able to efficiently and constitutively utilize any carbon source for cell growth (Abdel-Aziz 1999). Molasses is rich in minerals, heavy elements, vitamins, and amino acids. Sugarcane molasses is a preferred low-cost carbon source. Presence of sucrose and other reducing sugars in molasses obviously initiated the growth of strain NRC-14 as accessible simple substrate that enhanced growth, increased enzymes secretion and effectively degraded chitin. Initiation of the lag growth phase, in presence of molasses, was found to be most effective for NAG production. Cells were grown with molasses to an O.D660 of about 3.7 (Fig. 2 and 3) at a 4% concentration compared with O.D660 of 2.5 with flaked chitin alone as carbon source (Fig. 1A). Growth of strain NRC-14 in a high concentration (6%) reveal that the strain can tolerate high levels of heavy ions such as Cu2+, Mn2+, Fe2+, and Zn2+ as well as high levels of Na+ and K+ (Table 1). However, the cell growth obviously decreased at high (8%) concentration of molasses (Fig. 2). On other hand, cell growth decreased at high concentrations of flaked chitin (12 g/L) may be due the thickness of the media at this concentration (Mongkol *et al.* 2002).

Effect of Medium pH and Temperature:

Effect of incubation temperature and pH value of medium on cell growth and production of NAG was investigated. As shown in Fig. 4, maximum yield of NAG was obtained at pH value of 6.0 at 30oC. The pH of fresh cane molasses is 4.9-5.4. The maintenance of a favorable pH is very essential for the production of NAG. Effect of different pH values (3-9) on NAG production was studied and maximum yield was obtained when initial pH of the growth medium was kept at 6.0 (Fig. 4). Lower or higher initial pH caused reduction in NAG yield which might be due to the formation and accumulation of toxic metal ions affected bacterial growth.

When cultures of strain NRC-14 were grown at 40oC, low yield of NAG was obtained, while the culture broth exhibited inhibitory effect upon some pathogens (Fig. 5). The same behavior was observed with the strain when the growth temperature was shifted from 30 to 40oC, where the culture broth exhibited also inhibitory effects when flaked chitin was used as a carbon source (Abdel-Aziz et al. 2012). From our studies, it was clearly demonstrated that switching the incubation temperature to 40oC could be an effective method for production of bioactive compound (Abdel-Aziz et al. 2012). In the present study, when the temperature degree was elevated to inhibitory temperature during growth, low levels of enzymes were detected (accompanied with low yield of NAG) while formation of bioactive compound was observed (Fig. 5)

Effect of end-Product Inhibition:

Inhibition of cell growth by end-product (NAG) was also studied. Strain NRC-14 was strongly resistant to high levels of NAG. Inhibitory effects of NAG on strain NRC-14 cells were demonstrated by growing cells in a

medium containing different levels of NAG (Fig. 5). Growth was slightly inhibited by NAG at 40 g/ L. However, NAG at 60 g/L completely prevented cell growth (Fig. 5). Inhibitory effects of NAG may be occurred by NAG or by its degradation products. Similar results were reported by Deng et al. (2005) for production of NAG by *E. coli* 7107-18.

Fig. 5: Inhibitory effect of the bioactive compound produced by B. alvei NRC-14, during growth at 40oC, against: 1) *E.coli*; 2) *Staphylococcus aureus*; 3) *Aspergillus flavus*. To a prepared cell suspension of the strains, 0.2 ml of the bioactive compound was added and incubated at room temperature for 2 hrs.
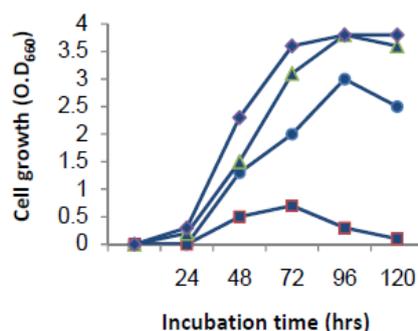

Fig. 6: Inhibition of cell growth of *B.alvei* NRC-14 by high levels of NAG in the medium: ♦) 10; 20 (▲); 40 (●) and 60 (■) g/L of NAG.

Enzymes Production:

The enzymes released during the incubation period of strain NRC-14 were estimated (Fig. 7). Chitosanase and ß-1,4 glucanase were firstly detected in first 24 hrs of growth, followed by chitinase activity (Maximum at 72hrs) whereas *N*-acetyl-*D*-glucosaminidase activity began after 72 hrs of growth and increased up to 120 hrs of growth when the initial pH of culture broth decreased, accompanied with increased yield of NAG. Microorganisms capable of enzymatic degradation of chitin primarily use their chitinolytic and/or chitosanolytic machinery to saccharify and consume the substrate as a nutritional source of carbon and nitrogen. Solubilization of chitin is essential as the first step. Hence, a microorganism produces a number of enzymes that convert chitin to soluble products for further degradation through different metabolic pathways. Enzymatic degradation of chitin can occur via different pathways: 1) the major chitinolytic pathway involves the hydrolysis of ß-1,4 glycosidic bonds of chitin by the endo-chitinases (EC 3.2.1.14); 2). Further hydrolysis of the ß-1,4- glycosidic bond of chitin is carried out by exo-chitinases (EC 3.2.1.30) and *N*- acetyl-*D*-glucosaminidase (EC 3.2.1.52) to yield NAG (Stine 2011); 3) an alternative pathway for chitin degradation involves deacetylation of chitin to form chitosan by a chitin-deacetylase (EC 3.5.1.41). Thus, in the present study, it could be suggested that, chitin may be degraded by endo-chitinase to yield chitooligosaccharides (COS) followed by exo-chitinase and NAGase activities to yield the monomer, NAG. Detection of such enzymes in the culture broth (Fig. 7), may confirm this suggestion.

The tightly packed chitin strands of α-chitin are known to have low susceptibility to enzymatic degradation. For solubilization of chitin, endo-chitinases cleave chitin randomly at internal sites on the glycosidic bonds between NAG residues, and such cleavage generates low molecular weight chitooligosaccharides (e.g. tetramers, trimers, and dimers). Then, the exo-chitinase and NAGase, in some microorganisms, catalyze the progressive release of dimers. Finally, NAGases cleave the oligomers and dimers which are produced by endo-

and exo-chitinases to generate NAG (Miro *et al.* 2010). The higher activity of NAGase, usually results in higher purity of NAG (Sashiwa *et al.* 2002). In the present study, the mechanism of α-chitin degradation included both endochitinase and *N*-acetylglucosaminidase activities, which were detected in the culture supernatant of strain NRC-14. The flaked α-chitin was completely degraded, so that, the culture supernatant seemed white. These results implied that endo-chitinase and *N*acetylglucosaminidase activities play crucial roles for chitin degradation to completion.
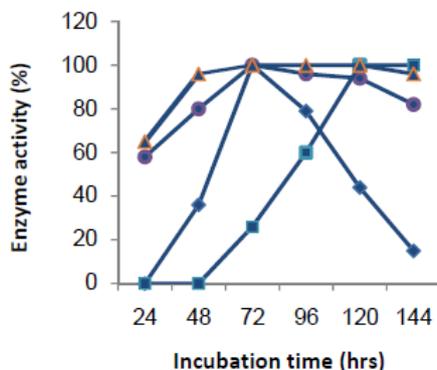

Fig. 7: Enzymes detected in the growth medium of B. alvei NRC-14, using flaked chitin (6 g/L) along with sugarcane molasses (4%▲) (chitosanase; (●) glucanase; (♦) chitinase; and (■) N - acetylglucosaminidase during the incubation period. Maximum values of enzymes activity were set as 100%.
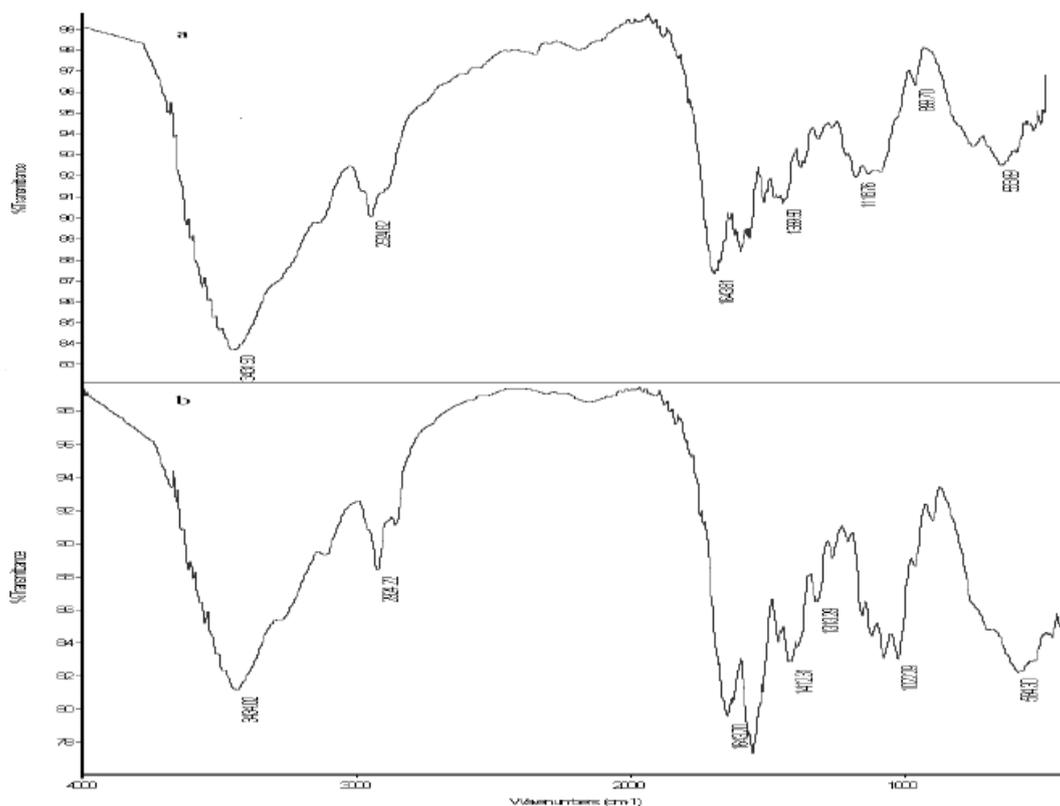

Fig. 8: IR-spectra for standard chitin (a) and the produced N-acetylglucosamine (b) from α-chitin degradation.

IR-spectra:

The infrared spectra (IR) for standard chitin and the produced NAG from chitin degradation are represented in Fig. 5. The spectra showed a hydroxyl band around 3450 cm-1 as characteristic band for chitin and its derivative NAG. Observed bands at 1643 cm-1 assigned to the amide group, indicating the presence of carboxyl groups (C=O). The absorption peaks observed at 1000-1100 cm-1 are generally known to be typical characteristics of aminosugar derivatives (Laura *et al.* 2006).

Glucosamine is a compound found naturally in the body, made from glucose and the amino acid glutamine. It is available as a nutritional supplement, often combined with chondroitin sulfate (a molecule naturally present in cartilage). Recently, NAG is widely used and become more preferable product as a food additive and for treatment of osteoarthritis due to its sweet taste (Miro *et al.* 2010). NAG was found to be an ideal alternative product since it is stable in solution at neutral pH (Deng *et al*. 2005). Over the years, numerous researchers have demonstrated that NGA is a more efficient, highly absorbed and utilized in the intestine (Wong 2007, Jared 2011). Moreover, no side effects for NAG have been reported, except for those persons with fish allergies because this product supplement (NAG) is produced from shellfish. The aminosugar, NAG, might also increase blood sugar levels in patients with diabetes. Benefits and applications of *N*-acetylglucosamine (Table 2) are well documented (Bissett *et al.* 2007; Wong *et al*. 2007; Chen *et al.* 2010). It has gained a lot of benefits which are supported by numerous clinical studies on thousands of patients about the absorption, distribution and elimination of orally administered NAG. Results of these studies have shown an absorption rate of as high as 98% and that once NAG absorbed, it is then distributed primarily to joint tissues where it is incorporated into the connective tissue matrix of cartilage, ligaments, and tendons (Chen *et al.* 2010, Wong 2011).

Table 2: Applications and benefits of N-acetyglucosamine.

Function	Object	Trial	Reference
Disease treatment	Cancer and metastasis	Animal, human	Xu *et al.* 2006
	Intestinal disease	Human	Xu *et al.* 2007
	Bowel disease	Human	Kanazawa & Fukudo 2006
	Joint damage	Human	Talent & Gracy 1996 Marcum & Seanor 2007
Disease prevention	Toxicosis of drugs and chemicals	Cell, animal	Xu *et al.* 2008
	Antibiotics	Bacteria	Xu *et al.* 2006
	Side effects of radio-therapy and chemo-therapy	Human	Xu *et al*. 2006
	Motion sickness	Human	Xu *et al.* 2005
Dermatology	Skin sanitary article preparation	Human	Xu *et al.* 1997
Food supplement	Additive in milk	Animal, human	Xu *et al.* 2004
	Additive in sports drinks	Animal, human	Wong 2007
Cosmetics	Maintaining skin moisture	Human	Wertz & van der Bergh, 1998
	Improve skin wrinkle and color	Human	Sayo *et al.* 2004
	Reduction of melanin production	Human	Bissett *et al.* 2007

Beneficial microorganisms are well known to contribute to the welfare of humanity**.** These microorganisms are widely used in food industry for production of a large number of fermented food products. Such microorganisms can also convert some food industrial wastes into value added products (Umar *et al.,* 2012). The ability of microorganisms to convert large and complex molecules into the simplest one depends upon the type of culture and the growth requirements. The selection of a suitable culture to convert a specific type of substrate into useful product, thus, plays a vital role in fermentation technology (Umar *et al.,* 2012). For the production of valuable products, cheap and low-cost substrates are being used to reduce the production costs. In the present study, we have evaluated the nutrient supplementation with sugarcane molasses and it was found that cane molasses could be used for enhancing efficacy of the growth and yield of NAG. Molasses contains essential nutrients for microorganisms as well as metal ions and amino acids. Reyed (2006) has reported that addition of molasses (5%) to skim milk (12%) supported and enhanced the growth of bifidobacteria and lactobacillus more than other dietary carbohydrates and that acetic acid and lactic acid production were enhanced when bifidobacteria were grown in the presence of molasses.

In fact, the novel strain *B. alvei* NRC-14 secretes a battery of extracellular metabolites: i) enzymes; ii) bioactive substances; iii) exopolysaccharide. In addition, the crude enzymes produced by the strain can degrade aminosugars (such as chitin and chitosan) to obtain NAG and chitooligosaccharides. When exposed to an abiotic stress (high degree of temperature), *B. alvei* NRC-14 secreted an inhibitor substance that showed antimicrobial

effects (Abdel-Aziz *et al*. 2012). When thrive in an extreme environment, microorganisms develop certain adaptation mechanisms which may be useful for their defense, and the resultant products, e.i. secondary metabolites, of these adaptations may be useful for human beings in many forms such as antibiotics (Ira and Kim 2010). Microorganisms, including certain bacteria, fungi and algae, produce secondary metabolites which have some degrees of bioactivity, either against another microorganism or acting against certain physiological states of a diseased body. These metabolites, otherwise known as bioactive compounds, may be inhibitor substances, antibiotics as well as enzymes, or even exopolysaccharides. Recently, it was reported that some polysaccharides are characterized to exert broad-spectrum biofilm inhibition activity (Peng et al. 2011). Nowadays, several arguments support the hypothesis that secondary metabolites improve the survival of the producer in competition with other living species. These arguments are as follows: 1) secondary metabolites act as an alternative defence mechanism; 2) they have sophisticated structures, mechanisms of action, and complex and energetically expensive pathways; 3) secondary metabolites act in the competition between microorganisms, plant and animals; and 4) the production of secondary metabolites with antibiotic activities is temporarily related with sporulation when the cells are particularly sensitivity to competitors and requiring special protection when a nutrient runs out(Demain and Fang 2000). Furthermore, the wide diversity of secondary metabolites suggests a broad range of functions. Nevertheless, these functions could depend on the conditions, optimal or not, surrounding the producer microorganism. Finally, due to their crucial importance the study and exploitation of secondary metabolites continue to progress despite the lack of agreement regarding why microbes produce such chemical diversity of antimicrobial compounds.

Conclusion:

The utilization of cane molasses by *B. alvei* NRC-14 for growth and production of NAG was investigated. Without pretreatment, molasses could support the strain for high production of NAG. The parameters such as carbon source, pH, temperature, and incubation time influenced the production of NAG. This study highlights the possibility of using *B. alvei* NRC-14 to deal with agro-industrial wastes to produce NAG. The strain has proved to be a valuable enzyme source for selective production of NAG which has generated interest as a new functional material with high potential uses in various pharmaceutical and medical fields. In fact, this strain is particularly interesting because of: 1) the large quantities and variety of extracellular metabolites it produces; 2) the possibility of controlling its growth conditions using a variety of substrates for production of enzymes, antimicrobial agents, bioflocculant has unique properties, or even aminosugar (NAG) that have great importance in medical and pharmaceutical fields. This bacterial strain has adopted special metabolic pathways to survive in extreme conditions and so has better capacity to produce special bioactive compounds.

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