The Effect of Some Sugars on the Growth of *Aspergillus niger*

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Received (Geliş Tarihi)**: 23.10.2014** **Accepted (**Kabul Tarihi)**: 23.12.2014**

Abstract: The majority of black Aspergilli, including *Aspergillus niger*. Here, we provide to evidence that the exploration of sugar uptake in the filamentous fungus *A. niger* as a sole carbon source. The goal of this research line is determination of the growth of fungi was evaluated every 24h, measuring the colony diameter (cm). *A. niger* was inoculated onto two culture media: PDA: for maintains a strain as pure while Czapeck Dox Agar was used in investigation into their carbon requirement, using five different carbon sources (vizs. glucose, fructose, sucrose, maltose, and starch). The fungus was tested grew sparsely on the basal medium lacking in carbon, which was the control. However this fungus was found to vary from their ability to use the supplied sources of carbon. Fructose and sucrose were found to be suitable sources of carbon for a fungal isolates, whereas glucose and maltose proved good carbon source to have a higher affinity. Starch as a polysaccharide, was a poor source of carbon for the growth of this isolate. Despite earlier claims, saccharides rather than monosaccharide were breakdowns extracellularly by means of a broad range of extracellular enzyme activities from *Aspergillus niger*.
Key words: *Aspergillus niger*, Saccharides utilization, fungal growth

INTRODUCTION

Fungi are significant environmental microorganisms especially in the ecosystem of the nature where they are responsible for spoilage, production of mycotoxins and in some cases desirable bioconversions. Consequently, it is important to know their requirements for nutrients and carbon-skeleton nutrient, water, temperature, oxygen and other factors for nutrient for fungi and use to energy production, carbohydrates are presented in fungi primarily as saccharide (Hashimoto *et al.*, 2005). Knowledge of structures and properties of sugar are classified as follows: (i) Monosaccharaide: it is available forms of absorption; it cannot be hydrolyzed into simple once, (ii) Disaccharide: it is hydrolyzed into two molecules of monosaccharaides forms, (iii) Oligosaccharide: the number of carbon atoms in their structure is 2–10 molecules, and (iv) Polysaccharide: it yields more than 10 molecules of monosaccharaides which may be linear or branched (Mcnaught, 1996).

Filamentous fungi represent a physiologically diverse group of micro-organisms. Their growth-form can be long, thin, branched threads of mycelium, but also compact mycelial pellets (Schrickx *et al.*, 1993).

In nature, filamentous fungi are able to utilize a great variety of carbon sources of secreting a wide range of different enzymes in large amounts of their environment (Fang *et al.*, 1998).

Aspergillus niger {it is a filamentous fungus growing aerobically composed of black-colonies (Frisvad *et al.*, 2002)} is a mold known for its ability to produce different kinds of enzymes and thus to degrade a big diversity of organic compounds. This characteristic allows the growth of the species in different media and environments (Schuster *et al.*, 2002).

On the other hand, *Aspergillus niger* is equipped for utilizing sugar as the sole source of carbon and energy for cell growth and metabolism. Growth and allocation of *A. niger* response to saccharides is well established and generally include increasing entire colonies, biomass, and reducing level of carbohydrates in the surrounding environment (Gupta and Neha, 2012).

In the present study, we describe the influence of various refined carbohydrate sources such as glucose, fructose, maltose, sucrose, and starch on growth and activity by *A. niger* was studied under colony diameter measurement technique.

MATERIALS and METHODS

Fungus Sample: In this study used *A. niger* and suitable to select; because it was a widely distributed over nature, easily growth on anywhere area, and produce considerable amount of circular colony. Also, the techniques of isolation, cultivation, and identification were simpler. This fungus was pre-served in Biology Department, College of Science, Salahaddin University, Erbil, Iraq.

Culture Media: Fungus selected depend upon the medium or substrate for synthesis their cellular constituents and obtain necessary energy for their life; the media which was used for the present work purposes includes: Potato Dextrose Agar (this medium was prepared according to the formula developed by Bilgrami and Verma, 1988), PDA is natural medium; and Czapek Dox Agar (this medium was prepared according to the formula developed by Thom and Church, 1926), CDA is synthetic medium and it had a defined chemical composition so that it was recommended for isolation of some fungi with similar physiological requirements (Eaton *et al.*, 1998). The

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medium was sterilized by autoclaving at 121ºC for 15 minutes. Fungi were plated on the agar medium and incubated at 30ºC for 5 days and pH 5.5 (Khokhar *et al.*, 2011). Although, fungal isolates could be kept and transferred to another agar plate in order to replenish their nutrient source (Henderson, 1961).

Growth Experiments: Puncture Test Method (it was used to determine the puncture, rupture, and propagation characteristics of a fungus) was used for inoculation. The organisms were growing a circle on modified Czapek Dox Agar after obtained fungus from pure culture of PDA and transferred onto central of a plate. Modified Czapek Dox Agar (NaNO3: 3g, MgS04.7H20: 0.5g, KCl: 0.5g, KH2PO4: 1.0g, sugar: 30g, agar: 20g, water: 1000ml) was using different types of sugar (glucose, fructose, maltose, sucrose, and starch) and apply at different concentrations (7.5 g/250 mL, 3.75 g/250 mL). The control was used with no sugar content of a medium (Henderson, 1961).

Estimations of growth: Growth was recorded by measuring the diameter of colonies in two directions at right angles with a rule. Growth rates were calculated for measuring when the colonies were growing at a constant rate (Trinci, 1969).

RESULTS

On observation in this work, it was apparent that all sugar's type used a deterring effect on the growth of fungal colonies but some saccharides were more effective than another one. There was done statistical analysis among utilization of all sugar substrate.

Typical results of the relation between the rate of *A. niger* growth and monosaccharaides uptake are shown in (Figure 1). Generally, the normal concentration (7.5 g sugar/ 250 mL solution) of fructose was the most useful to enhance fungal growth than glucose. Besides that, utilization of these sugars at half value (3.75 g sugar/ 250 mL solution) was same result but it was smaller in growth of colony and diameter measurement.

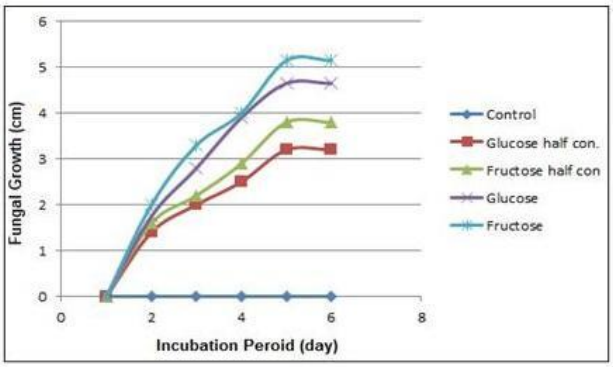

Figure 1. Illustrates the effect of the monosaccharaides on the growth of *Aspergillus niger*

After incubation *A. niger* with media was containing disaccharides; as shown in (Figure 2). Sugar was splitting may be via hydrolytic processes or enzymatic activity. Sucrose supports maximum growth and good sources of carbon for the growth when compare with maltose utilization at both concentration (7.5 g sugar/ 250 mL solution) and (3.75 g sugar/ 250 mL solution) but variation occurred in colony diameter.

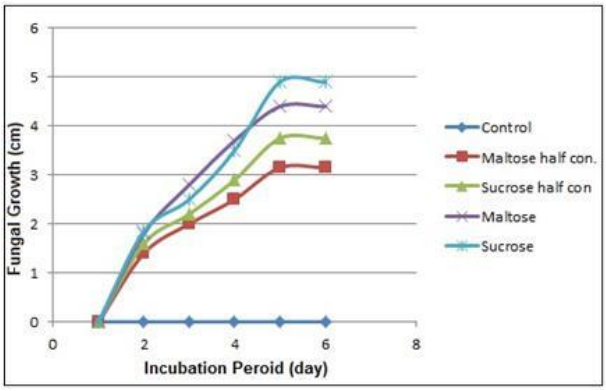

Figure 2. Illustrates the effect of the disaccharides on the growth of *Aspergillus niger*

In (Figure 3) represent the screening of *A. niger* with the media contained starch as a sloe of carbon sources. Whatsoever, *A. niger* produced α-amylase and glucoamylase. The results of both concentrations (7.5 g sugar/ 250 mL solution) and (3.75 g sugar/ 250 mL solution) showed higher enzyme activities but not similar in growth rates and/or mycelium size development.

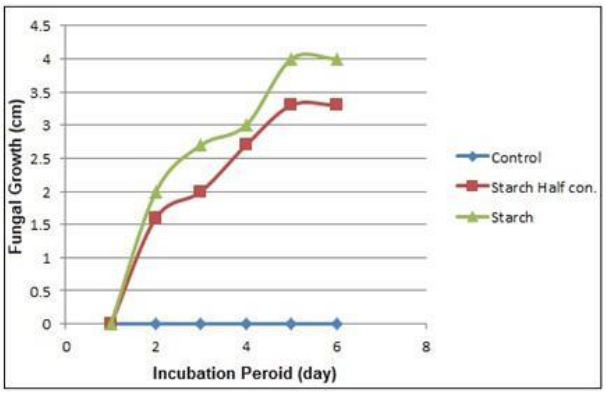

Figure 3. Illustrates the effect of the polysaccharide on the growth of *Aspergillus niger*

DISCUSSION

Aspergillus niger is a filamentous fungi. It is able to digest a wide range of carbohydrates when substrate was containing saccharides which available as the sole source of carbon and energy. All the cellular components must be synthesized from these compounds via appropriate metabolic pathways. Among the practical problem that utilization effect of saccharides for energy and as carbon source by fungus, are: (1) physical availability of sugar, (2) cultural condition, and (3) adaptation of the strain on the substrate.

There are several known transporters capable of transporting the simple sugar such as hexose and pentose into the cell for subsequent phosphorylation and conversion into mainly biomass and CO2. Moreover, in the study Mchunu *et al.*, (2013) indicates that monosaccharaides showed good assimilation by *Trichoderma reesei* and *Aspergillus niger*.

The absorption of the hexoses (glucose and fructose) by *A. niger* was an active process of the sense that it was dependent on the rate of normal metabolism of the mycelium. Glucose was more rapidly absorbed at all concentrations than fructose. Therefore, the fungus appears to have a higher affinity with glucose than fructose; but the growth rate was often rapid and extensive of fructose as more as glucose. Fructose application had a greatly colony diameter when compared the usage of glucose and the ratio of fructose to glucose several times as much fructose as glucose present; because of during glycolysis, glucose was phosphorylated to form glucose-6-phosphate then by isomerization reaction in which glucose-6-phosphate was converted to fructose-6-phosphate; there was during a time periods here used. In addition, the main destination of fructose absorption was into higher saccharide molecules. There was escaping a part of the conversion: glucose to fructose, so fructose directly made phosphorylation and formed fructose-6-phosphate (Figure 4). In each case, the estimation of colony size of fructose was greater than the glucose in the media was inoculated by *A. niger*.

Mehrotra and Kumar (1961) observed that the carbon utilization studies was a quick approach to studying and assessing filamentous fungi for specific activities and showed that the fructose was earlier and faster according to different behavior from the rest of the organisms; therefore, inducing the highest growth and increasing the biomass of fungi.

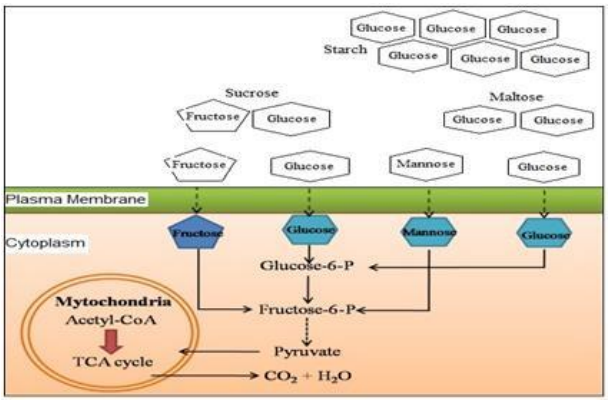

Figure 4. Illustrates the assimilation of simple sugar and cellular respiration

Absorption of disaccharides by *A. niger* was generally depended upon the production of necessary hydrolytic enzymes. On the other hand, the integration and coordination of metabolic processes in the filamentous fungi, and their modulation in response to environmental and developmental changes, is brought about by regulation of the activities of enzymes.

The uptake of sugar by *A. niger* from sucrose solutions was found to be dependent on normal metabolism and hence to be an active process of the same sense of the uptake of hexose. The evidence suggests that the process involved extracellular sucrose breakdown (invertase) to hexose. It also resembles the uptake from mixtures of glucose and fructose in which the glucose moiety was selectively absorbed and an excess of fructose over glucose appeared in the medium. Much more glucose was selectively absorbed; but fructose was supporting agent and made rapid growth.

In the following descriptions of maltose utilization which was used to denote the disappearance of maltose from the external solution and degraded sugar in order to release of glucose into environment. Maltose on hydrolysis yields glucose only (maltase). Therefore, the fungus appears to have a higher affinity with glucose. It was observed that spore germination occurred. After spore germination, the cells grew rapidly then the maximum specific growth rates were found on the surface of the plate.

The comparisons among literature and our findings, Yuan *et al.* (2008), who suggested that *A. niger* utilize maltose by means of extracellular hydrolysis secretion followed by glucose uptake and metabolism. Salzer and Hager (1991) demonstrated that some fungi could not use sucrose directly but glucose and fructose were readily consumed. Lamb (1974) observed growth of fungi on sucrose, caused by introduction of 'starter' monosaccharide.

Polysaccharides was cleaved extracellularly by means of a broad range of extracellular enzymes (amylases). *A. niger* was able to grow on all the tested carbon sources e.g.: starch. There were significant differences in the yield of the biomass and amylase production. Degrading of starch was produced mono, di, and oligosaccharides; these were required additional digestion to form available units of absorption. For this reason, growth was becoming slower; meanwhile, the structure of mycelia was less development. These findings are in line with the work conducted by various workers (Omemu *et al.*, 2005; Pandey *et al.*, 2006 and Sasi *et al.*, 2010) where the selection of potent species was made by plate method. However, zonation was correlated with the amount of enzyme produced (amylase) during the isolation of fungi using starch as a sole of carbohydrates.

Generally, among chemical parameters, carbon sources plays a very important role in inducing enzyme secretion and high-affinity of simple sugar transportation and digestion in the medium which was inoculated *Aspergillus niger*.

CONCLUSION

In the present study, the growth parameter of *A. niger* was calculated for the observed data showed

significant variations in the growth rate with different carbon sources (Figure 5). The order of usability of substrate was fructose > glucose > sucrose > maltose > starch. There were recorded high increases in biomass yields and diametric size of colony or mycelia (Table 1).

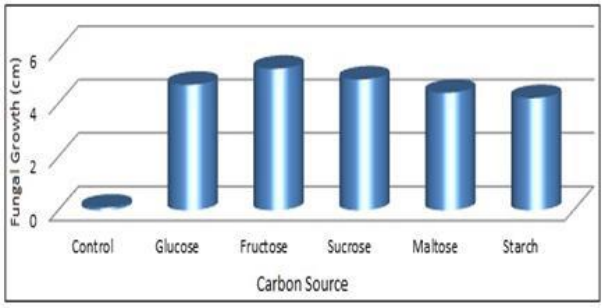

Figure 5. Effect of carbon sources on *A. niger*, incubation for 5 days at 30 °C and pH 5.5.

STATISTICAL ANALYSIS

The experimental results were carried out in triplicates and it was expressed by mean ± Standard Error. Statistical analysis was performed using Data Entry: ANOVA; There was considered statistically significant level $p \leq 0.05$.

ACKNOWLEDGMENT

The authors are grateful to Head of Biology Department at Salahaddin University (Iraq) for providing encouragement and laboratory facilities for carrying out this work.

Table 1. Illustrates the effect of saccharides on the growth of *Aspergillus niger*

Substrates		Sugar Normal Concentration*				Sugar Half Concentration*			
		Mean*	SD	Low	Hi	Mean*	SD	Low	Hi
Monosaccharide	Glucose	4.65 ± 0.03	0.05	4.60	4.70	3.20 ± 0.13	0.20	3.00	3.40
	Fructose	5.15 ± 0.10	0.15	5.00	5.30	3.75 ± 0.13	0.20	3.55	3.95
Disaccharide	Maltose	4.40 ± 0.13	0.20	4.20	4.60	3.15 ± 0.20	0.30	2.85	3.45
	Sucrose	4.90 ± 0.06	0.10	4.80	5.00	3.80 ± 0.06	0.10	3.70	3.90
Polysaccharide	Starch	4.00 ± 0.06	0.10	3.90	4.10	3.30 ± 0.20	0.30	3.00	3.60

* Mean = Diameter of colony growth (cm).
* Sugar Normal Concentration = 7.5 g sugar/ 250 mL medium; Sugar Half Concentration = 3.75 g sugar/ 250 mL medium
* There was significant biologically efficiencies of all substrates ($P \leq 0.05$).

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