

Full Length Research Paper

Factors affecting yeast growth and protein yield production from orange, plantain and banana wastes processing residues using *Candida* sp.

A. Adoki

Health Safety and Environment, Shell Petroleum Development Company Limited, Old Aba Road, Port Harcourt, Nigeria.
E-mail: akuro.adoki@shell.com.

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Agricultural residues rich in carbohydrates can be utilized in fermentation processes to produce microbial protein which in turn can be used to upgrade both human and animal feeds. Studies to determine the factors influencing cell biomass production with *Candida* sp. using citrus fruit wastes showed that the test strain was capable of meeting its amino acid requirements in culture when supplied with inorganic nitrogen sources. The organism was capable of growth at 37°C. Supplementation of media with 0 – 15% and 0 - 6% w/v combination of dextrose and ammonium nitrate, respectively, resulted in optimal growth at a pH of 4.6 (optimum pH) after 6.0 h. However, supplementation with phosphorus was not a critical condition for growth.

Key words: *Candida*, Yeast, microbial, protein, production, factors, banana, plantain, orange, wastes.

INTRODUCTION

Increasing world population and the resultant food crisis has shifted emphasis to the availability of 'waste' products of agriculture that could be utilized for alleviating food shortages (Bhattacharjee, 1970). In many of the developing countries where major nutritional problems exist, excess of materials rich in carbohydrates are produced. These materials can be utilized in fermentation processes to produce microbial protein which in turn can be used to upgrade both human food and animal feeds. Traditional protein sources are relatively more expensive and the absence of well-developed technological facilities have contributed to losses incurred through spoilage of even the limited available sources. Consequently, there is need to explore alternative ways of meeting the protein demands. Compared to the developed countries (mainly the U.S.A, U.K. and Japan) the less developed countries appear to have more abundant supply of agro-waste substrate raw materials that could be converted to additional protein sources (Moo- Young, 1977).

Various factors are known to influence fermentation processes. These include carbon and energy source requirements, oxygen demand and supply, temperature, pH, nitrogen, phosphorus and potassium requirement (Pirt, 1975). In this study some factors affecting the yield of cell biomass production from citrus wastes by *Candida*

sp. are ascertained.

MATERIALS AND METHODS

Isolation

The yeast strain used for this study was isolated from ripe banana. Fifty grams of fresh ripe banana pulp was mashed in a pre-sterilized mortar, and transferred aseptically to 100 ml sterile distilled water in a 250 ml conical flask stoppered loosely with a cotton wool plug. This was left to stand for 48 h and a loopful cultured on streptomycin-containing potato dextrose agar (PDA) as described by Cruickshank et al. (1975) at 25°C for 24 h. Stock cultures of the isolated strain were maintained on agar slants of streptomycin-containing PDA in screw-capped bottles and stored at 4°C.

Morphological and biochemical characterization of test strain

The morphological characteristics of the isolate were determined by noting colony size, shape, colour and cellular morphology by micro-copy. Biochemical tests involving the utilization of dextrose, fructose, maltose, mannitol, lactose and sucrose were carried out as described by Cruickshank et al. (1975) and Bradshaw (1979). The amino acid requirements of the test strain were determined using modified minimal medium as outlined by Cruickshank et al. (1975). One litre of the complete medium contained the following

supplements: 20 mg each of adenine, arginine, histidine, isoleucine, leucine, lysine, methionine, tyrosine, uracil and valine, phenylalanine (40 mg), tryptophan (30 mg) and threonine (200 mg). Selective omission media were prepared by omitting individual amino acids or bases from the complete medium. The amino acids and the preparation of base solutions, complete and selective omission media have been described previously (Adoki, 1987). Plates containing complete and omission media sp. were incubated at 25°C in an incubator for 24 h. This allowed for identification of the growth requirements of the test strain. (Hawthorne and Mortimer, 1960).

The ability of the test strain of *Candida* sp. to grow at 37°C was determined by streaking duplicate plates of potato dextrose agar (PDA) with yeast cells from a slant culture and incubating the plates at 37°C for 24 h, after which the plates were observed for growth by the development of visible colonies. The optimum pH of growth of the test strain was determined by first preparing various suspensions (100 ml) of the fruit waste to give a pH range of 3.0 - 6.2. Preparation of culture suspensions have been described previously (Adoki and Adoki, 1995). The pH of the basal medium was adjusted to the desired range by using citrate-phosphate buffer as described by Cruickshank et al. (1975). After sterilization, flasks were cooled to ambient temperature then inoculated with a suspension of yeast cells (about 10^3 cfu/ml) in sterile distilled water. These were then incubated at room temperature on an orbital shaker at 150 rpm. An uninoculated flask was also included in the determination as a control. At regular intervals, 1 ml sample of the culture suspensions were withdrawn aseptically, serially diluted in sterile distilled water and plated in triplicates on PDA plates. The plates were incubated at 25°C in an incubator and counts of viable organisms recorded after 24 h.

Optimum substrate concentration

The basal media for the fermentation/growth studies of the test strain were prepared using dry ground orange, plantain and banana wastes. A range of fruit waste concentrations of 1.0 - 2.0% (w/v) were prepared by suspending variable amounts of the dry ground wastes in 100 ml of de-ionized water and the adjusted as required, using citrate-phosphate buffer as described by Cruickshank et al. (1975). Preliminary investigations had shown that substrate concentrations of up to 3.0% (w/v) or above were not suitable for growth since the growth medium became very viscous after autoclaving. After autoclaving flasks were cooled to ambient temperature prior to inoculation with 1 ml portions of a suspension of yeast cells (about 10^3 cfu/ml) in sterile distilled water. These were then incubated at room temperature (about 25°C) on an orbital shaker at 150 rpm. An uninoculated control flask was also included in the determination. At regular intervals, 1 ml samples of the culture suspensions were withdrawn aseptically and serially diluted in sterile distilled water and then plated by surface spreading technique on antibiotic-containing PDA plates. The plates were then incubated at 25°C in an incubator, and counts of variable organisms recorded after 24 - 36 h.

Effects of carbon, nitrogen and phosphorus

The effects of supplementation with inorganic forms of carbon, nitrogen and phosphorus were determined by supplementing growth media in flasks with glucose as a source of carbon, ammonium sulphate and ammonium nitrate as sources of nitrogen, and potassium dihydrogen phosphate as source of phosphorus. Liquid culture (100 ml) suspensions containing citrus fruit residues were supplemented with appropriate volumes of filter-sterilized stock solutions of the carbon and nitrogen sources. Supplemented flasks were then inoculated with 1 ml portions of a suspension of

yeast cells (about 10^3 cfu/ml) in sterile distilled water. The inoculated flasks were incubated at room temperature on an orbital shaker at 150 rpm. Growth was measured as viable counts as described earlier (Cruickshank et al., 1975; Bradshaw, 1979; Adoki, 1987).

Effect of substrate type on cell biomass production

Biomass production in supplemented and unsupplemented orange, plantain and banana waste media were compared. Basal media (100 ml) were prepared as described for other tests and supplemented with the optimal combination of carbon and nitrogen sources. Flasks were prepared in duplicates and sterilized at 121°C for 15 min. When cool, each flask was inoculated with 1 ml suspensions of the test strain of *Candida* sp. and incubated at room temperature on an orbital shaker at 150 rpm. for 60 h. Uninoculated controls were also included (Adoki, 1987). The solids fraction of duplicate set of uninoculated flasks were harvested by centrifugation and the supernatant decanted off. The harvested solid was then washed three times with sterile distilled water with centrifugation carried out intermittently between washings. The washed solid was then dried in a hot air oven at 80°C until constant weight was obtained. This was then recorded as the initial weight (Adoki, 1987). For the incubated flasks, after the incubation period the solids fractions were harvested and treated as described above. The increase in the solids fraction by weight difference was taken as the quantity of microbial biomass produced (obtained by subtracting the initial weight from the final weight).

RESULTS AND DISCUSSION

Biochemical characterization

The *Candida* sp. used for this study produced discrete colonies on PDA on incubation at room temperature but failed to grow at 37°C. Since one of the requirements of human microbial pathogens is that they must be capable of growth at 37°C, this result presumably indicates the safety of the use of this organism for animal feed supplement production.

During growth in culture media one of the factors controlling the level of growth of any selected strain is the pH. The pH would also determine the occurrence and types of particular contaminants in the culture medium. It is therefore necessary that the pH of growth of any selected strain be such that is optimal for this strain and at the same time reduces growth of contaminants. During this study, it was found that the test strain was capable of growth over a wide pH range of 3.0 to 6.2 (Figures 1 and 2). The pH value considered as appropriate for further studies was 4.6 as below this level (3.0 and 3.4) and above (5.8) mixing by rotary shaking was difficult as the media tended to be viscous. Since most saprophytic bacteria have wide pH growth range and can thrive at pH as low as 4.4 (Cruickshank et al., 1975), the optimum pH of 3.0 selected for the test strain has discriminating advantage.

The test strain was also studied for its amino acid requirements and results obtained (Table 1) show that the organism was capable of growth in the absence of the

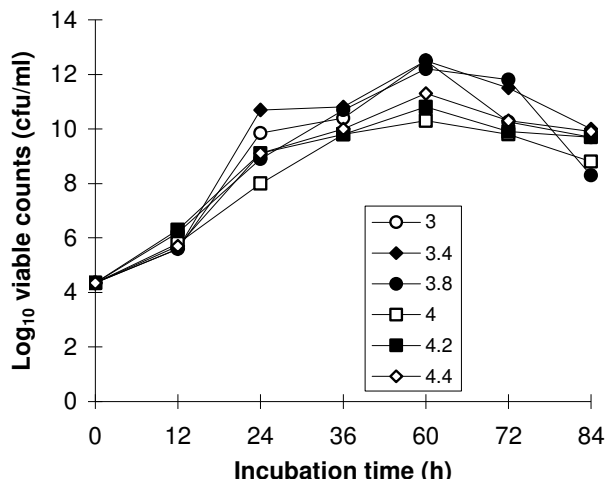


Figure 1. Effect of pH (3.0 - 4.4) on growth of *Candida* sp. in orange waste medium at 25°C.

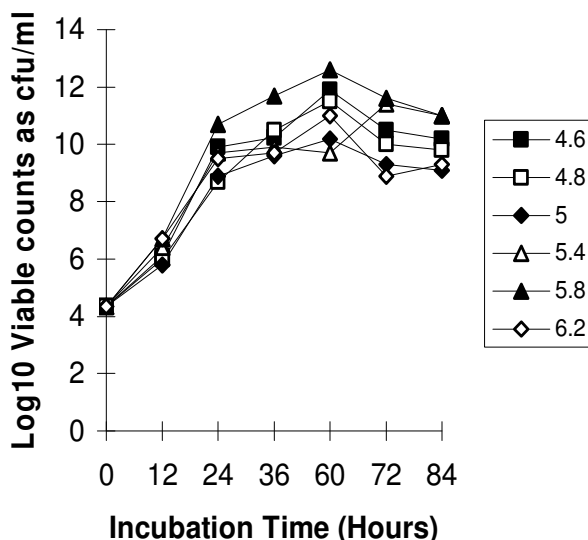


Figure 2. Effect of pH (4.6 - 6.2) on growth of *Candida* sp. in orange waste medium at 25°C

Table 1. Amino acid requirements of *Candida* sp.

Amino acid deficiency	Growth
Tryptophan	+
Methionine	+
Isoleucine	+
Arginine	+
Histidine	+
Threonine	+
Adenine	+
Uracil	+
Phenylalanine	+
Tyrosine	+
Leucine	+
Lysine	+
Valine	+

amino acids and bases screened. This indicates that the test strain when grown in culture is able to supply its amino acid requirements and could therefore be cultivated and harvested as a nutritional source of these amino acids and bases.

Influence of optimum substrate concentration

Results of tests with the selected strain of *Candida* sp. for the determination of optimum substrate concentration for growth as illustrated in Figure 3 show that growth, as total viable counts, was highest (12.5) for the substrate concentration of 2.0% (w/v). On the other hand, the growth levels were approximately equal at substrate concentrations of 1.2 and 1.5% (11.8) and least at 1.0%. The results also show that for the substrate concentrations 1.2, 1.5, 1.7 and 2.0% w/v the peak growth was

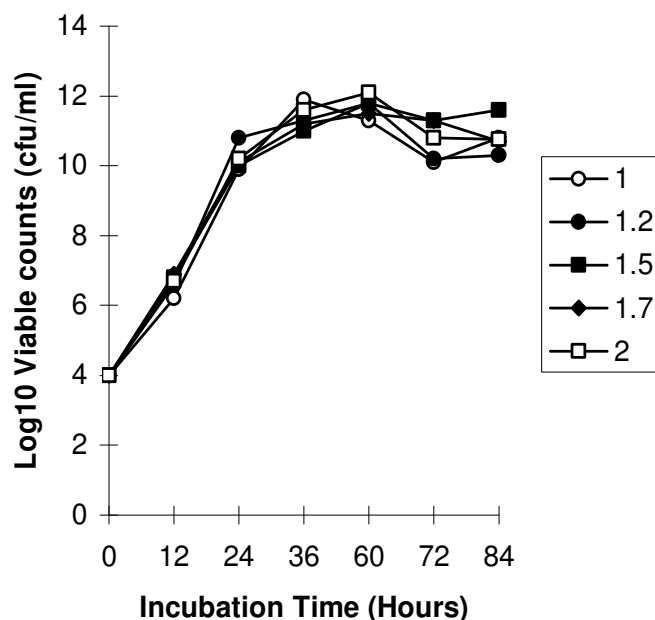


Figure 3. Effect of substrate concentration (% w/v) on growth of *Candida* sp. in orange waste medium at 25°C

obtained after 60 h. Comparatively, for the 1.0% concentration peak growth was observed after 40 h. Also, an increase in viscosity of the culture medium was noticed as substrate concentration was progressively increased. The substrate concentration, and therefore medium viscosity, would influence the growth of the test strain in terms of agitation/aeration efficiency. Solomons (1983) has also reported that substrate concentration affects the yield of

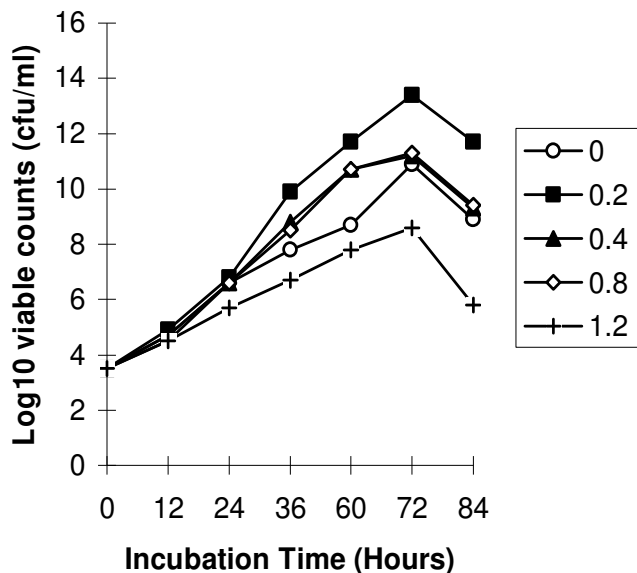


Figure 4. Effect of substrate supplementation (% w/v) with dextrose on growth of *Candida* sp. in orange waste medium.

Saccharomyces cerevisiae when grown on an assimilable carbohydrate such as glucose or sucrose. For this organism he reported that substrate levels in batch culture have to be kept very low, otherwise the substrate is oxidized to carbon dioxide and heat. He has similarly stated that this is one of the reasons why *Candida* species are preferred for single cell protein (SCP) production since they have a better regulation between anabolic and catabolic pathways which prevent waste of substrates.

Effects of supplementation with carbon, nitrogen and phosphorus

Substrate supplementation tests using varying concentrations of dextrose as illustrated in Figure 4 showed that the highest level of growth (13.4) determined as log₁₀ of total viable counts, was obtained with the 0.2% w/v supplementation. Comparatively, growth (11.3) at supplementation levels of 0.4 and 0.8% w/v was approximately two log cycles lower. The lowest growth was recorded at the 1.2% supplementation level. Growth in the unsupplemented medium was higher than the levels obtained with 0.4 and 0.8% (w/v). However, statistical analysis (ANOVA) of data obtained showed that at the incubation periods of 36, 48 and 60 h, supplement concentration has significant effect on growth of the test strain (at $n - 1 = 4$ d.f and 95% significance level). This indicated that supplementation with dextrose had a positive effect on the growth of the organism. This effect was higher with increase in incubation period.

Results of further supplementation tests using ammo-

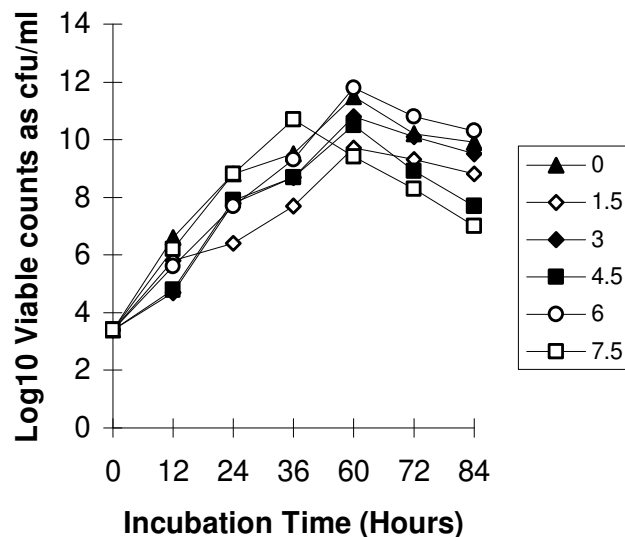


Figure 5. Effect of substrate supplementation (%v) with nitrogen as ammonium sulphate on growth of *Candida* sp. in orange waste medium at 25°C

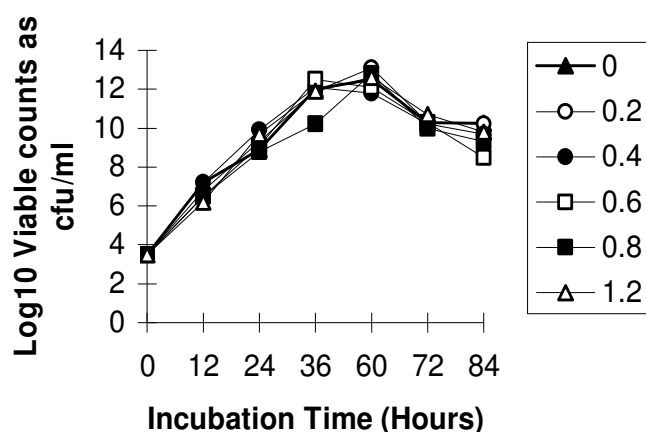


Figure 6. Effect of substrate supplementation (% w/v) with nitrogen as ammonium nitrate on growth of *Candida* sp. in orange waste medium

niun sulphate $[(\text{NH}_4)_2\text{SO}_4]$ are as illustrated in Figure 5. The results show that the growth levels (11.6) in the unsupplemented medium and 6.0% (w/v) supplementation were approximately equal but correspondingly lower at 1.5, 3.0 and 4.5% (w/v) (9.75, 10.85 and 10.7, respectively). At incubation time of 48 h, the level of growth (10.5) observed for the 7.5% supplementation was higher than that (9.75) at 1.5% (w/v) after 60 h by approximately one log cycle. A relatively lower growth was recorded for the 9.0% (w/v) supplementation.

Growth tests investigating the effects of supplementation with NH_4NO_3 showed that optimum growth (13.1) was obtained with 0.6% (w/v) level (see Figure 6). Com-

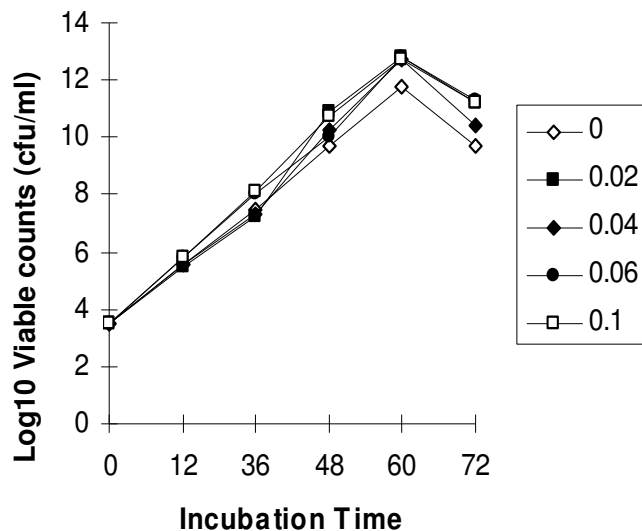


Figure 7. Effect of substrate supplementation with phosphorus (as KH_2PO_4) on growth of *Candida* sp. in orange waste medium.

paratively, a lower growth was recorded for the 1.2% level of supplementation. The result also showed that for the supplementation levels of 0.0 - 0.8%, maximum growths were recorded after 54 h. On the other hand, this time was reduced to 36 h in the case of the 1.2% (w/v) ammonium nitrate addition. Generally, the results showed that better growth (12.8 - 13.1) occurred at the supplementation levels of 0.0 - 0.3 percent.

Data on results of supplementation tests with KH_2PO_4 as shown in Figure 7 indicate that although growth in the unsupplemented medium was lower (11.8) compared to that recorded in the supplemented medium (12.8), the difference between the individual supplementation levels was much lower. For example, the difference in the level of growth (as cfu/ml) recorded for the supplementation levels 0.4, 0.06 and 0.10% was much lower than one log cycle at the peak growth point.

Further tests investigating the effects of mixed substrate supplementation on growth of the test strain of *Candida* sp. were also carried out and results are presented in Figure 8. The highest growth was recorded at the 0.15%/0.6% (w/v) dextrose/ammonium nitrate combination, and this was more than one log growth cycle greater than the unsupplemented medium at peak growth point. On the other hand the lowest growth was obtained with the 0.05%/0.2% (w/v) combination. In all cases, optimal growth was recorded after 60 h incubation at room temperature.

One criterion that is crucial in the selection of a yeast strain for protein production is its ability to grow in unsupplemented substrates or where supplementation is made it is simple and cheap. This criterion is satisfied with the results obtained where the test strain was found to grow well even in unsupplemented substrates. The higher level of added nitrogen indicates that the level of

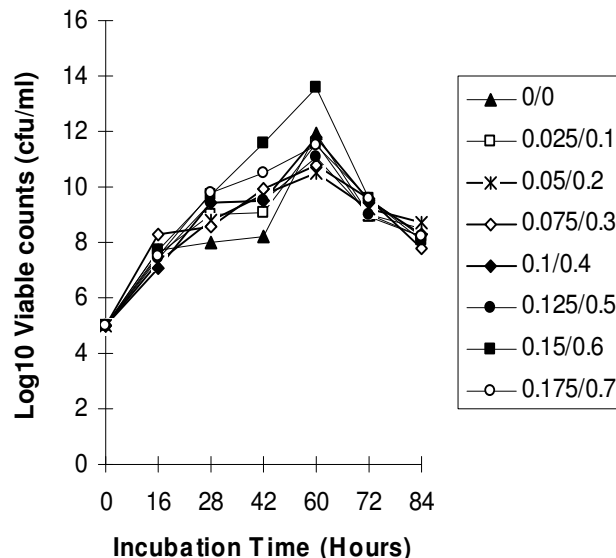


Figure 8. Effect of mixed supplementation (% w/v C & N) on growth of *Candida* sp. in orange waste medium.

nitrogen in the growth substrate was more limiting than that of the sugars. Whereas supplementation with nitrogen source showed an increase in growth of the test strain, no such marked effect was observed when the production medium was supplemented with a source of phosphorus. This result could be explained in part by the relatively high levels of phosphorus in the substrates, and compared to carbon and nitrogen the level of phosphorus required by growing organisms is lower.

Effect of substrate type on cell biomass production

A comparison of biomass production by the test strain in the three substrates as presented in Table 2 revealed that productivity was highest for orange in the supplemented and unsupplemented conditions. This confirms results obtained when growth was studied under unsupplemented conditions (pH unadjusted) in earlier experiments. Similarly, the absence of any marked advantage in supplementation in the case of banana was also observed when viable counts were monitored.

Similar to the biomass measurements, the increase in crude protein content of the test substrates was also studied. Results indicated that protein production for orange was approximately double that recorded for plantain, and more than double that for banana. The level recorded for orange is comparable to that reported by Lequerica and Lafuente (1977). They showed that using *Candida* sp. in semi-solid fermentation of orange peels, the protein content was increased from 7.3 to 18.5%.

Conclusion

This study showed that *Candida* sp. when employed in

Table 2. Comparison of biomass/protein production by *Candida* sp. in unsupplemented and supplemented fruit wastes media after 60 h incubation (pH 4.6, 25°C).

Substrate	Biomass production (Kg cell wt/Kg fruit waste)		Crude protein content (%)	
	Unsupplemented	Supplemented	Initial	Final
Orange	1.33 ± 0.1	1.60 ± 0.05	8.8 ± 0.3	17.5 ± 0.1
Plantain	0.67 ± 0.2	1.33 ± 0.1	5.4 ± 0.24	8.5 ± 0.15
Banana	0.67 ± 0.1	0.67 ± 0.1	5.1 ± 0.08	7.9 ± 0.01

cell biomass production using citrus fruit wastes was capable of meeting its nutritional requirements in culture when supplied with inorganic nitrogen sources. The organism grew at 37°C. Supplementation of media with 0 - 15% and 0 - 6% (w/v) combination of dextrose and ammonium nitrate, respectively, resulted in optimal growth at a pH range of 3.0 – 5.8 after 6.0 h. However, pH of 3.0 should be employed to reduce growth of saprophytic contaminants. Also, supplementation with phosphorus is not critical for growth. The relatively low growth temperature and minimal supplementation requirements makes this strain of *Candida* sp. a good candidate for crude cell biomass production. This property could be employed in the protein enrichment of animal feed supplements.

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