Research Article

# Natural Sources of Coconut Component Used for Microbial Culture Medium (NSM)

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**ABSTRACT**

Coconut extract (coconut liquid endosperum), with its many application is one of the world's most versatile natural product. Coconut are associated by a number of fungi and bacteria pathogens such as *Mucor*sp, *Rhizopus*sp, *Fusarium*sp, *Trichoderma*sp and *Klebsiella pneumonia, Staphylococcus aureus, Streptococcus pneumonia*, *Shigella*sp *and proteus*sp. We developed a semi solid media, termed natural source medium (NSM), to selectively and rapidly isolate fungi and bacteria pathogenic to and associated with coconut and some other fruits. Most strains of interest grow sufficiently on NSM in 24hrs at 37°C for bacteria and 48hrs at room temperature for fungi tentative identification based on colony morphology, Gram staining and Biochemical characteristic.

**Keywords:** Coconut extract, Coconut pathogenic microbes, Microbial growth rate, NSM medium formulation.

## INTRODUCTION

The edible part of the coconut fruit (coconut meat and coconut water) is the endosperm tissue. Endosperm tissues undergo one of three main modes of development, which are the nuclear, cellular and helobial modes[1] and the development of coconut endosperm belongs to the nuclear mode. Initially the endosperm is a liquid containing free nuclei generated by a process in which the primary endosperm nucleus undergoes several cycles of division without cytokinesis (the process in which the cytoplasm of a single eukaryotic cell is divided to form two daughter cells). Cytokinesis then occurs, progressing from the periphery towards the centre, thus forming the cellular endosperm layer. At first, the cellular endosperm is translucent and jelly-like, but it later hardens at maturity to become white flesh (coconut meat). Unlike the endosperms of other plants (e.g., wheat and corn), the cellularization process in a coconut fruit does not fill up the entire embryo sac cavity, but instead leaves the cavity solution-filled. This solution is commonly known as coconut water and it is of cytoplasmic origin.[2] Coconut (Cocosnucifera L) water also referred to as coconut juice is a refreshing natural drink common and mostly consumed in the tropical regions of the world.[3] It is a clear, colourless, sweet, naturally flavoured slightly acidic drink, with reported pH ranging between 4.2 and 6.0.[4-5] Over six decades of research has shown that coconut water contains proteins, fats, and is rich in carbohydrates and nutritionally important elements (potassium being the most abundant).[6] It is also a rich source of essential amino acids (lysine, histidine, tyrosine and tryptophan), fatty acids, glucose, fructose, cellulose, sucrose, and organic acids such as tartaric, citric and malic acids.[7] Coconut water's rich enzyme systems include very effective and selective reductase, polyphenol oxidase (PPO) and peroxidase (POD). These are involved in its development of a brownish colour when it is exposed to air for a long time.[8] Based on its content and properties, coconut water has been used in the treatment of child and adult diarrhoea, and gastroenteritis as well as for urinary stone dissolution, short-term intravenous hydration and protecting against gastrointestinal tract infections.[9] Assessing the risk of bacteria infections through the consumption of coconut water is made a difficulty since there are limited reports that show the survival and growth of pathogenic bacteria in coconut water. Considering the risk of bacteria contamination of coconut water in Ghana, the possibilities of survival and growth of bacteria in coconut water and the potential for the use of coconut water as a bacterial growth media in resource limited countries/laboratory like ours, we initiated this study. We studied some characteristics of coconut contaminated microbial pathogen.

Most bacteria, yeasts, and fungi do best when glucose is provided as the primary energy source because they may not be able to digest other carbohydrates. All bacteria found on and in animals and most of those found on plants and soils do well on glucose as the sole carbon and energy source. However, bacteria which use light or oxidation of minerals for their energy source may do better in relatively organic-free media. In water we find many little studied bacteria which do better in media containing only 0.5 gram of peptone and 0.5 gram of starch. Many general media such as nutrient agar(NA), LB agar, tryptic soy agar, yeast extract peptone agar(YP), and yeast extract calcium carbonate agar(YDC) have been used to isolate coconut pathogenic bacteria from coconut surface.[10-15] Studied the use of potato processing waste as a fermentation substrate for the production of single cell

proteins (SCP) for use in supplementation of animal feeds.[16] Comparisons were conducted using raw and steamed potato waste; both fermented using a single microbial strain and also the solid-state fermentation of wastes with a mixed microbial culture. Composition before and after fermentation was determined and this showed that the crude protein contents were 13.4, 18.53 and 22.16%, for the raw steamed and solid-state treatments respectively. As the current research shows, the protein of raw potato wastes has been usable much more than steamed or solid-state wastes for microorganismsgrowth.[16] A new marine medium was used by[17] and a common commercial medium were evaluated for their effectiveness for promoting growth of different bacteria. Comparisons between the media were centred on the most important kinetic parameters of the corresponding cultures, that is, maximum biomass and specific growth rate, calculated by applying two widely accepted mathematical models (logistic and Gompertz equations) to measure data both in terms of dry weights and cell numbers. The parametric estimations allowed a classification of the results that demonstrated the effectiveness of all the media derived from fishery residues to meeting the proposed objectives. Growths were generally higher (up to 10 times in terms of cell numbers) than those from the common commercial medium, with the best results obtained from tuna.[17] The conventional medium palm kernel agar (PKA) for the recovery of aflatoxigenic fungi from mixed cultures and the detection of aflatoxigenic fungi and direct visual determination of aflatoxins in agricultural commodities was assessed by.[18] The medium was able to efficiently detect aflatoxin production through direct visual observation of fluorescence. It can be routinely used as an alternative culture medium for screening aflatoxigenic fungi and direct visual determination of aflatoxins in agricultural commodities since it is faster and has a unique pink background for easy identification. Culture media formulations for industrial application were patented by.[19] The invention related to formulations of culture mediums for the industrial development of liquid starter cultures, is characterized by a larger number of microbial cells per volume unit of fermentation medium than the one of traditional liquid. The method for preparing a culture medium includes the addition of a suitable amount basic neutralizing agent preferably to any traditional culture medium, depending on the microorganisms. Potentials of cellulosic wastes in media formulation were investigated by.[20] Two agar media, Czapek-Dox and Sabouraud, were modified by substituting their carbon sources with cellulose, sawdust and sugarcane pulps. The modified Sabouraud's agar containing sawdust (Wood-Pep agar) and sugarcane pulps (Cane-Pepagar) yielded 84.4 – 100% of the maximum growth on Sabouraud's agar. Cellulosecontaining media gave a lower level of growth (60.0 to 66.7%) of that obtained for the unmodified media.

We aimed to develop a semi salt medium and broth to isolate, characterize and presumptively identify all mentioned coconut-pathogenic bacteria from coconut fruits.

## MATERIALS AND METHODS

### Coconut fruit micro flora preparation

Coconut fruit (coconut meat and coconut water) is the endosperm tissue. Two to three pieces of unwashed coconut fruits were put in 200 ml sterile nutrient broth (NB) culture media and incubated on a laboratory shaker (95 rpm) at 37°C for 24hrs to increase the population of coconut fruit micro flora. Aliquots 0.1 ml of the NB culture was inoculated on the surface of plate count agar and incubated at 37°C for 24hrs. Microbial colonies were isolated and sub-cultured using NB and SDS as reported earlier. The procedure was carried out in duplicate for each isolate studied. Ultimately, the selected colonies were characterized by morphological and biochemical techniques.

### Development of coconut extract medium (NSM)

Coconut extract was the main source of nutrients for (NSM), its natural source (NS). The pH of the medium was adjusted to 7.2 by addition of potassium phosphate salts, and sodium chloride was added to increase osmotic concentration.[21-22] The medium was prepared as follows: coconut fruits washed with normal distil water and grained, then filtered through coarse filter paper, the following were added to the filtrate: 1 g of $K_2HPO_4$ (anhydrous), 3.8 g of $KH_2PO_4$ (anhydrous). The volume was made up to 1 L by add it ion of high purity (HP) water. The pH was checked and adjusted to 7.2, if needed, by adding $KH_2PO_4$ or $K_2HPO_4$. Agar (20 g) was added before autoclaving at 121°C for 35 min. The medium was cooled to 55°C and poured into 15 mm-deep X 100 mm-diameter plastic Petri plates (20 ml/plate). Finally, the media was inoculated with the isolated micro flora of the coconut (five replicates /examination) according to agar dilution method as a recommended standard method.[23]

Result produced from coconut sets compared with classic culture media containing (SDS) for fungi and plate count agar (NA) for bacterial cultures. The results were recorded after the incubation interval.

### Microbial growth rate in coconut broth

### Bacterial growth kinetic of different hours

Bacterial growth level in different incubation time, consentient temperature at 37°C, was performed with 100 ml of coconut broth in 500 ml of Erlenmeyer flasks were inoculated with different incubation times (6, 12, 18, 24, 30, 36 and 42hrs). Samples were collected after different hours growth rate measured at 480nm.[24]

### Fungi growth kinetic of different hours

Fungi growth level in different incubation times was performed with 100 ml of coconut broth in 500 ml of

Erlenmeyer flasks were inoculated with different incubation times (20, 40, 60, 80 and 100hrs). Samples were collected after different hours growth rate measured at 480nm.

## RESULTS AND DISCUSSION

### Development of NS Medium

A broth derived from autoclaving diced coconut extract, adjusted in pH and salt concentration and supplemented with certain growth promoter and agar was found suitable for the medium and rapid growth of coconut-pathogenic and fruits-associated bacteria in Petri plates. NSM was suitable for initial isolation of bacteria from a coconut fruits and some food pathogenic microorganism.

Nutrition content of coconut extract such as water 94.99(g/100g), protein 0.54(g/100g), lipid 0.15(g/100g), sucrose 9.18(mg/ml), glucose 7.25 (mg/ml), fructose 5.25 (mg/ml), calcium Ca 31.64(g/100g), Iron Fe 0.02(g/100g), magnesium Mg 9.44(g/100g), phosphorus P 12.77(g/100g), potassium K 257.52(g/100g), sodium Na 16.10(g/100g).[25] These nutrients were sufficient to support the growth of coconut pathogenic, fruits-associated bacteria and fungi.

### Bacteria

### Gram positive bacteria

*Staphyolcoccusaureus*

This gram positive bacterium was susceptible to the coconut media culture components and its population was increased approximately by 76% after the incubation period (Figure 1).

*Streptococcus pneumonia*

The *Streptococcus pneumonia* was susceptible to the coconut media culture components its population was increased approximately by 68% after the incubation period (Figure 2)

### Gram negative bacteria

*Klebsiella pneumonia*

*Klebsiella pneumonia*growth was increased in the coconut media by almost 88% in comparison with NA media (Figure 3). Therefore, it appears that inexpensive coconut media can be used as an effective alternative to commercially prepared media for cultivation of *Klebsiella pneumonia.*

*Shigella*sp

This gram negative bacteria's population, was somewhat restricted in its growth by the coconut components (Figure 4). The observed growth level was decreased in coconut medium.

*Proteus*sp

*Proteus* growth was decreased in the coconut media by almost 25% in comparison with NA media (Figure 5). Therefore, it not suitable for cultivation of*proteus*spin coconut media.


**Figure 1:** *Staphyolcoccusaureus*growth in NMS


**Figure 2:** *Streptococcus pneumonia*growth in NMS

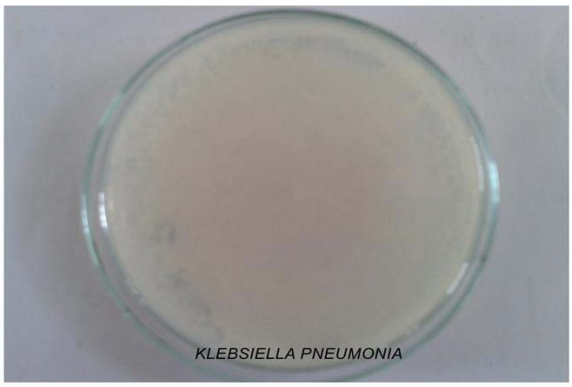



**Figure 3:** *Klebsiella pneumonia*growth in NMS


**Figure 4:** *Shigella*spgrowth in NMS


**Figure 5:** *Proteus* sp growth in NMS

**Fungi**

*Mucorsp*

The results showed that coconut fruit components induced *Mucor*sp3.4 times more than (SDA). These results suggest that *Mucor*sp is an important fungus for *Mucor*sp fruit infection and spoilage; coconut had more suitable ingredients for *Mucor*sp growth as a culture media than SDA. Therefore, it appears that inexpensive coconut-based media can be used as an effective alternative to commercially prepared media for cultivation of *Mucor*sp (Figure 6).

*Rhizopus*sp

Coconut extract components helped *Rhizopus*sp growth 2.1 times more than SDS. Hence, it is concluded that *Rhizopus*sp is a major infection factor for coconut and fruits products, coconut extract media can be used for the enrichment of *Rhizopus*sp culture media in microbiological analysis.

*Fusarium*sp

The results showed that coconut and SDA had a similar effect on the growth of*Fusarium*sp and coconut media did not have any additional effects. The population of this organism was the same in both culture media. Therefore indicating that, *Fusarium*sp was resistant to components of coconut extract. Coconut media can be used as a selective media for this microorganism.

*Trichoderma*sp

The culturing results showed that *Trichoderma*sp was susceptible to coconut extract components. The microbial populations were increased by 20% in coconut-base media when compared to SDA. It was concluded that, coconut extract has sufficient nutrition for fruits pathogenic microorganisms.

**Microbial growth kinetic**

The incubation time an important role in microbial cell growth. The microbial growth was tested with different incubation hours (6, 12, 18, 24, 30, 36 and 42hrs) for bacterial culture and (20, 40, 60, 80 and 100hrs) for fungi culture, it measured 480nm spectrophotometer. Further, the higher levels of bacterial population were recorded at 24hrs (Figure 7) and higher level of fungi population were recordedat 80hrs (Figure 8).

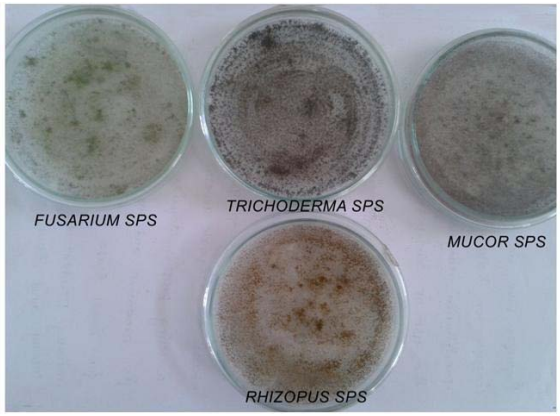
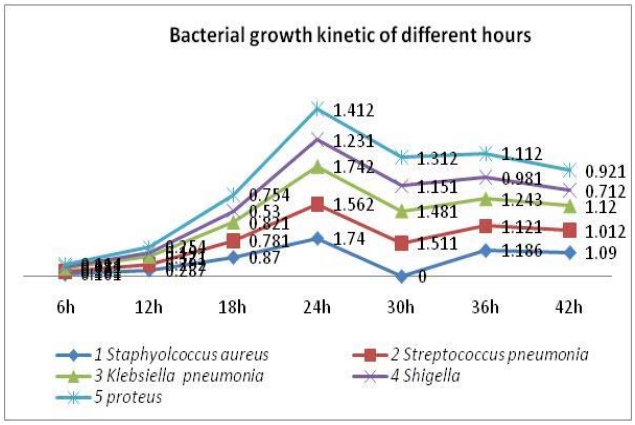
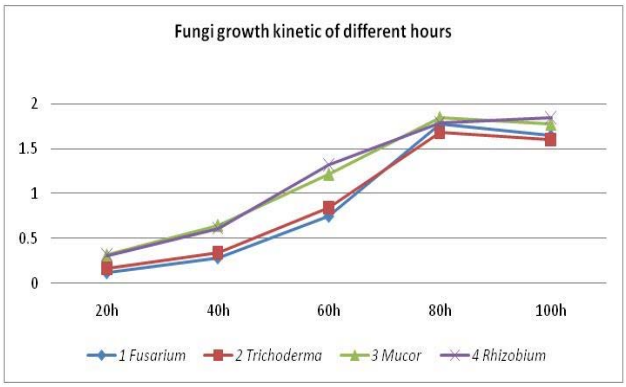



**Figure 6:** Fungi growth in NMS




X axis = Time (hrs); Y axis = Number of bacterial growth

**Figure 7:** Bacterial growth kinetic of different hours




X axis = Time (hrs); Y axis = Number of cell growth

**Figure 8:** Fungi growth kinetic of different hours

## CONCLUSION

Coconut extract media that are the focus of this work have been shown to possess sufficient amounts of nutrients for support of the growth of microorganisms such as *Mucor*sp, *Rhizobium* sp and *Klebsiella pneumonia, Staphylococcus aureus, Streptococcus pneumonia.* It was also shown that, coconut extracts are capable of insufficient the growth of other fungi and bacteria. For example, the growth of the fungi *Fusarium*sp, *Trichoderma*sp and the gram negative bacteria *shigella*sp

and *proteus*sp were growth decreased compared to NA and SDA media. It is postulated that the decreased of the growth of some susceptible microorganisms may be due to the tartaric, citric, malic acids compounds existing in coconut components.

The NS media naturally containing sufficient amount of glucose, fructose, cellulose and sucrose for bacterial fungi growth used in this study, can play an important role in the formulation of NS culture media for fungi as well as bacteria. This work has shown that, coconut extract products can be used efficaciously and economically for the cultivation of the fungi and bacteria that were reported in this work. Moreover, due to equal effect of NA, SDA and coconut culture media on the growth of *Mucor*sp, *Rhizobium* sp and *Klebsiella pneumonia, Staphylococcus aureus, Streptococcus pneumonia* sp, it is suggested that, coconut extract media can be used for cultivation of bacteria and fungi in research laboratory and industrial technology.

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