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Natural Sources of Gooseberry Component used for Microbial Culture Medium (NSM)

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ABSTRACT

Gooseberry are plagued by a number of bacterial and fungi pathogens including *Mucor sps*, *Rhizopus sps*, *Fusarium sps*, *Trichoderma sps* and *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Shigella sps ,and proteus.* We developed a semi solid media, termed natural source medium (NSM), to selectively and rapidly isolate fungi and bacteria pathogenic to and associated with gooseberry and some other fruits. Most strains of interest grow sufficiently on NSM in 24h at 37^0C for bacteria and 48h at room temperature for fungi tentative identification based on colony morphology, Gram staining and Biochemical characteristic.

INTRODUCTION

The gooseberry is a small round fruit that comes in hundreds of varieties. Most plant experts suggest the earliest cultivars were from in Northern Africa, but the fruit is now grown widely throughout Northern Europe and in North America. Gooseberries grow on a bush that stands about 3 - 5 feet (0.91 - 1.52 m) high. The bush has spines, making picking the fruit a little harsh on the hands. In North America, the gooseberry's season extends from May to August. One finds them most frequently in June, but this depends on temperature and location. Gooseberries also seem to withstand harsher temperatures. The makes them easy to grow in areas with frosts and snow. Nutrition content of gooseberry such as Energy 184kj, Carbohydrates 10.18g, Dietary fiber 4.3g, Fat 0.58, Protein 0.88g, Water 87.87, vitamin C (Ghosal et al., 1996). Microorganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce. In the environment, microbes adapt to the habitats most suitable for their needs while in the laboratory, these requirements must be met by a culture medium. So growth media are used for various purposes including the identification of unknown microorganisms, as well as the production of large quantities of microbial populations for commercial uses as in biotechnology. Numerous types of media are available commercially including some that may have added compounds that either enhance growth or suppress outgrowth of competing organisms. Complex media are rich in nutrients; they contain water soluble extracts of plant or animal tissue. Usually, sugar or glucose is added to serve as the main carbon and energy source. The combination of extracts and sugar creates a medium which is rich in minerals and organic nutrients, but since the exact composition is unknown the medium is called complex. Selection of preferred media is based on how it affects the microorganism's growth and other physiological functions and the purpose of research. 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 gooseberry pathogenic bacteria from gooseberry surface (Azad et al., 2000; Coutinho et al., 2002;Coutinho and venter, 2009; Walcott et al., 2002; Kido et al., 2008. Qiyun and Liang, (2004) studied the use of potato processing waste as a fermentation substrate for the production of single cell

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proteins (SCP) for use in supplementation of animal feeds (Qiyun and Liang, 2004). 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 microorganisms' growth (Qiyun and Liang, 2004).

A new marine medium was used by Vazquez et al. (2004) and a common commercial medium were evaluated for their effectiveness for promoting growth of different bacteria. Comparisons between the media were centered 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 (Vazquez et al., 2004).

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 Atanda et al., (2006). 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 (Atanda et al., 2006).

Two representative vegetable-based tryptic soy formulations were used to culture a range of bacteria and fungi by Cleland et al. (2007). Then the growth characteristics of them were compared with each other. All the representative of microorganisms grew well on the vegetable based media and the media provided suitable recoveries of the organisms following simulated storage. Subtle phenotypic changes were observed between cultures grown on different media, but these did not significantly change the strain identification (Cleland et al., 2007).

Culture media formulations for industrial application were patented by Giovanni (2008). 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 (Giovanni, 2008).

Potentials of cellulosic wastes in media formulation were investigated by Nwodo-Chinedu et al. (2009). 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. Cellulose containing media gave a lower level of growth (60.0 to 66.7%) of that obtained for the unmodified media (Nwodo-Chinedu et al., 2009).

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

MATERIALS AND METHODS

Gooseberry fruit micro flora preparation

Two unwashed gooseberry fruits were put in 200 ml sterile nutrient broth (NB) culture media and incubated on a laboratory shaker (95 rpm) at 37°C for 24 hrs; to increase the population of gooseberry fruit micro flora. Aliquots (0.1 ml) of the NB culture were inoculated on the surface of plate count agar and incubated at 37 °C for 24 hrs. 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 markers as gooseberry fruit micro flora.

Development of gooseberry extract medium (NSM)

Gooseberry extract was the main source of nutrients for (GEM), 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.

The medium was prepared as follows: Gooseberry fruits washed with normal distall water and grained, then filtered through coarse filter paper (CAT # 28331–081 VWR Scientific Products, West Chester, PA 19380). The following were added to the filtrate: 5 g NaCl, 1 g of K_2HPO_4 (anhydrous), 3.8 g of KH2PO4 (anhydrous), and 250 mg of cycloheximide. 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 gooseberry fruit (five replicates /examination) according to agar dilution method as a recommended standard method (Brown.1994).

Result produced from gooseberry 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 GOOSEBERRY BROTH

Bacterial growth kinetic of different hours

Bacterial growth level in different incubation time, consentient temperature at 37^0C, was performed with 100 ml of gooseberry broth in 500 ml of Erlenmeyer flasks were inoculated with different incubation times (6, 12, 18, 24, 30, 36 and 42h). Samples were collected after different hours growth rate measured at 480nm (Sathiyavimal et al., 2013).

Fungi growth kinetic of different hours

Fungi growth level in different incubation times, was performed with 100 ml of gooseberry broth in 500 ml of Erlenmeyer flasks were inoculated with different incubation times (20, 40, 60, 80 and 100h). Samples were collected after different hours growth rate measured at 480nm.

RESULT

Development of NSM

A broth derived from autoclaving diced gooseberry fruits 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 gooseberry-pathogenic and fruits-associated bacteria in Petri plates. NSM was suitable for initial isolation of bacteria from a gooseberry fruits and some food pathogenic microorganism.

Nutrition content of gooseberry such as Energy 184kj, Carbohydrates 10.18g, Dietary fiber 4.3g, Fat 0.58, Protein 0.88g, Water 87.87, vitamin C (Ghosal et al., 1996). These nutrients were sufficient to support the growth of gooseberry-pathogenic, fruits-associated bacteria and fungi.

BACTERIA

Gram positive bacteria

Streptococcus pneumonia

This gram positive bacterium was susceptible to the gooseberry media culture components and its population was increased approximately by 86% after the incubation period (Figure 1).

Staphyolcoccus aureus

The *Streptococcus pneumonia* was susceptible to the gooseberry media culture components its population was increased approximately by 75% after the incubation period (Figure 2).

Gram negative bacteria

Shigella sps

Klebsiella pneumonia growth was increased in the gooseberry media by almost 89% in comparison with NA media (Table 3). Therefore, it appears that inexpensive gooseberry media can be used as an effective alternative to commercially prepared media for cultivation of *Shigella sps*.

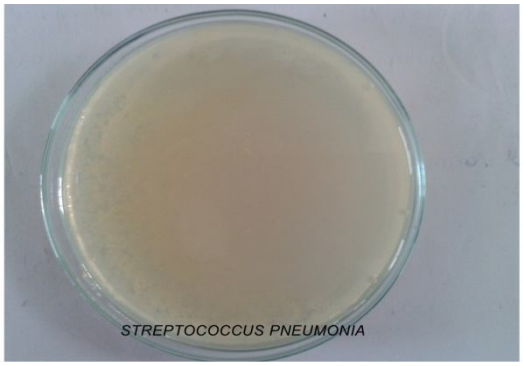

Fig. 1: *Streptococcus pneumonia* Growth in NSM.

Fig. 2: *Staphylococcus aureus* Growth in NMS.

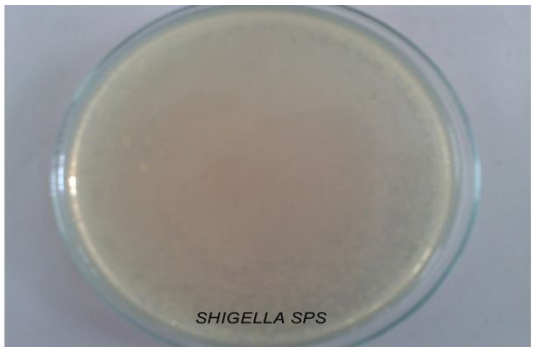

Fig. 3: *Shigella sps* Growth in NMS.

Klebsiella pneumonia

This gram negative bacteria's population, was somewhat induced in its growth by the gooseberry components (Figure 4). The observed growth levels was increased in gooseberry medium.

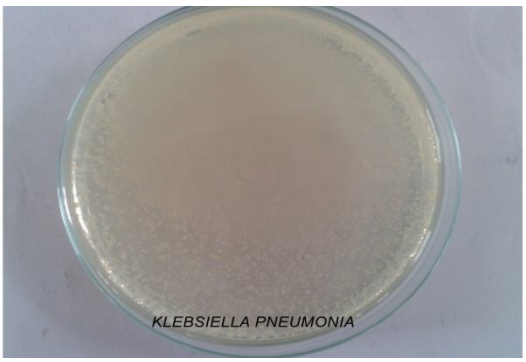

Fig. 4: *Klebsiella pneumonia* Growth in NMS.

Proteus sps

Proteus growth was decreased in the gooseberry media by almost 36% in comparison with NA media (Figure 5). Therefore, it not suitable for cultivation of *proteus sps* in gooseberry media.

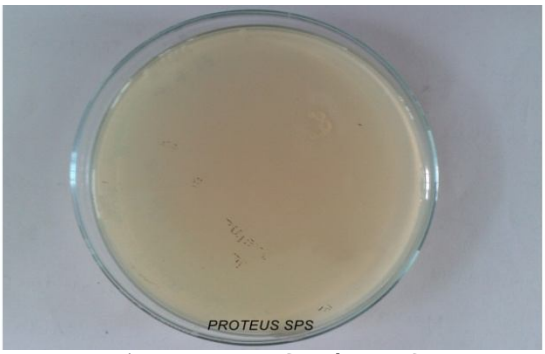

Fig. 5: *Proteus sps* Growth in NMS.

Mucor sps

The results showed that gooseberry fruit components induced *Mucor sps* 2.6 times more than (SDA). These results suggest that *Mucor sps* is an important fungus for fruit infection and spoilage; gooseberry fruit had more suitable ingredients for *Mucor sps* growth as a culture media than SDA. Therefore, it appears that inexpensive gooseberry-based media can be used as an effective alternative to commercially prepared media for cultivation of *Mucor sps* (Figure 6).

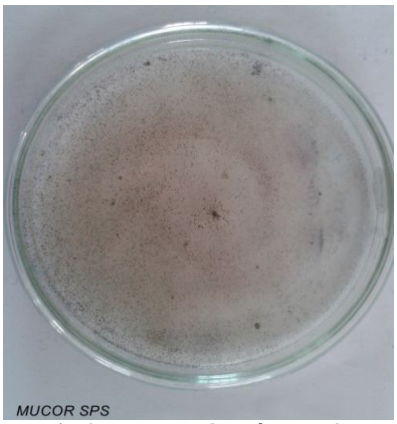

Fig. 6: *Mucor sps* Growth in NMS.

Rhizopus sps

Gooseberry extract components helped *Rhizopus sps* growth 2.3 times more than SDS. Hence, it is concluded that *Rhizopus sps* is a major infection factor for gooseberry and fruits products; gooseberry extract media can be used for the enrichment of *Rhizopus sps* culture media in microbiological analysis (Figure 7).

Trichoderma sps

The results showed that gooseberry and SDA had a similar effect on the growth of *Trichoderma sps* and gooseberry media did not have any additional effects. The population of this organism was the same in both culture media. Therefore indicating that, *Trichoderma sps* was resistant to components of gooseberry extract. Gooseberry media can be used as a selective media for this microorganism (Figure 8).

Fusarium sps

The culturing results showed that *Fusarium sps*. Was susceptible to gooseberry extract components. The microbial populations were increased by 21% in gooseberry-base media when compared to SDA. It was concluded that, gooseberry extract has sufficient nutrition for fruits pathogenic micro Organisms (Figure 9).

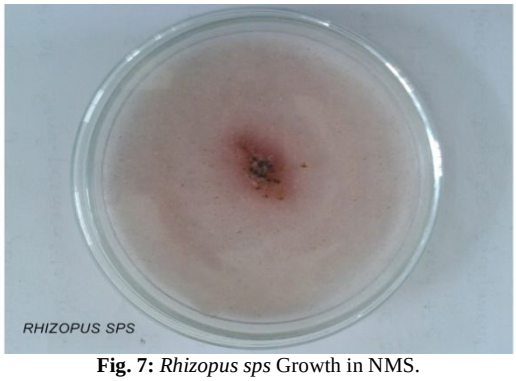

Fig. 7: *Rhizopus sps* Growth in NMS.

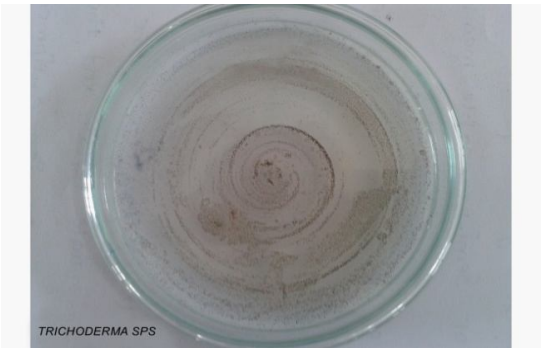

Fig. 8: *Trichoderma sps* Growth in NMS.

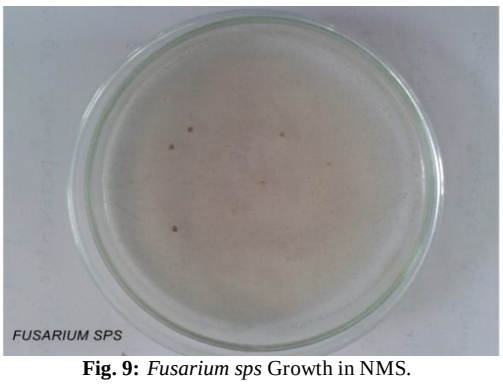

Fig. 9: *Fusarium sps* Growth in NMS.

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 42h) for bacterial culture and (20, 40, 60, 80 and 100h) for fungi culture, it measured 480nm spectrophotometer. Further, the higher levels of bacterial population were recorded at 24h (Figure 10) and higher level of fungi population were recorded at 80h (Figure 11).

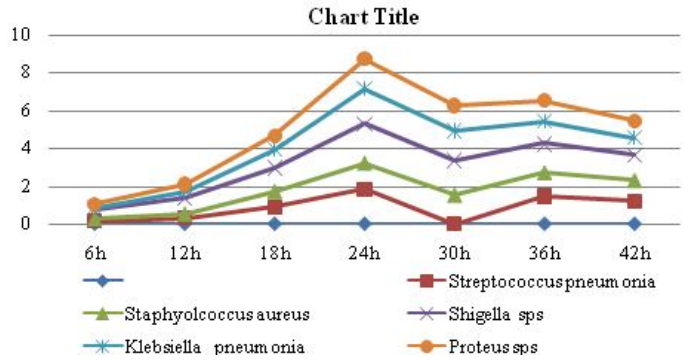

Fig. 10: Bacterial Growth kinetic in NSB.

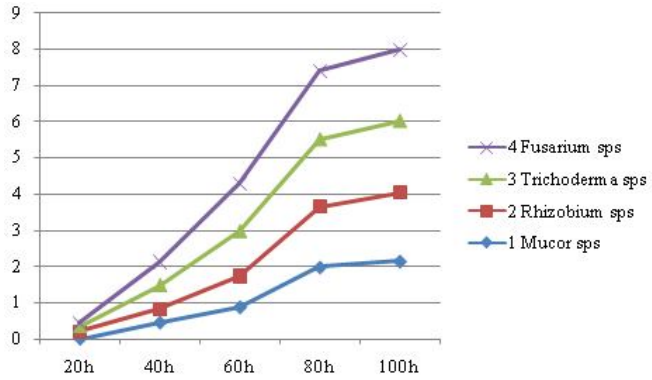

Fig. 11: Fungi Growth kinetic in NSB.

CONCLUSION

Gooseberry extract media that is the focus of this work have been shown to possess sufficient amounts of nutrients for support of the growth of microorganisms such as *Mucor, Rhizopus sps* and, *Staphylococcus aureus, Streptococcus pneumonia, shigella sps.* It was also shown that, gooseberry extracts are capable of insufficient the growth of other fungi and bacteria. For example, the growth of the fungi *Fusarium sps, Trichoderma sps* and the gram negative bacteria *Klebsiella pneumonia* and *proteus sps* 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 phenolic compounds existing in gooseberry components (Vinita Puranik., et al 2012).

The NS media naturally containing sufficient amount of Energy, Carbohydrates, Dietary fiber, Fat, Protein, Water, vitamin and sucrose for bacterial fungi growth used in this study, can play an important role in the formulation of NSM culture media for fungi as well as bacteria. This work has shown that, gooseberry 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 gooseberry culture media on the growth of *Mucor sps*, *Rhizopus sps* and *shigella sps*, *Staphylococcus aureus*, *Streptococcus pneumonia sps*, it is suggested that, gooseberry extract media can be used for cultivation of bacteria and fungi in research laboratory and industrial technology.

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