Selection of Most Potent *A. niger* Isolates Growing on Different Carbohydrate by-Products for Citric Acid Production

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Abstract: Twenty strains isolates of *A. niger* were isolated from different sources, screened for their capacity to produce citric acid. All the isolated strains were able to produce All isolates were able to produce citric acid in different quantities at different time intervals i.e. 4, 8 and 12 days on indicator medium. The best incubation period for production for all isolates was 12 days. The best production obtained by isolates A1, A4 & A5, while A8, A16, A18 & A19 recorded weak production on that medium. Citric acid productivity was obtained by all strains isolates when using different concentrations of four carbohydrate by-products (maize straw, potato peel wastes, sugar beet pulp and molasses) when each used alone without any additions after 12 days incubation and the production enhanced when the fermentation medium amended with the same concentrations of the mentioned substrates. Type and concentration of carbohydrate by-product affect the production of citric acid by *A. niger* strains isolates under the study. Increasing substrate concentration led to increase in production, the best concentration for production was 25% for all carbohydrate by-products. As recorded with indicator medium, A1, A4 & A5 recorded the best production when growing on the four carbohydrate by-products supplemented to the basal medium, while A8, A6, A16 A18 & A19 recorded the weak production with the carbohydrate by-products used. Further studies will be carried out as a trail to improve the production using the obtained (most potent & the weak) isolates after exposure to mutagenic agents.

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1. Introduction:

Citric acid occurs naturally in various fruits such as lemons, oranges, gooseberries, pears, figs etc. It was isolated from lemon juice and has since been known as natural plant substance in many citric fruits. Citric acid obtained from fruits in known as natural citric acid as compared to synthetic produced by microbial fermentation (Prescott and Dunns 1987, Ranya *et al*., 1999). Citric acid (a tricarboxylic acid) is of industrial importance because it is widely used in dairy, medicine and biochemical industries. *A. niger* has been the organism of choice for citric acid production due to its ease of handlings, ability to use a variety of cheep raw material and high yields of citric acid (Mourya and Jauhri 2000; Vandenberghe *et al*., 2000; Sankpal *et al*., 2001; Asad ur-Rehman *et al*., 2003; Papagianni 2007; Torres and Garcia 2009 and Alagarsamy & Nallusamy 2010). Considerable attention has been shown in using agriculture wastes for citric acid production (Prado *et al*., 2005; Khosravi & Zoghi 2008 and Khosravi *et al*., 2008). Different agro-industrial residues including, applepomace, wheat straw, coffehusk, pineapple waste, cassava bagasse, banana, sugar beet cosset and Kiwi fruit peel, etc., have been investigated with SSF technique for their potential to be used as substrates for citric acid production (Hang and Woodams 1995; Tran and Mitchell 1995; Pintado *et al*., 1998; Fatemi and Shojaosadati 1999; Luciana *et al*., 2000; Ali *et al*., 2002 and Abdulrahman *et al*., 2006). This investigation aims to isolate *A. niger* from different sources for citric acid production using various concentrations of different carbohydrate by-products in a fermentation medium to select the most potent isolates for production and also to detect the weak isolates, both for further studies

2. Materials and methods

Microorganisms:

Twenty isolates of *A. niger* were isolated from different rotted sources (Table 1).

Media used:

Potato Dextrose agar (PDA) was used for isolation, purification and maintenance the isolates which were incubated at 28^{o}C for 7 days. Indicator medium was used for screening the isolates and citric acid production (Chopra *et al*., 1983).

Carbohydrate by-products:

Maize straw and potato peel wastes were cut to small pieces, sugar beet pulp was grounded and

molasses was diluted by water (1:3), they were used as substrates for growing the isolates under the study without any additions at different concentrations and also used as carbon source in basal medium instead of glucose.

Inoculums preparation and cultivation:

Spore suspension (10^7 spores/ml) of isolates were prepared for inoculation, samples were taken after 6 and 12 days incubation at 30°C for citric acid production.

Assay of citric acid:

It was determined according to Marrier and Boulet (1958). Data recorded are the mean of three replicates.

3. Results and Discussion:

Twenty isolates of *A. niger* were isolated from different sources, screened for their capacity to produce citric acid. All isolates were able to produce citric acid in different quantities at different time intervals i.e. 4, 8 and 12 days on indicator medium. The best incubation period was 12 days for all isolates. A1, A4 & A5 recorded maximum production on indicator medium, while A8, A16, A18 & A19 recorded weak production on the same medium as shown from (Table 2). Citric acid productivity were obtained by all isolates when using different concentrations i.e 5, 10, 15, 20 and 25% of four carbohydrate by-products (maize straw, potato peel wastes, sugar beet pulp and molasses) when each used alone without any additions after 12 days incubation (Tables 3, 5, 7 and 9) and the production enhanced when the fermentation medium amended with the same concentrations of the mentioned substrates (Tables 4, 6, 8 and 10). This means that citric acid production in basal medium amended with the substrates was better than with using the substrate alone in the four carbohydrate by-products under the study.

It can be noticed that increasing the substrate concentration led to increase the production and the best concentration for production was 25% for all carbohydrate by-products. Mourya and Jauhri (2000) reported that increasing concentration of substrate (maize starch hydrolysate) corresponding increase of citric acid by *A. niger*. As recorded with indicator medium, A1, A4 & A5 are also the most potent strains isolates for production when growing on the basal medium amended with the four carbohydrate by-products, while A8, A16, A18 & A19 recorded the weak production.

Table (1):. Source of *A. niger* isolates.

Code number of *A. niger* isolates	Source of isolates
A_1	Maize grains
A_2	Cheese
A_3	Peanut
A_4	Banana
A_5	Apple
A_6	Potato
A_7	Bread
A_8	Phylloplane of *Solanum persicum*
A_9	Orange
A_{10}	Peach
A_{11}	Bagasse
A_{12}	Tomato
A_{13}	Peach
A_{14}	Pickles
A_{15}	Yoghurt
A_{16}	Garlic
A_{17}	Lemon
A_{18}	Potato
A_{19}	Tomato sauce
A_{20}	Bread

Table (2): Diameter of clear zone (mm) and production of citric acid (mg/ml) by the *Aspergillus niger* isolates using indicator medium at different time intervals.

Code number of *A. niger* isolates	Diameter of clear zone (mm)		Citric acid production (mg/ml)		
	4 days	8 days	4 days	8 days	12 days
A_1	50	90	0.384±0.04	0.670±0.03	0.950±0.08
A_2	35	55	0.250±0.09	0.557±0.06	0.810±0.09
A_3	45	80	0.361±0.07	0.648±0.10	0.906±0.13
A_4	48	85	0.378±0.03	0.663±0.05	0.935±0.07
A_5	51	92	0.390±0.06	0.682±0.08	0.986±0.12
A_6	25	45	0.212±0.07	0.515±0.04	0.760±0.10
A_7	27	47	0.239±0.09	0.530±0.09	0.771±0.06
A_8	30	38	0.204±0.09	0.496±0.09	0.735±0.04
A_9	37	70	0.309±0.12	0.603±0.11	0.878±0.11
A_{10}	40	75	0.342±0.04	0.624±0.07	0.883±0.08
A_{11}	36	55	0.255±0.05	0.561±0.06	0.817±0.06
A_{12}	35	60	0.265±0.03	0.574±0.06	0.840±0.07
A_{13}	23	42	0.204±.09	0.510±0.05	0.763±0.09
A_{14}	38	65	0.289±0.10	0.590±0.13	0.850±0.09
A_{15}	37	65	0.292±0.11	0.594±0.06	0.857±0.11
A_{16}	22	30	0.198±0.09	0.477±0.08	0.711±0.04
A_{17}	39	72	0.322±0.09	0.612±0.04	0.899±0.07
A_{18}	25	30	0.194±0.07	0.471±0.09	0.683±0.03
A_{19}	17	22	0.172±0.03	0.446±0.05	0.624±0.05
A_{20}	38	62	0.260±0.03	0.578±0.07	0.845±0.08

Table (3):. Citric acid production (mg/ml) by the *A. niger* isolates grown on different concentrations of maize straw after 12 days incubation.

. Code number of *A. niger* isolates	Citric acid production (mg/ml)				
	Maize straw (%)				
	5	*10*	*15*	*20*	*25*
A_1	0.26±0.07	0.31±0.02	0.45±0.07	0.55±0.05	0.68±0.04
A_2	0.19±0.02	0.25±0.05	0.38±0.05	0.40±0.03	0.51±0.07
A_3	0.24±0.06	0.28±0.03	0.40±0.06	0.53±0.05	0.63±0.03
A_4	0.22±0.08	0.30±0.09	0.44±0.03	0.54±0.11	0.64±0.05
A_5	0.27±0.03	0.33±0.08	0.46±0.07	0.58±0.09	0.70±0.09
A_6	0.22±0.05	0.24±0.06	0.33±0.09	0.39±0.07	0.51±0.08
A_7	0.21±0. 10	0.27±0.04	0.34±0.10	0.41±0.10	0.53±0.08
A_8	0.16±0.09	0.21±0.07	0.31±0.12	0.35±0.09	0.48±0.06
A_9	0.20±0.07	0.24±0.03	0.34±0.07	0.43±0.05	0.59±0.11
A_{10}	0.20±0.07	0.25±0.09	0.37±0.05	0.50±0.06	0.62±0.12
A_{11}	0.20±0.06	0.24±0.12	0.37±0.09	0.40±0.11	0.53±0.10
A_{12}	0.20±0.03	0.24±0.04	0.31±0.08	0.41±0.05	0.55±0.04
A_{13}	0.17±0.03	0.22±0.05	0.32±0.11	0.38±0.07	0.50±0.05
A_{14}	0.23±0.12	0.24±0.11	0.33±0.09	0.44±0.04	0.56±0.07
A_{15}	0.23±0.07	0.23±0.08	0.32±0.09	0.44±0.08	0.56±0.08
A_{16}	0.15±0.09	0.20±0.04	0.29±0.08	0.32±0.09	0.47±0.04
A_{17}	0.21±0.06	0.23±0.07	0.35±0.08	0.47±0.05	0.61±0.03
A_{18}	0.15±0.09	0.21±0.05	0.30±0.04	0.33±0.07	0.47±0.03
A_{19}	0.13±0.11	0.17±0.04	0.27±0.07	0.30±0.07	0.45±0.05
A_{20}	0.20±0.03	0.22±0.03	0.30±0.09	0.44±0.03	0.56±0.07

Table (4): Citric acid production (mg/ml) by the *A. niger* isolates growing on basal medium amended with different concentrations of maize straw after 12 days incubation.

Code number of *A. niger* isolates	Citric acid production (mg/ml)				
	Maize straw (%)				
	5	*10*	*15*	*20*	*25*
A_1	0.35±0.07	0.48±0.05	0.61±0.3	0.70±0.08	0.73±0.04
A_2	0.28±0.04	0.39±0.08	0.45±0.07	0.51±0.06	0.56±0.03
A_3	0.31±0.07	0.51±0.09	0.57±0.05	0.60±0.03	0.69±0.07
A_4	0.35±0.06	0.45±0.12	0.59±0.04	0.65±0.03	0.70±0.09
A_5	0.36±0.06	0.53±0.05	0.62±0.04	0.73±0.07	0.84±0.11
A_6	0.24±0.09	0.33±0.07	0.42±0.03	0.50±0.09	0.53±0.08
A_7	0.23±0.12	0.31±0.06	0.42±0.08	0.49±0.09	0.55±0.05
A_8	0.21±0.08	0.30±0.09	0.38±0.06	0.41±0.03	0.48±0.03
A_9	0.29±0.08	0.39±0.09	0.48±0.07	0.53±0.08	0.61±0.07
A_{10}	0.31±0.04	0.41±0.08	0.53±0.09	0.57±0.09	0.67±0.06
A_{11}	0.28±0.03	0.38±0.04	0.47±0.06	0.54±0.04	0.57±0.09
A_{12}	0.27±0.07	0.34±0.11	0.44±0.05	0.51±0.04	0.58±0.08
A_{13}	0.21±0.09	0.30±0.06	0.37±0.05	0.42±0.08	0.51±0.03
A_{14}	0.20±0.03	0.32±0.06	0.45±0.04	0.53±0.07	0.59±0.09
A_{15}	0.21±0.03	0.33±0.09	0.45±0.11	0.53±0.06	0.59±0.04
A_{16}	0.17±0.05	0.22±0.04	0.33±0.03	0.40±0.06	0.48±0.11
A_{17}	0.35±0.08	0.40±0.03	0.54±0.03	0.53±0.03	0.65±0.010
A_{18}	0.18±0.06	0.23±0.05	0.35±0.13	0.40±0.07	0.48±0.04
A_{19}	0.14±0.09	0.19±0.05	0.30±0.05	0.33±0.09	0.46±0.07
A_{20}	0.28±0.11	0.35±0.07	0.44±0.12	0.51±0.11	0.58±0.03

Table (5): Citric acid production (mg/ml) by different isolates of *A. niger* grown on different concentrations of potato solid wastes after 12 days incubation.

Code number of *A. niger* isolates	Citric acid production (mg/ml)				
	Potato solid wastes (%)				
	5	*10*	*15*	*20*	*25*
A_1	0.37±0.11	0.48±0.08	0.55±0.04	0.67±0.07	0.75±0.06
A_2	0.23±0.04	0.35±0.07	0.44±0.12	0.53±0.10	0.60±0.08
A_3	0.33±0.09	0.46±0.10	0.54±0.02	0.64±0.04	0.71±0.05
A_4	0.35±0.03	0.47±0.07	0.54±0.08	0.65±0.04	0.74±0.09
A_5	0.38±0.07	0.49±0.09	0.57±0.03	0.68±0.04	0.76±0.07
A_6	0.22±0.11	0.31±0.08	0.48±0.07	0.57±0.06	0.59±0.04
A_7	0.22±0.07	0.32±0.05	0.47±0.03	0.56±0.09	0.59±0.05
A_8	0.21±0.08	0.28±0.09	0.42±0.04	0.51±0.03	0.56±0.03
A_9	0.28±0.06	0.44±0.07	0.51±0.08	0.57±0.04	0.65±0.07
A_{10}	0.30±0.06	0.45±0.05	0.53±0.03	0.61±0.04	0.68±0.03
A_{11}	0.23±0.09	0.29±0.03	0.44±0.06	0.57±0.03	0.61±0.09
A_{12}	0.24±0.04	0.40±0.03	0.47±0.07	0.59±0.05	0.63±0.10
A_{13}	0.21±0.07	0.30±0.06	0.45±0.09	0.51±0.12	0.57±0.9
A_{14}	0.27±0.03	0.42±0.07	0.49±0.09	0.58±0.14	0.64±0.11
A_{15}	0.26±0.03	0.42±0.09	0.47±0.12	0.57±0.08	0.64±0.04
A_{16}	0.20±0.11	0.25±0.07	0.43±0.03	0.50±0.09	0.55±0.07
A_{17}	0.29±0.09	0.45±0.06	0.52±0.08	0.60±0.05	0.67±0.03
A_{18}	0.20±0.03	0.26±0.09	0.43±0.06	0.50±0.07	0.53±0.06
A_{19}	0.18±0.04	0.22±0.08	0.40±0.09	0.48±0.03	0.51±0.05
A_{20}	0.25±0.05	0.42±0.70	0.49±0.04	0.57±0.03	0.64±0.07

Table (6): Citric acid production (mg/ml) by different isolates of *A. niger* grown on basal medium amended with different concentrations of potato solid wastes after 12 days incubation.

Code number of *A. niger* isolates	Citric acid production (mg/ml)				
	Potato solid wastes (%)				
	5	*10*	*15*	*20*	*25*
A_1	0.36±0.05	0.58±0.04	0.77±0.07	0.85±0.09	0.94±0.03
A_2	0.29±0.07	0.48±0.08	0.51±0.06	0.55±0.11	0.69±0.09
A_3	0.34±0.03	0.56±0.08	0.74±0.04	0.78±0.12	0.88±0.10
A_4	0.34±0.08	0.57±0.05	0.76±0.07	0.80±0.06	0.91±0.08
A_5	0.37±0.06	0.58±0.04	0.77±0.08	0.89±0.10	0.98±0.09
A_6	0.26±0.03	0.37±0.09	0.46±0.08	0.54±0.03	0.65±0.07
A_7	0.26±0.07	0.38±0.08	0.48±0.04	0.56±0.04	0.66±0.03
A_8	0.25±0.10	0.36±0.12	0.43±0.03	0.54±0.07	0.62±0.08
A_9	0.30±0.08	0.41±0.04	0.65±0.03	0.69±0.06	0.79±0.05
A_{10}	0.32±0.07	0.43±0.05	0.69±0.06	0.75±0.06	0.84±0.12
A_{11}	0.29±0.03	0.47±0.09	0.51±0.09	0.55±0.09	0.68±0.09
A_{12}	0.30±0.07	0.46±0.11	0.58±0.04	0.63±0.03	0.75±0.04
A_{13}	0.28±0.05	0.37±0.04	0.44±0.03	0.54±0.09	0.63±0.03
A_{14}	0.33±0.04	0.48±0.05	0.61±0.07	0.65±0.04	0.77±0.05
A_{15}	0.33±0.03	0.49±0.06	0.62±0.09	0.66±0.07	0.78±0.05
A_{16}	0.24±0.03	0.37±0.08	0.41±0.09	0.52±0.07	0.60±0.07
A_{17}	0.31±0.08	0.51±0.09	0.66±0.10	0.70±0.10	0.81±0.11
A_{18}	0.25±0.09	0.38±0.03	0.41±0.013	0.51±0.08	0.60±0.09
A_{19}	0.23±0.09	0.35±0.03	0.38±0.04	0.49±0.07	0.58±0.05
A_{20}	0.30±0.11	0.43±0.07	0.65±0.05	0.71±0.07	0.80±0.06

Table (7): Citric acid production (mg/ml) by different isolates of *A. niger* growing on different concentrations of sugar beet pulp after 12 days incubation.

Code number of *A. niger* isolates	Citric acid production (mg/ml)				
	Sugar beet pulp (%)				
	5	*10*	*15*	*20*	*25*
A_1	0.50±0.12	0.68±0.04	0.84±0.05	0.91±0.07	1.10±0.09
A_2	0.37±0.06	0.52±0.09	0.66±0.04	0.79±0.03	0.90±0.10
A_3	0.39±0.07	0.63±0.07	0.84±0.08	0.91±0.11	1.00±0.04
A_4	0.48±0.09	0.69±0.03	0.86±0.06	0.93±0.05	1.05±0.03
A_5	0.49±0.04	0.70±0.09	0.89±0.09	0.92±0.03	1.08±0.08
A_6	0.35±0.03	0.60±0.07	0.72±0.12	0.83±0.04	0.95±0.11
A_7	0.38±0.13	0.65±0.06	0.77±0.10	0.88±0.03	0.98±0.09
A_8	0.34±0.05	0.48±0.08	0.64±0.03	0.74±0.04	0.91±0.07
A_9	0.41±0.06	0.53±0.07	0.70±0.07	0.83±0.03	1.00±0.09
A_{10}	0.44±0.09	0.58±0.03	0.76±0.07	0.80±0.04	1.03±0.08
A_{11}	0.39±0.06	0.57±0.07	0.64±0.10	0.82±0.03	0.95±0.03
A_{12}	0.41±0.09	0.59±0.11	0.70±0.18	0.85±0.07	0.99±0.04
A_{13}	0.32±0.03	0.45±0.04	0.60±0.09	0.73±0.08	0.84±0.07
A_{14}	0.42±0.04	0.63±0.07	0.74±0.03	0.89±0.04	1.02±0.09
A_{15}	0.40±0.08	0.60±0.03	0.72±0.03	0.84±0.09	1.00±0.06
A_{16}	0.35±0.11	0.44±0.12	0.56±0.09	0.65±0.04	0.72±0.07
A_{17}	0.41±0.07	0.60±0.03	0.74±0.10	0.87±0.03	0.94±0.04
A_{18}	0.30±0.04	0.40±0.06	0.52±0.05	0.64±0.09	0.69±0.10
A_{19}	0.31±0.03	0.38±0.06	0.53±0.07	0.60±0.06	0.65±0.09
A_{20}	0.42±0.05	0.59±0.09	0.74±0.07	0.89±0.07	0.95±0.11

Table (8): Citric acid production (mg/ml) by different isolates of *A. niger* growing on basal liquid medium amended with different concentrations of sugar beet pulp after 12 days incubation.

Code number of *A. niger* isolates	Citric acid production (mg/ml)				
	Sugar beet pulp (%)				
	5	*10*	*15*	*20*	*25*
A_1	0.55±0.06	0.75±0.09	0.97±0.07	1.15±0.05	1.56±0.03
A_2	0.43±0.08	0.69±0.03	0.88±0.04	0.93±0.07	1.28±0.03
A_3	0.45±0.09	0.75±0.03	0.91±0.09	0.97±0.12	1.40±0.04
A_4	0.51±0.05	0.72±0.03	0.92±0.04	1.06±0.03	1.46±0.05
A_5	0.53±0.11	0.73±0.05	0.93±0.09	1.09±0.07	1.52±0.06
A_6	0.40±0.09	0.66±0.09	0.78±0.03	0.89±0.04	1.02±0.09
A_7	0.42±0.09	0.71±0.07	0.80±0.07	0.90±0.06	1.08±0.06
A_8	0.38±0.03	0.57±0.04	0.72±0.06	0.80±0.09	0.94±0.07
A_9	0.47±0.09	0.69±0.07	0.81±0.03	0.92±0.04	1.24±0.08
A_{10}	0.50±0.04	0.73±0.03	0.87±0.06	0.96±0.07	1.33±0.09
A_{11}	0.45±0.08	0.62±0.07	0.74±0.03	0.90±0.06	1.11±0.04
A_{12}	0.49±0.11	0.64±0.08	0.77±0.09	0.92±0.04	1.20±0.07
A_{13}	0.37±0.12	0.53±0.05	0.66±0.014	0.84±0.06	0.92±0.05
A_{14}	0.50±0.06	0.71±0.03	0.83±0.04	0.94±0.09	1.32±0.02
A_{15}	0.46±0.05	0.65±0.06	0.79±0.07	0.91±0.11	1.15±0.04
A_{16}	0.42±0.07	0.51±0.03	0.67±0.04	0.86±0.08	0.90±0.06
A_{17}	0.47±0.06	0.69±0.06	0.79±0.07	0.92±0.08	1.16±0.09
A_{18}	0.38±0.12	0.54±0.11	0.60±0.08	0.70±0.09	0.85±0.04
A_{19}	0.34±0.09	0.46±0.05	0.57±0.07	0.67±0.04	0.82±0.03
A_{20}	0.48±0.04	0.67±0.03	0.82±0.09	0.93±0.07	1.25±0.08

Table (9): Citric acid production (mg/ml) by different isolates of *A. niger* growing on different concentrations of molasses after 12 days incubation.

Code number of *A. niger* isolates	Citric acid production (mg/ml)				
	Molasses (%)				
	5	*10*	*15*	*20*	*25*
A_1	0.45±0.06	0.60±0.08	0.75±0.07	0.83±0.04	0.94±0.11
A_2	0.31±0.05	0.45±0.03	0.52±0.07	0.67±0.08	0.77±0.09
A_3	0.35±0.04	0.52±0.09	0.70±0.08	0.74±0.04	0.82±0.03
A_4	0.30±0.03	0.49±0.04	0.70±0.05	0.75±0.07	0.84±0.06
A_5	0.43±0.08	0.56±0.07	0.71±0.03	0.78±0.03	0.86±0.09
A_6	0.27±0.03	0.36±0.05	0.49±0.04	0.57±0.05	0.76±0.10
A_7	0.29±0.05	0.41±0.08	0.52±0.05	0.63±0.08	0.77±0.08
A_8	0.30±0.06	0.39±0.07	0.59±0.08	0.66±0.06	0.72±0.04
A_9	0.38±0.04	0.46±0.14	0.62±0.16	0.71±0.03	0.83±0.03
A_{10}	0.42±0.08	0.49±0.09	0.67±0.03	0.78±0.05	0.87±0.05
A_{11}	0.34±0.06	0.46±0.10	0.53±0.04	0.67±0.03	0.87±0.08
A_{12}	0.39±0.07	0.54±0.07	0.66±0.09	0.77±0.04	0.89±0.07
A_{13}	0.26±0.10	0.37±0.07	0.51±0.05	0.66±0.03	0.72±0.09
A_{14}	0.35±0.12	0.54±0.03	0.65±0.09	0.81±0.07	0.98±0.04
A_{15}	0.33±0.06	0.55±0.07	0.61±0.08	0.79±0.03	0.87±0.10
A_{16}	0.28±0.03	0.35±0.09	0.49±0.06	0.58±0.09	0.67±0.03
A_{17}	0.39±0.09	0.54±0.04	0.69±0.12	0.85±0.10	0.90±0.04
A_{18}	0.24±0.04	0.33±0.07	0.44±0.08	0.56±0.09	0.65±0.65
A_{19}	0.23±0.03	0.31±0.08	0.45±0.07	0.51±0.06	0.59±0.04
A_{20}	0.38±0.09	0.51±0.03	0.64±0.04	0.85±0.10	0.90±0.07

Table (10): Citric acid production (mg/ml) by different isolates of *A. niger* growing on basal liquid medium amended with different concentrations of molasses after 12 days incubation.

Code number of *A. niger* isolates	Citric acid production (mg/ml)				
	Molasses (%)				
	5	*10*	*15*	*20*	*25*
A_1	0.52±0.04	0.69±0.03	0.87±0.06	0.98±0.09	1.17±0.04
A_2	0.39±0.03	0.54±0.05	0.63±0.09	0.76±0.07	0.92±0.08
A_3	0.43±0.03	0.67±0.09	0.82±0.04	0.90±0.07	0.98±0.06
A_4	0.41±0.07	0.65±0.09	0.84±0.06	0.94±0.09	1.04±0.03
A_5	0.51±0.10	0.70±0.07	0.87±0.03	0.86±0.04	1.15±0.09
A_6	0.30±0.04	0.48±0.03	0.61±0.09	0.77±0.08	0.85±0.07
A_7	0.34±0.03	0.50±0.11	0.66±0.08	0.79±0.03	0.87±0.08
A_8	0.33±0.07	0.45±0.07	0.63±0.06	0.74±0.12	0.80±0.03
A_9	0.45±0.06	0.64±0.05	0.74±0.07	0.90±0.05	1.02±0.11
A_{10}	0.50±0.09	0.68±0.10	0.79±0.03	0.86±0.03	1.09±0.09
A_{11}	0.40±0.11	0.52±0.09	0.64±0.03	0.75±0.13	0.94±0.10
A_{12}	0.43±0.08	0.60±0.08	0.71±0.05	0.87±0.03	0.99±0.05
A_{13}	0.31±0.04	0.42±0.05	0.60±0.03	0.70±0.06	0.78±0.14
A_{14}	0.41±0.05	0.62±0.03	0.80±0.06	0.89±0.16	1.03±0.04
A_{15}	0.40±0.08	0.61±0.13	0.76±0.08	0.88±0.04	0.97±0.05
A_{16}	0.31±0.03	0.44±0.09	0.59±0.08	0.70±0.08	0.78±0.09
A_{17}	0.48±0.03	0.67±0.08	0.78±0.04	0.92±0.09	1.05±0.11
A_{18}	0.28±0.04	0.41±0.07	0.56±0.03	0.65±0.06	0.76±0.07
A_{19}	0.29±0.06	0.38±0.04	0.50±0.05	0.58±0.10	0.65±0.10
A_{20}	0.44±0.05	0.63±0.08	0.78±0.10	0.91±0.05	1.00±0.09

Production of citric acid (mg/ml) by the most potent isolates (A1, A4 & A5) after 12 days incubation respectively was:

On indicator medium: 0.95, 0.94 and 0.99
On basal medium with 25% maize straw: 0.73, 0.70 and 0.84
On basal medium with 25% potato peel wastes: 0.94, 0.91 and 0.98
On basal medium with 25% sugar beet pulp: 1.56, 1.46 and 1.52
On basal medium with 25% molasses: 1.17, 1.04 and 1.15

Production of citric aid (mg/ml) after 12 days by weak isolates (A8, A16, A18 & A19 respectively

On indicator medium: 0.74, 0.71, 0.68 and 0.62
On basal medium with 25% maize straw: 0.48, 0.48, 0.48 and 0.46
On basal medium with 25% potato peel wastes: 0.62, 0.60, 0.60 and 0.58
On basal medium with 25% sugar beet pulp: 0.94, 0.90, 0.85 and 0.82
On basal medium with 25% molasses: 0.80, 0.78, 0.76 and 0.65

It can be noticed that sugar beet pulp and molasses giving the highest yield of citric acid, while maize straw giving the lowest yield. The yield on indicator medium and potato peel wastes almost equal. This means that type of carbohydrate by-product affect the production of citric acid by *A. niger* isolates under the study. Vandenberghe *et al.* (2000) compared citric acid production from cassava bagasse and two other substrates (sugar cane bagasse and coffee husk) in SSF using a culture of *A. niger*, cassava bagasse giving the highest yield of citric acid among the tested substrates.

Further studies will be carried out using the most potent isolates A1, A4 & A5 and also the weak isolates A8, A16, A18 & A19 in a trail to improve the production after exposure to mutagenic agents.

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