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Full Length Research Paper

Waste to wealth: Production of oxytetracycline using streptomyces species from household kitchen wastes of agricultural produce

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The production of oxytetracycline by *Streptomyces speibonae* OXS1 in solid-state fermentation from cocoyam peels (household kitchen wastes of agricultural produce) was investigated. The proximate analyses of peels of the two cocoyam species showed that *Colocasia esculenta* had higher protein (1.39%) and fibre (15.70%) contents than *Xanthosoma esculenta* with protein and fibre contents of 0.91 and 6.95%, respectively. Oxytetracycline was detected on the first day of fermentation and reached its peak on the third day. The optimum moisture content of the substrate for the production of the antibiotic was 65% at room temperature, and a pH range of 5.8 to 6.0. Seven days fermentation gave higher biomass weight (140.86 g) for C. *esculenta* than *X. esculenta* (101.62 g), indicating a possible higher presence of the anticipated fermentation product (oxytetracycline) in the fermentation jar of the former. Bioassay for determination of the antibiotic presence confirmed that oxytetracycline was present in both species of the cocoyam peels, but in higher amounts in *C. esculenta* at every instance. Cocoyam peels- a common household kitchen wastes, that otherwise would have become breeding foci for disease pathogens, are by the outcome of this study shown to be bioconvertible into substrates for the production of oxytetracyclines, an important class of antibiotics that are vitally useful both for the health care delivery system and agro-poultry industry, etc. The fact that cocoyam peels could become very useful substrate for the production of this very important class of drugs is indeed a big plus both for the pharmaceutical industry and public health programme of environmental health and safety. This application also offers an alternative waste management option for this class of household kitchen wastes of agricultural produce that is usually present in great abundance in our environment. By so-doing, these wastes with great potential for environmental degradation, pollution and disease causation, are turned into raw materials for the pharmaceutical industry, thus, becoming a veritable resource for industrial growth, with possible positive impacts of this exploitation on job and wealth creations for national economic prosperity. Added to these are the public health impacts of a safer and healthier environment likely to be secured through the indirect waste management option so offered.

Key words: Oxytetracycline production, streptomyces, household kitchen wastes.

INTRODUCTION

Antibiotics are substances produced by microorganisms, which selectively suppress the growth or kill other microorganisms at very low concentrations (Tripathi, 2008). They can also be defined as the complex chemical substances the secondary metabolites that are produced by microorganisms and act against other micro-

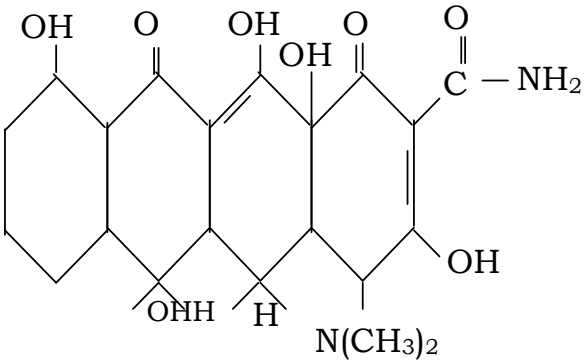

Figure 1. Structure of tetracyclines (Henry, 2007).

organisms (Dubey, 2008). They are classified based on:

Chemical structure: Sulfonamides and related drugs; diaminopyrimidines; quinolones, β – lactam antibiotics; tetracylcines including oxytetracycline, chlortetracycline, doxycycline etc; nitrobenzene derivatives; amino-glycosides; macrolide antibiotics, lincosamide antibiotics; glycopeptide antibiotics; oxazolidinone; polypeptide antibiotics; nitrofuran derivatives; nicotinic acid derivatives; polyene antibiotics; azole derivatives and others including rifampin, spectinomycin, cycloserine, griseofulvin etc (Tripathi, 2008).

Types of organisms against which they are primarily active: Antibacterial for example, penicillins, aminoglycosides, erythromycin etc; antiviral for example, acyclovir, amantudine, zidovudine etc; antifungals for example, griseofulvin, amphotericin B, ketoconazole; antiprotozoal: chloroquine, pyrimethamine, diloxanide etc; antihelminthic for example, mebendazole, pyrantel, niclosamide etc.

Mechanism of action: Inhibition of cell wall synthesis for example, penicillins, cephalosporins; causing leakage from cell membranes for example, the polypeptide including polymxins, colistin, and polyenes like amphotericin B etc; inhibition of protein synthesis for example, tetracyclines, chloramphenicol, and erythro-mycin, etc; causing misreading of m-RNA code and affecting permeability for example, aminoglycosides including streptomycin, gentamicin, neomycin etc; inhibition of DNA gyrase for example, fluoroquinolones such as ciprofloxacin, ofloxacin, levofloxacin; interference with DNA synthesis for example, Acyclovir, zidovudine; interference with DNA function for example, rifampin, metronidazole; and interference with intermediary meta-bolism for example, sulfonamides, sulfones, ethambutol, etc (Tripathi, 2008).

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Spectrum of activity: Some are narrow spectrum for example, penicillin G, streptomycin, erythromycin, oxazolidinones, ciprofloxacin; while others are broad spectrum for example, chloramphenicol, levofloxacin, tetracyclines.

Types of action: Some are primarily bacteriostatic for example, sulfonamides, tetracyclines, erythromycin, clindamycin etc. Some bacte-riostatic drugs may become bacteriocidal at higher concentrations for example, erythromycin. Others are primarily bacteriocidal for example, penicillins, cephalo-sporins, vancomycin, ciprofloxacin, isoniazid, rifamipin etc (Tripathi, 2008). Again, some bacteriocidal drugs may become bacteriostatic under certain conditions for example, streptomycin and clotrimoxazole.

Tetracyclines and oxytetracyclines

Tetracyclines are a class of antibiotics having a nucleus of four cyclic rings (Figure 1). Examples include chlortetracycline, oxytetracycline, demeclocycline, metha-cycline, rolitetracycline, iymecycline, doxycycline, minocycline, etc.

All are obtained from soil actinomycetes. The first to be introduced was chlortetracycline in 1948 under the name aureomycin (Dellit and Hoofen, 2006). It contrasted markedly from penicillin and streptomycin (which were the antibiotic available at that time) in being active orally, and in affecting a wide range of microorganisms, hence called broad spectrum antibiotics. Oxytetracycline soon followed; others were produced later either from mutant strains or semi synthetically. All tetracyclines are slightly bitter solids which are weakly water soluble, but their hydrochlorides are more soluble. The subsequently developed members have high lipid solubility, greater potency and some other differences (Henry, 2007).

In terms of mechanism of action, the tetracyclines are primarily bacteriostatic; inhibit protein synthesis by binding to 30S ribosomes in susceptible organisms. Sub-sequent to such binding, attachment of aminoacyl RNA to the mRNA ribosome complex is interfered with, thus the peptide chain fails to grow (Tripathi, 2008). The sensitive organisms have an energy dependent active transport process which concentrates tetracycline intracellularly.

The more lipid-soluble members (doxycycline, mino-cycline) enter by passive diffusion. Two factors are responsible for the selective toxicity of tetracycline for the microbes and these include: the absence of the carrier involved in active transport of tetracycline in the host cells and the less sensitivity of protein-synthesizing apparatus of the host cell to tetracycline (Black and Allan, 2007). Talking about their antimicrobial activities, the tetracyclines are broad spectrum.

All Gram positive and Gram negative cocci were originally sensitive, but now many *streptococcus*

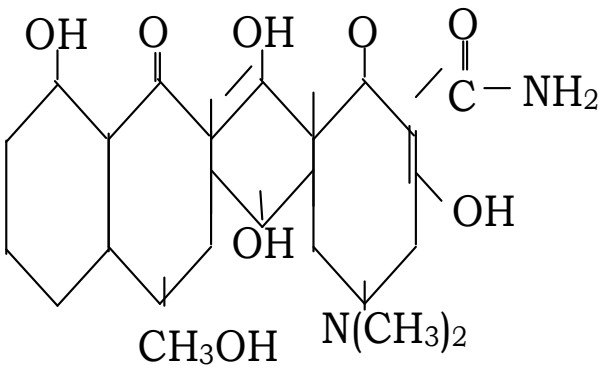

Figure 2. Oxytetracycline, with tetracycline nucleus.

pyogenes, Staphylococcus aureus and *enterococii* have become resistant. Tetracyclines (especially miocycline) are non active against few *Neisseria gonorrhoeae*, and *Neisseria meningitides.* Most Gram-positive bacilli for example, *Clostridia* and other anaerobes *Listeria*, *Corynebacteria*, *Propionibacterinum acnes* are inhibited but not Mycobacteria, except some atypical ones. Sensitive Gram negative bacilli include *Calymmatobacterium granulomatis, Vibro cholerae, Yersinia pestis, Helicobacter pylori*, *Pasteurella multocida, Brucella* and many anaerobes.Spirochetes (*Treponema pallidium* and *Borrelia*) are also quite sensitive, while all rickettsiae (typhus) and chlamydiae are highly sensitive. However, *Entamoeba histolytica* and plasmodia are inhibited at high concentration (Tripathi, 2008). Resistance to tetracycline develops slowly in a graded manner. In such bacteria, usually the tetracycline concentrating mechanism becomes less efficient or the bacteria acquire capacity to pump it out. Another mechanism is plasmid mediated synthesis of a protection protein which protects the ribosomal binding site from tetracycline.

In terms of pharmacokinetics, tetracyclines are most commonly administered through the oral route, and capsule, the most common dosage form, should be taken ½ h before or 2 h after food. Tetracyclines are not recommended by intramuscular route because it is painful and absorption from the injection site is poor (Elmer et al., 1996). Slow intravenous injection may be given in severe cases but it is rarely required. A variety of topical preparations (ointments, creams) are available but rarely used due to high risk of sensitization (Archer et al., 2001). However, ocular application is not contradicted. The older tetracyclines are incompletely absorbed from gastro intestinal tract, absorption is better if taken in empty stomach. Doxycycline and minocycline are completely absorbed irrespective of food. Tetracyclines have chelating property, form insoluble and unabsorbable complexes with calcium and other metals (Henry, 2007). Milk, iron preparations, non-systemic antacids and sucralfate reduce their absorption. Tetracyclines are widely distributed in the body (volume of distribution >1 L / kg) (Tripathi, 2008). Variable degree of protein binding is exhibited by different members. They are concentrated in liver, spleen and bind to the connective tissue in bone and teeth. Intracellularly, they bind to mitochondria. Mincocycline accumulates in body fat. The cerebrospinal fluid (CSF) concentration of most tetracycline is about ¼ of plasma concentration (Steinman, 2003). Most tetracycline is excreted in urine by glomerular filtration. They are partly metabolized and significant amounts enter bile, some are secreted in milk in amounts sufficient to affect the suckling infant. Enzyme inducers like phenobarbitone and phenytoin enhance metabolism and reduce the t1/2 of doxycycline.

The broad spectrum nature of tetracycline and therefore their wider application meant that a large quantity of this drug is being consumed, with the possibility of hitherto sensitive organisms becoming resistant. Hence there is urgent need to search for new forms of the same drug that will overcome the short-coming of a shorter half life and of resistance by some species of organism hitherto sensitive to the drug. Oxytetracyclines are among the new brand of tetracyclines resulting from that search.

Oxytetracyclines

Oxytetracycline was the second member of the tetracycline group to be discovered in 1950 from soil Actinomyces, *Streptomyces rimosus* (Henry, 2007). Its systematic IUPAC name is 2-(amino-hydroxy-methylidene)-4-dimethylamino–5, 6, 10, 11, 12 a hexahydrotetracene –1, 3, 12- trione. The structure of oxytetracycline contains nucleus tetracycline as shown in Figure 2.

Oxytetracycline is a bacteriostatic antibiotic that inhibit protein synthesis by binding reversibly to the 30S ribosomal subunit of the microorganism (Tripathi, 2008). Oxytetracycline is a broad-spectrum antibiotic (Archer et al., 2001). It is therefore a very important class of antibiotics. Oxytetracycline is amphoteric and forms salts with both acids and bases, may be in capsule, tablet, liquid or injectable forms (Scott, 2004). The base is slightly soluble in water but the hydrochloride is readily soluble. Both base and hydrochloride are readily soluble in dilute hydrochloric acid, fairly soluble in methyl and ethyl alcohol but insoluble in chloroform and ether (Henry, 2007). In the dry state, it is stable for at least two years at room temperature and relatively stable in acidic solution especially below pH 2.5. Solutions of oxytetracyclines are affected by temperature. At pH 2.5 and room temperature, unbuffered solutions are quite stable, but at the same pH and 37°C, the half life of the antibiotic is about 5½ days (Scott, 2004).

Sources

Oxytetracycline and other antibiotics had been produced by variety of methods. Some may be obtained from a semi solid culture where low water content and high degree of aeration at the surface favours the production of antibiotics (Yang and Swei, 1996). Oxytetracyclines had been produced from various strains of streptomyces organism predominantly found in the soil and decaying vegetation. Several agro-industrial waste and by-products such as sweet potato residue (Yang and Ling, 1989), saw dust, rice hulls and corn cob (Yang and Swei, 1996), cassava peel, corn pomace, corncob and groundnut shell (Asagbra et al., 2005) are effective substrates for the production of antibiotics by solid-state fermentation. Solid-state fermentation is defined as the process in which microbial growth and products formation occur on the surfaces of solid substrates in the near absence of water. Oxytetracycline, a quinone antibiotic commonly called terramycin, is used in human and veterinary medicine and as a supplement in poultry and swine production, preservation of fish, meat and poultry (Yang and Swei, 1996; Humber, 2001). It is also used in non-therapeutics for the control of plant diseases, stimulation of amino acid fermentation and inhibition of material bio-deterioration (Archer et al., 2001; Asagbra et al., 2005).

Streptomyces, the largest genus of actinobacteria are a group of Gram-positive bacteria that generally have high guanine-cytosine (GC) content (Walve et al., 2001; Hopwood, 2007). They are predominantly found in the soil, decaying vegetation and noted for their distinct earthly odour which result from the production of a volatile metabolite geosmin (Kieser et al., 2000). They make use of wide range of organic compounds as sole sources of carbon for energy and growth (Brawner et al., 2001). The optimum temperature is 25 to 35°C; some species grow in temperatures within the psychrophilic and thermophilic ranges. The optimum pH range for growth is 6.5 to 8.8. They have over 500 species including *Streptomyces ambofaciens, Streptomyces noursei, Streptomyces griseus, Streptomyces hygroscopicus, Streptomyces rimosus*, *Streptomyces speibonae*, etc (Kieser et al., 2000), with a few species being pathogenic for animals, plants and humans.

Their ability to produce oxytetracycline from a widerange of organic compounds has been quite phenomenal. Equally phenomenal is the wider applications/ consumption of this antibiotic because of its broad spectrum nature. This means that for a long time to come there would continue to be a need for this drug. Therefore, the search for alternative substrates for enhanced production base of oxytetracycline would possibly remain an endless one for a long time to come. Here lies the justification for the present study- to explore the potentials of cocoyam peels as possible substrate for the production of oxytetracyclines. Many organic compounds such as corncob, groundnut shell, cassava peel, corn pomace (Asagbra et al., 2005); Saw dust, rice husks and corn cobs (Yang and Swei, 1996) had served as production substrates for oxytetracycline.

Cocoyams belonging to the genera *colocasia* and *xanthosoma* and family Araceae, are tropical flowering plant grown primarily for the starchy corms. Cocoyams have over the years been a permanent feature of the farming system in South East and South West, Nigeria. It is the third most important tuber crop cultivated after yam and cassava. The starchy corms serves as a dietary fibre, it can be boiled, consumed with palm oil or used in making sauce. It can also be fried and served as chips using the species *X. esculenta*. In all these instances or applications, their peels remain wastes which must be disposed off immediately or it decomposes, becoming nuisance and a source of environmental degradation/ pollution. Thus, a major aim of this study is to explore cocoyam peels as possible substrate for the production of oxytetracycline antibiotics. Success in this regard would become another way of adding value to this vital resource of agricultural produce, thereby also curbing the environmental menace likely to occur from improper disposal of these household kitchen (cocoyam peel) wastes, just as was previously established for orange peel wastes (Ezejiofor et al., 2011).

MATERIALS AND METHODS

Sources

Both the chemical reagents and materials used were all obtained from the Biotechnology Division, Federal Institute of Industrial Research, Oshodi, Nigeria. The media used was nutrient agar and nutrient broth (oxide Limited, Basingstoke Hampshire, England). Cocoyam, the raw material used was purchased from Agege market, Lagos, Nigeria. The cocoyam provided the peels used for fermentation substrate.

Proximate analyses

The two species of cocoyam *X. esculenta* and *C. esculenta* were analyzed for their moisture content, ash content, crude fibre and crude protein as described by AOAC (1990)

Bulk density

The dry weight or wet weight of the samples per unit volume (1 ml) was the bulk density in dry weight or wet weight respectively (Baver, 1956).

Sterilization of material

All glass wares were thoroughly washed with detergent rinsed several times over with tap water and then distilled water and sterilized in a hot air oven at 160°C for 1 h. All media used were sterilized at 121°C for 15 min before dispensing aseptically into Petri dishes. The surface of the table upon which all experimental work was performed was properly disinfected using purified ethanol before and after the experiment to avoid contamination.

Plate 1. Residue of two species of cocoyam (*X. esculenta* and *C. esculenta (*substrates for fermentation).

Microbiological media preparation

Nutrient agar was prepared according to the specification of the manufacturer (Oxide Limited, Basingstoke Hampshire England); approximately 0.5 ml of cyclohexamide was added to inhibit the growth of fungi. The medium was then poured into sterile Petri dishes and allowed to cool.

Subculturing of streptomyce

A flamed and cooled wire loop was used to pick colonies of *S. speibonae OXS1* from a stock culture to inoculate the nutrient agar in Petri dish. The Petri dish was then incubated at 28 to 30°C in an incubator for 48 h.

Preparation of growth media/inoculation of culture

Different salts components were weighed, dissolved with distilled water and sterilized for the growth of streptomyces, which requires rich nutritive medium (starch casein media). A flamed and cooled wire loop was used to pick discrete colonies of streptomyces from the nutrient agar plate and plated out on the sterilized starch casein medium and then incubated in a shaker incubator for aeration and agitation (since adequate supply of oxygen and proper mixing are necessary for maximum growth) at a speed of 240 rpm and temperature of 25°C for four days.

Preparation of fermentation medium

Cocoyams purchased from Agege market, Lagos, Nigeria, were peeled. The peels were weighed and treated by sun-drying in a sun box drier for 3 days. The dried peels were weighed and hammer-milled. This provided a suitable substrate which served as the sole carbon source for the organism to be used subsequently (Plate 1). Fifty gram (50 g) of milled *X. esculenta* and *C. esculenta* respectively was weighed out and transferred into two beakers each, each beaker containing 1 g of $(NH_4)_2S0_4$, 1 g of $MgSO_4.H_20$, 0.25 g K_2HP0_4 and 0.5 g of $CaC0_3$. The four beakers were respectively covered with aluminum foils and each held with a rubber band and sterilized at 121°C for 1 h in an autoclave and then allowed to cool.

Preparation of fermentation culture

This is the inoculation of the fermentation medium with streptomyces inoculum. The percentage moisture of the two species of cocoyam which was noted as 8% for *X. esculenta* and 6.5% for *C. esculenta* respectively were adjusted to 65% required for the fermentation using the inoculums and sterilized distilled water. Twenty-five milliter (25 ml) of the inoculums and 3.5 ml of the sterilized distilled water were added into each of the two beakers containing *X. esculenta*. Equal volume of the inoculums and 4.25 ml of sterilized distilled water were added into each of the two beakers containing *C. esculenta*. Each of the beakers was thoroughly stirred covered with flamed aluminum foil and incubated at 28°C for 7 days. Stirring of the beakers was carried out every day for seven days (Plate 2).

Isolation of antibiotics

At regular intervals, during the fermentation process, the culture mass was extracted with 4 volume distilled water by shaking at room temperature for 5 min and then filtering with whatman filter paper. The filtrate was then transferred and stored in a sterile

Plate 2. Fermenting substrates.

sample bottle (Plate 3).

Determination of antibiotic activity

Antibiotic activity of the extract was measured by the agar well diffusion method using *Bacillus subtilis* an indicator organism for oxytetracycline. Total concentration of oxytetracycline was calculated based on the diameter of the zone of inhibition.

RESULTS

Results of proximate analyses of the two species of cocoayam peels used for fermentation are shown in Table 1. Except for fat and carbohydrate, the percentage contents of all other analytes including fibre and protein were higher in *C. esculenta.*

In Table 2, bulk density weights of both loose and packed samples of the two species of cocoyam peel wastes are presented. Though packed samples of both species of cocoyam peel wastes gave higher bulk density weights than the loose ones, that of *X. esculenta* (32.5 g) was higher than that of *C. esculenta* (28.3 g).

Table 3 shows the result of 7-day fermentation in fermentation culture jars for *X. esculenta* (sample A) and *C. esculenta* (sample B) respectively. Sample B gave higher biomass weight (140.86 g), indicating a higher fermentation product than sample A (101.62 g). Tables 4 and 5 show the results of seven days oxytetracycline determinations using bioassay method in both finely-milled and coarsely-milled peels of both species of cocoyam. In all instances, finely-milled samples showed higher presence of oxytetracycline than the coarsely milled samples, and *C. esculenta* also showed elevated presence of the antibiotic than *X. esculenta* for both finely- and coarsely-milled samples.

DISCUSSION

Proximate analyses showed that substantial amount of agricultural wastes still contain several reusable substances of high value such as soluble sugars, protein, moisture, ash and fibre, all of which are of variable concentrations as shown in Table 1. Heavy sporulation was obtained after 7 days of fermentation on both sample A and B fermentation culture jars, though packed samples of both species of cocoyam peels wastes gave higher bulk density weights than the loose ones, that of *X. esculenta* (32.5 g) was higher than that of *C. esculenta* (28.3 g) (Plate 1 and Table 2). In this study, the production of oxytetracycline from isolated *S. speibonae OXS1* by solid-state fermentation using cocoyam residue substrate at room temperature, pH of 5.8 to 6.0 and moisture content of 65% progressed steadily as was evidenced by the steady increase from the initial weight

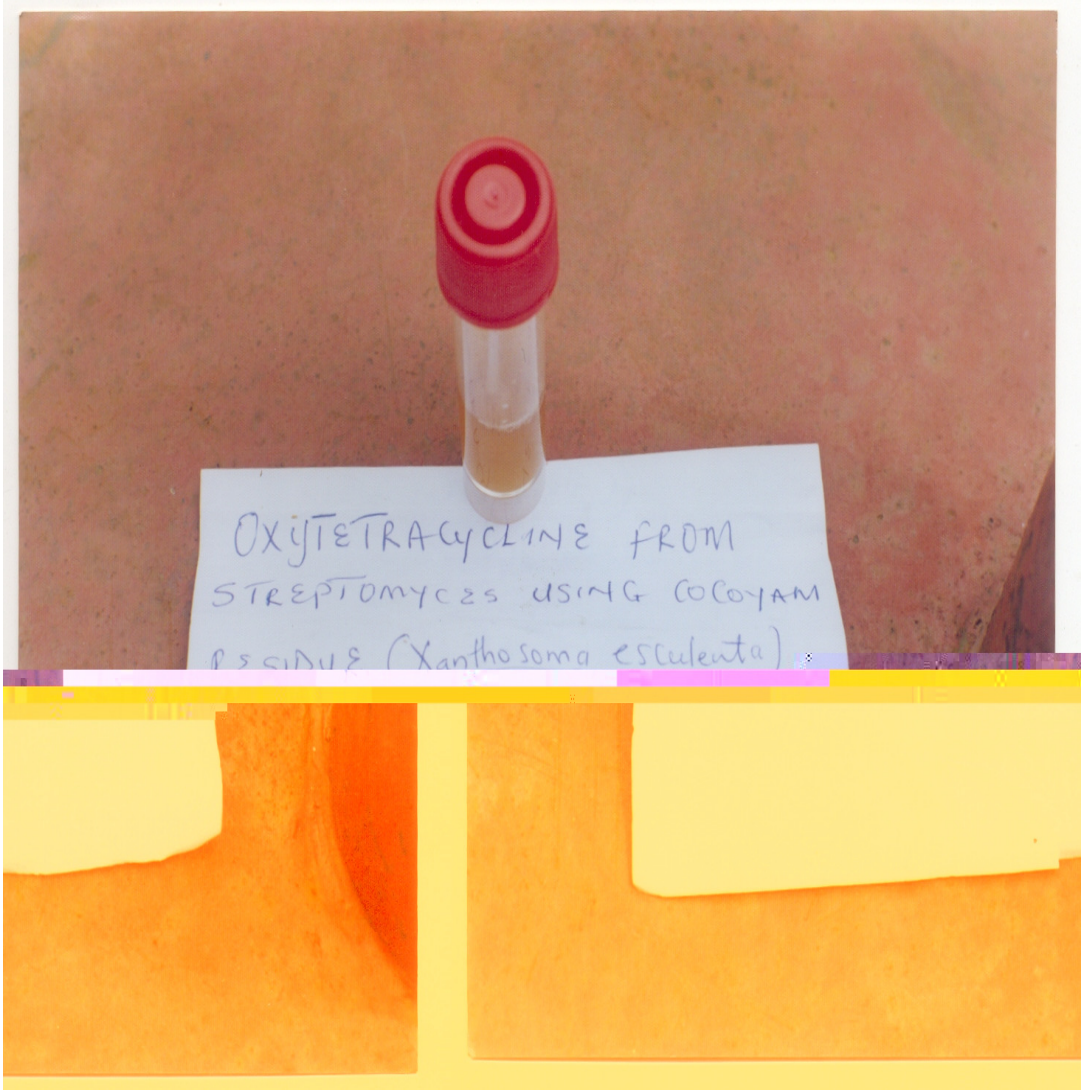

Plate 3. Extracted oxytetracycline in a sterile sample bottle.

of 150.12 g (fermentation jar alone for each of the fermenting species) to a final weight of 251.74 and 290.98 g (fermentation jar and culture) for *X. esculenta* and *C. esculenta*, that is, a gain of biomass weight of 101.62 and 140.86 g for the fermenting species respectively (Table 3). The higher value indicates a possible higher presence of the anticipated fermentation product (oxytetracycline) in the fermentation jar of the *C. esculenta.* Antibiotic activity of the oxytetracycline extract (Plate 3) was measured by the agar well diffusion method using *B. subtilis,* an indicator organism for oxytetracycline, in which total concentration of oxytetracycline was calculated based on the diameter of the zone of inhibition. This bioassays for the determination of anticipated antibiotics truly confirmed the presence of oxytetracycline, with peak activity occurring on the third day in all the fermentation jars (Tables 4 and 5); and *C. esculenta* continued to exhibit higher concentrations of this antibiotics in all instances, including finely- and coarsely-milled cocoyam peels substrates (Plate 1, and Tables 3 to 5). Though the reason for the optimum microbial growth obtained in sample B as against sample A is not clear, it may be due to the large surface area of *C. esculenta* compared to that of *X. esculenta*; it may also not be unconnected with the peculiarity of its natural constituents including higher protein (1.39 g), fibre (9.70 g) and ash (16.07 g) contents among others, as revealed by the proximate analyses (Table 1), all of which may be acting singly or synergistically to bring about a better oxytetracycline production. Higher protein concentrations in *C. esculenta* are reportedly helpful in the production of oxytetracycline (Asagbra et al., 2005; Yang and Yuan,

Table 1. Proximate analyses of the two species of cocoyam used for fermentation.

Analyte (%)	*Xanthosoma esculenta*	*Colocasia esculenta*
Moisture	6.12	11.58
Ash	11.28	16.07
Fat	1.20	0.56
Fibre	6.95	9.70
Protein	0.91	1.39
Carbohydrate	73.54	60.70

Table 2. Bulk density weights of the cocoyam peel waste.

Sample	Bulk density (g/100 ml)	
	Loose	Packed
Xanthosoma esculenta (sample A)	23.2	32.5
Colocasia esculenta (sample B)	15.9	28.3

Table 3. Biomass weight of 7-day fermentation culture for both species.

Parameter	*Xanthosoma esculenta* (sample A)	*Colocasia esculenta* (sample B)
Weight of beaker (g)	150.12	150.12
Weight of fermentation culture and beaker (g)	251.74	290.98
Weight of biomass (g)	101.62	140.86
4 volume distilled water used for extraction (ml)	406.48	563.44

1990). In our study, the conidia in solid media started germinating on the first day, mycelia grew abundantly on the third day and reached stationary phase on the fifth-sixth day (Plate 2 and Table 3). This observation was corroborated by the findings of the bioassays (Tables 4 and 5) in which oxytetracycline was detected on the first day of fermentation, and this is quite different from what was reported for earlier works (Asagbra et al., 2005; Yang and Swei, 1996) in which oxytetracycline production became manifested only from the second day. Though the reason for this variation in antibiotic production initiation time is not clear, earlier commencement of production observed in this study could simply be suggestive of an optimized fermentation conditions in our study, and this requires further verification for reproducibility. However, the activities of post-fermentation extracted oxytetracycline (Plate 3), as in other cases, decreased within a few days of production. These decreases, according to Asagbra et al. (2005), were due to instability in acidity of the fermenting medium.

Direct disposal of cocoyam peels waste to neighbourhood farms, soil or landfill as commonly practiced around is hazardous, as it has potential to cause serious environmental problem. Thus, the development of potential value-added management process for these wastes is highly attractive. Given that cocoyam peels, like orange and other peels, are common and readily available wastes seeking immediate disposal, an imperative consideration for industrial economy generally would be the cost benefits derivable from exploring the industrial raw materials potentials of the various wastes, as was previously evaluated for orange peels wastes (Ezejiofor et al., 2011). Cocoyam peel materials could and have been transformed by the use of biotechnological techniques into veritable raw material for the pharmaceutical industry. Oxytetracycline production using cheap and regularly available waste material as is done in this study is a sure way of reducing production costs for this class of pharmaceuticals. Here lies the potential benefit expected from the production of this antibiotic from this source for the pharmaceutical industry, as production cost per unit of oxytetracycline activity can be reduced drastically through selection of raw materials, which this study suggests a readily available alternative.

In conclusion, cocoyam peels- a common household kitchen wastes, that otherwise would have become breeding foci for disease pathogens, are by the outcome of this study shown to be bioconvertible into substrates for the production of oxytetracyclines, an important class of antibiotics that are vitally useful both for the health care delivery system and agro-poultry industry, etc. The fact

Table 4. Measurements of zones of inhibition of *B. subtilis* in a seven-day determination of oxytetracycline presence in coarsely milled cocoyam peel wastes using bioassay method.

Day	*Xanthosoma esculenta* (sample A) (mm)	*Colocasia esculenta* (sample B) (mm)
0	0.0	0.0
1	16.4	20.0
2	24.0	34.8
3	32.0	36.0
4	28.0	28.8
5	25.2	28.0
6	21.2	23.8
7	17.2	19.6

Table 5. Measurements of zones of inhibition of *B. subtilis* in a seven-day determination of oxytetracycline presence in finely milled cocoyam peel wastes using bioassay method.

Day	*Xanthosoma esculenta* (sample A) (mm)	*Colocasia esculenta* (sample B) (mm)
0	0.0	0.0
1	19.6	24.4
2	32.8	38.0
3	36.6	40.8
4	30.0	37.2
5	27.2	32.0
6	25.2	30.2
7	23.2	28.4

that cocoyam peels could become very useful substrate for the production of this very important class of drugs is indeed a big plus both for the pharmaceutical industry and public health programme of environmental health and safety. This application also offers an alternative waste management option for this class of household kitchen wastes of agricultural produce that is usually present in great abundance in our environment. By so-doing, these wastes with great potential for environmental degradation, pollution and disease causation are turned into raw materials for the pharmaceutical industry, thus becoming a veritable resource for industrial growth, with possible positive impacts of this exploitation on job and wealth creations for national economic prosperity. Added to these are the public health impacts of a safer and healthier environment derivable through the indirect waste management option so offered.

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