A Survey of Ascorbic Acid Producing Fungi In The Soil Environment Of Keffi Metropolis, Nasarawa State, Nigeria

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Abstract: A survey was carried out to determine the presence of ascorbic acid producing fungi in the soil environment of Keffi metropolis, Nigeria. Soil samples were obtained from ten different locations by random sampling technique. Soil dilution and direct soil plate methods were employed for the isolation of the soil fungi. The species isolated included *Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Cladosporium* spp, *Curvalaria lunata, Penicillium brevicompactum, Rhizopus stolonifer* and *Trichoderma viride.* The results further revealed that five of the isolates, *A. niger, R. stolonifer, A. flavus, P. brevicompactum,* and *T. viride* were found to produce ascorbic acid at concentration levels of 4.9mg/ml, 2.9mg/ml, 1.3mg/ml, 1.0mg/ml and 0.8mg/ml, respectively. The results of this survey demonstrated that ascorbic acid producing fungi are present in the soil environment of Keffi, and the fact that *A. niger* and *R. stolonifer* produced 4.9mg/ml and 2.9mg/ml of ascorbic acid in the fermentation broth suggests that these fungi species could be harnessed locally for large scale industrial production of ascorbic acid.

Keywords: survey**,** ascorbic acid, fungi, soil, Keffi, Nigeria.

Introduction

Ascorbic acid commonly called vitamin C is a dietary factor which must be present in the human diet to prevent scurvy, and as an antioxidant has been identified as an agent that increases resistance to infection (Null and Garry, 2001). Ascorbic acid was discovered after scientist had searched for centuries for the cure of a disease known as scurvy (Huskey, 2000). The name ascorbic acid came from the word “anti-scurvy” acid; because it was known to dramatically cure this diseases. This disease was caused by a serious deficiency of vitamin C, and it makes the victim’s small blood vessels to rapture, bones to weaken, and joint to swell, among other symptoms (Null and Garry, 2001). The symptoms of this deficiency was due to the fact that without a source of vitamin C, one develops severe problems concerning the body’s connective tissues, which are found in bones, skin, muscles, teeth, blood vessels, and cartilage (Ronzio, 1997). According to Thurlow *et al*. (1991), the deficiency of ascorbic acid would eventually lead to death if it goes untreated, and this is not uncommon, especially during the winter months of the year.

Ascorbic acid is an important metabolite for most living organisms. In higher organisms, it is indispensable for different physiological functions and thus becomes an essential nutrient for animals (like humans) lacking its biosynthetic pathway. From the variety of cellular functions affected by ascorbate (Padh, 1990; Padh, 1991), its role in the synthesis of collagen has been clearly established. Beneath the maintenance of metal ions in their reduced form, ascorbic acid acts as a scavenger ofreactive oxygen molecules. Hence, an important function is to protect tissues from harmful oxidative products. It is this capacity that attracts attention to this compound and that leads to an increasing demand for ascorbic acid as a food additive.

Industrially produced ascorbic acid is widely used in the feed, food, and pharmaceutical sector as nutritional supplement and preservative, making use of its antioxidative properties. Ascorbic acid is traditionally manufactured chemically in a complex step of reactions from glucose or intermediates of ascorbic acid such as 2 keto-L-gulonic acid. Until recently, the Reichstein-Grüssner process, designed in 1933, was the main industrial route. Here, D-sorbitol is converted to L-ascorbic acid via 2-keto-L-gulonic acid (2KGA) as key intermediate, using a bio-oxidation with *Gluconobacteroxydans* and several chemical steps (Ji and Gao, 2001).

The industrial uses and application of ascorbic acid depend on its chemical properties as an antioxidant or on its health related properties (Null and Garry, 2001). About one-third of total production is used for vitamin C preparation in the pharmaceutical industry. The rest is mainly applied as an additive to food and feed to enhance product quality and stability. Derek (1996) reported that ascorbic acid added to food stuffs during processing or before packing do protect colour, aroma and nutrient contents.

Fungi are diverse group of either single-celled or multicellular organisms that obtain food by direct absorption of nutrients (Cooke and Whipps 1993). Fungi are vital for their ecosystem functions, while some are used in the processing and flavouring of foods, and others are used in the production of antibiotics and organic acids. Some species of fungi are used for the commercial production of different kinds of organic acids such as ascorbic acid, citric acid, gluconic acid, and gallic acid (Roland *et al*., 1986; Onofri *et al*., 1997). Yet others are useful in the

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manufacturing of inks and dyes, commercially usable oils have been obtained from species of several genera of fungi (Bomstein and Johnson, 1992).
This investigation therefore aims at isolating ascorbic acid producing fungi present in the soil environment of Keffi, and to evaluating the level of ascorbic acid produced by each isolate with the view to harnessing the isolates for industrial production of ascorbic acid.

Materials and Methods

Study Area

This study was carried out in Keffi metropolis, Nasarawa State, Nigeria.Keffi lies between latitude 7^{0}45N and 9^{0}25N of the equator and between longitude 7^{0} 5'E and 9^{0} 37E. Keffi is about 68km from Abuja the Federal Capital Territory of Nigeria and 128 km from Lafia the state capital, it is situated on an altitude of 850meters above sea level, (Akwa *et al.*, 2007).

Sample Collection

Soil samples of 250g each were obtained aseptically using spatula from ten (10) different locations in Keffi town, this includeAngwanLambu, AngwanTiv, B.C.G Area, DadinKowa, High court, G.R.A, Nasarawa State University (main campus), Pyanku campus, Total round about, and Yelwa, into sterile plastic containers of 300g capacity.The samples were taken immediately to the microbiology laboratory, Nasarawa State University, Keffi, Nigeria, where they were analyzed.

Physico-Chemical Properties of the Soil Sample

Soil Types

The soil samples from each site were sieved characterized by the Unified Analysis Techniques as recommended by Pettijohn (2000) and Whitbread *et al.* (1996).

pH

The pH values of the soil were determined by digital pH meter using standard method of Watson and Brown (1998). Using this method, 3g of soil sample was weighed into a beaker containing 3ml of distilled water, which was stirred for 5 seconds and allowed to stand for 10 minutes. The electrode of the pH was then inserted into the slurry and swirled carefully. The reading was taken thereof and the average of three consecutive reading were recorded for each site.

Temperature

The temperature of the soil samples was determined by the use of field thermometer. The thermometer was inserted into the soil to the depth of 5cm and allowed to stay for 10 minutes, after which the temperature reading was obtained. The averages of three consecutive readings were recorded for each site (Dix and Webster, 1995).

Isolation and Identification Of Fungi

Serial dilutions of soil samples from each site were prepared. One milliliter (1ml) of the diluted sample (10^{3}) was transfered into a petri dish containing a solid medium of Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA), to which 30mg/L of streptomycin and penicillin were incorporated just before pouring into petri dishes. The plates were incubated at 30°C for 7days and observations were made daily to determine the presence of filamentous fungi (Nester *et al.,*1998)The fungal isolates were identified based on their cultural, morphological and biochemical characteristics using standard methods of Domsch *et al*. (1980) and Barnet and Hunter (1998) The morphological features were determined by staining the mycelium with lacto phenol cotton blue and observed under the microscope and comparing with compendium of soil fungi (Domsch *et al*., 1980).

Determination of Ascorbic Acid Production by Fungal Isolates

Iodine titration method of Morris and Jacobs (1999) was employed for the determination of ascorbic acid production by the fungal isolates. The fungal isolates were inoculated into nutrients broth and incubated at 30°C for 4 days (Manguson and Linda, 2007). The medium (Nutrient broth) was centrifuge after the incubation period. The supernatant (5ml) was obtained and diluted with 20ml of sterile distilled water. The solution was titrated rapidly with an accurately standardized 0.7g iodine solution containing 8g of potassium iodide per 500ml. One percent soluble starch was used as indicator, and a characteristic blue-black colour change indicated the presence of ascorbic acid (Morris and Jacobs, 1999). Each milliliter of iodine is equivalent to 0.88g of ascorbic acid. Therefore, the milligram of ascorbic acid per milliliter (mg/ml) was then quantified by their relationship.

Statistical Analysis

Averages, mean deviation and test for least significant difference were employed for analyses. Percentage occurrence for each species of fungi isolated was determined using the methods of Sampo *et al.* (1997).

Results

The results of Physico-Chemical Properties of Soil Samples of the different locations in Keffi Metropolis are shown in Table 1, while The Total Fungal Counts of Soil Samples are shown in Table 2. Tables 3 shows the Percentage Frequency of Occurrence of Fungal isolates, while Table 4 shows the Cultural Characteristics of Fungal isolates on Malt Extract Agar (MEA), whereas Table 5 shows the Fungal isolates and their Ascorbic acid production level.

Table 1: Physico-chemical properties of soil sample of different locations in Keffi town

Locations	Soil Types	pH	Temperature (^{o}C)
AngwanLambu	Loamy	8.4±0.2	26±0.4
AngwanTiv	Loamy	6.0±0.1	25±0.1
B.C.G Area	Loamy	8.4±0.2	26±0.4
DadinKowa	Clay	7.0±00	26±0.4
G.R.A	Loamy	6.1±0.1	25±0.1
High Court	Loamy	7.0±0.0	25±0.1
NSUK (MainCamp)	Sandy	6.5±0.0	26±0.4
Pyanku Campus	Sandy	5.5±0.1	26±0.4
Total Round About	Sandy	6.4±0.3	26±0.4
Yelwa	Sandy	5.4±0.1	25±0.1

Table 2: Total Fungal Counts of soil samples from different locations of Keffi metropolis

Locations	Total Fungal count (TFC/g)
AngwanLambu	2.0 x 10^{4}±1.0
Angwan Tiv	2.9 x 10^{4}±0.1
B.C.G Area	1.8 x 10^{4}±1.2
DadinKowa	1.5 x 10^{4}±1.5
G.R.A	3.6 x 10^{4}±0.6
High Court	2.8 x 10^{4}±0.2
NSUK (Main Campus)	4.5 x 10^{4}±1.5
Pyanku Campus	1.6 x 10^{4}±1.4
Total round about	3.9 x 10^{4}±0.9
Yelwa	5.2 x 10^{4}±1.2

Table 3: Percentage Frequency of fungal isolates

Fungal Isolates	Sites A B C D E F G H I J	Occurrence (%)
Alternaria alternata	+ - - - - + - - - -	20
Aspergillus flavus	- - + - - - + + - -	30
Aspergillus fumigates	- - + - - - - - - -	10
Aspergillusniger	+ + - - + + - + - +	60
*Cladosporium*Spp	+ - + - - + - - - -	30
Curvularialunata	+ - - - - - - - - -	10
Penicillium Brevicompactum	+ - + - + - - - - +	40
Rhizopus stolonifer	+ - - + - + - + + -	50
Trichoderma viride	+ - + + - - + - - -	40

Key: A = Angwan Lambu, B = AngwanTiv, C = B.C.G Area,D = DadinKowa, E = G.R.A, F = High Court, G = NSUK Main Campus, H = Pyanku Campus, I = Total Round About, J = Yelwa

Table 4: Cultural characteristics of fungal isolates on malt extract agar

Isolates	Surface	Reverse
Alternaria alternata	Greenish black	Brownish
Aspergillus flavus	Greenish yellow	Reddish brown
Aspergillus fumigatus	Blue – Green	White
Aspergillus niger	Black	Yellowish
Cladosporium spp	Milkish brown	Brownish black
Cuvularia lunata	Pinkish grey	Brownish black
Penicillium brevicompactum	Greenish	Yellowish
Rhizopus stolonifer	Grayish white	Whitish pale
Trichoderma viride	Whitish green	Pale yellowish

TABLE 5: Ascorbic acid production by fungal isolates

Fungal Isolates	Ascorbic Acid Production	Quantity (mg/ml)
Alternaria alternate	-	0.00±0.0
Aspergillus flavus	+	1.32±0.2
Aspergillus fumigates	-	0.00±0.0
Aspergillus niger	+	4.87±0.3
Cladosporuim spp	-	0.00±0.0
Cuvularia lunata	-	0.00±0.0
Penicillium brevicompactum	+	1.02±0.1
Rhizopus stolonifer	+	2.91±0.3
Trichoderma viride	+	0.84±0.2

Key: + = Present, - = Absent

Discussion

The soil pH of Keffi ranged from 5.4 and 8.4, which implies that the soil of Keffi at some locations is acidic, while at other locations it is alkaline. The temperature of the soil at the time of this investigation (May/June) had a very narrow temperature range of between 25^0C and 26^0C, and this most likely because the investigation was carried out in rainy season. These results are similar to those reported earlier (Makut and Godiya, 2010; Makut and Owlewa, 2011)

Among all the fungi isolated, *Aspergillus niger* is the most abundant, followed by *Rhizopus stolonifer*, *Penicillium brevicompactum, Trichoderma viride*, *Aspergillum flavus, Cladosporium* spp., *Alternaria alternata*, *Aspergillus fumigatus* and *Curvularia lunata* lowest in this order respectively. The identity of these isolates were established to be in confirmomity with the report of Barnett and Hunter (1998), and also agrees with the previous reports of Makut and Owolewa (2011).

Ascorbic acid production was highest in *A. niger*, followed by *R. Stolonifer*, *P. brevicompactum* and *T. viride*. However, low production were observed in *A. alternata*, *A. fumigatus*, *Cladosporium* spp, and *C. lunata.* This is in line with the report by other workers that fungi are known to produce diverse kinds of organic acids including ascorbic acid (Roland *et al.,* 1986; Onofri *et al.,* 1997)

Conclusion

This investigation demonstrated that *Aspergillus niger*, *Aspergillus flavus*, *Penicillium brecompactum, Rhizopus stolonifer* and *Trichoderma viride* do produce some appreciable quantities of ascorbic acid, and such potential can be harness for the use of locally isolated strains of fungi for the industrial production of ascorbic acid.

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