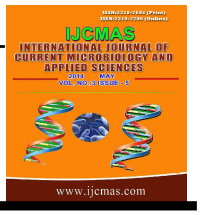
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 5 (2014) pp. 501-512**
http://www.ijcmas.com



Original Research Article

Isolation of Microorganisms associated with Deterioration of Tomato (*Lycopersicon esculentum*) and Pawpaw (*Carica papaya*) Fruits

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Keywords

Eudrilus eugeniae, cocoon, abnormal bulging, emancipation

A B S T R A C T

Evaluation of microorganisms associated with deterioration of tomato and pawpaw fruits were carried out. Analysis revealed that various bacteria and fungi in high densities participated in the spoilage processes. Analysis was conducted at 24 hours interval for four days. In tomato, bacterial population of 2.6 x 10^3cfu/g on the first day increased to 2.5 x 10^6cfu/g on the second day and reduced to 4.1 x 10^5cfu/g on the third day. In pawpaw, the load was 5.0 x 10^6cfu/g on the first day and increased to 1.9 x 10^6cfu/g on the second day but reduced to 1.3 x 10^5cfu/g on the 3rd day. Pathogenic bacteria (*Listeria monocytogenes, Micrococcus varians*) were isolated on the 1st day and 2nd day in the samples. *Lactobacillus fermenti* came in the second day. Total yeast (non-filamentous fungi) ranged from 3.00 x 10^3cfu/g on the first day to 9.8 x 10^7cfu/g on the 4th day in tomato, while in pawpaw it increased from 3.5 x 10^3cfu/g to 1.0 x 10^8cfu/g on the 1st to 4th day respectively. In tomato, mould count was 3.2 x 10^3cfu/g on the first day but increased to 7.8 x 10^7cfu/g on the fourth day. In pawpaw, it was ranged from 5.0 x 10^4 to 1.5 x 10^8cfu/g on the fourth day. No mould was found on the first day. Microbial species encountered were bacteria such as *Micrococcus varians, Lactobacillus fermenti, Bacillus subtilis, Rothia sp* in pawpaw and, *Lactobacillus fermenti, Pseudomonas stutzeri, Leuconostoc species, Rothia species* in tomato, and fungi such as *Penicillium nalgiovense, Penicillium notatum, Saccharomyces sp, R. stolonifer, Humicola sp, A. niger and Candida tropicalis* in pawpaw. *Rhizopus stolonifer, Botrytis cineria, Penicillium notatum, Saccharomyces species, Rhodotorula species, Vorticillium albo-atrum, Penicillium expansium, Mucor mucido, Monilia sp* were isolated from tomato. Washing of fruits with chlorinated water, fruit storage room kept at high sanitary conditions, treatment of fruits with antimicrobial agents, as well as some forms of refrigeration are necessary to increase shelf-life and reduce the risks of microbial toxins which are deleterious to human health.

Introduction

Food spoilage refers to various changes in which the food becomes less palatable or even toxic to consumers these changes may be accompanied by alterations in

taste, smell, appearance or texture. Numerous microbial defects of agricultural crops are characterized by the types of microorganisms responsible for their deterioration (Akinmusire, 2011).

A fruit is the edible part of a mature ovary of a flowering plant. It is usually eaten raw. When mature, they may be either fleshy or dry. Fleshy fruit are further classified into berry (orange, tomato, pineapple, pawpaw, and banana), drupes (plume, coconut, almond, cherry) and pomes such as apple and pear. The dry fruits, unlike the fleshy fruits which have unlayered pericarp, are classified into dehiscent (pod, follicle and capsule) and indehiscent fruits like achene, samara, cashew etc (Jay 2000).

Fruits and vegetables are vital sources of nutrients to human beings. They give the body the necessary vitamins, fats, minerals, and oil in the right proportion for human growth and development. Fruits and vegetables however, have serious challenges to their existence. These include changes in climatic condition, pests and microbial attack. Over the years, there has been an increase in the need to identify and isolate the microorganisms associated with the spoilage as a way of finding a means of controlling it (Akinyele and Akinkunmi, 2012).

Susceptibility of fruits and vegetables is largely due to differential chemical composition such as pH and moisture contents are associated with greater predisposition to microbial spoilage. The occurrence of fungal spoilage of fruits is also recognized as a source of potential health hazard to man and animal. This is due to their production of mycotoxins (naturally occurring toxic chemical often of aromatic structure) which are capable of producing aflatoxin in man, following ingestion or inhalation.

These fruits are usually displayed on benches and in baskets for prospective customers in the open markets until sold, thereby exposing them to further microbial infection beside those associated with these whole fruit surface and those from adjacent infected fruits (Baiyewu *et al*, 2007). In developing countries, post harvest deterioration are often more severe due to inadequate storage and transportation facilities. Microbial fruits infection may occur during the growth season, harvesting, handling, transport and post harvest storage and marketing conditions, or after purchasing by the consumer. Fruits contain high levels of sugars and nutrient elements and their low pH values make them particularly desirable to fungal decay (Singh and Sharma, 2007). Studies by Li-Cohen and Bruhn (2002) shows that fungi can survive and/or grow on fresh produce and that the nutrient content (carbohydrate, protein and fat) of fresh produce support pathogens.

Fruits are affected by a wide array of microorganisms causing its decay. Spoilage microorganisms can be introduced to the crop on the seed itself, during crop growth in the field, during harvesting and post-harvest handling or during storage and distribution (loading and offloading) (Barth *et al*, 2009). Those types of soil-borne spoilage microbes that occur on produce are the same spoilage microorganisms that are present on harvesting equipments, on handling equipment, in the packaging house, in the storage facility, and on food contact surfaces throughout the distribution chain. Therefore, early intervention measures during crop development and harvesting through the use of good agricultural

practices (GAP) will provide dramatic reductions in the yield loss due to deterioration at all subsequent steps in the food (Barth *et al*, 2009).

Tomato fruits are very rich in mineral, vitamins, and carbohydrate. Udoh *et al* (2005) reports that the tomato fruit has 94% water and 4.3% carbohydrate. In view of these, the fruit is often attacked by microorganisms especially after harvest, thus a fast and high rate of spoilage is often observed in storage. Barth *et al* (2009) says that fungal species destroys fruits more than bacterial, he isolated the following fungal species from soft rot infected tomatoes: *Fusarium oxyporum, Fusarium muniliforme, Aspergillus niger and Rhizopus stolonifer*.

Materials and Methods

Materials

Materials used for these analyses were standard microbiological materials and were gotten from the microbiology laboratory except tomato and pawpaw samples that will purchased from the market.

Preparation of pre-digested leaflitter-Sample collection

Pawpaw and tomato fruits were purchased from Umuahia Market, Abia State. Eight samples (4 each) were collected from fruit vendors and transported within 24 hours to the Microbiology laboratory. Sampling was conducted at 24 hours intervals for 96 hours.

Preparation of media

Culture media used for this evaluation were Nutrient Agar (NA), MacConkey Agar (MAC), Sabouraud Dextrose agar (SDA), Potato Dextrose agar (PDA) and Tomato juice agar (TJA). The media were prepared according to manufacturer's recommendation and directions.

Isolation of bacteria

The pour-plate method according to Harigan and McCane (1990) was adopted. Using standard Microbiological technique (serial dilution), a tenfold dilution of 1g of the sample was carried out in 9ml of sterile water (this was the aliquot). Precisely, lml of the aliquot (supernatant) was pipetted and mixed in another 9ml of sterile distilled water in a test-tube. The test-tube was shaken vigorously to homogenize. The exponential dilution continued to the fourth factor (10^{-4}). 1ml of the fourth factor was aseptically transferred and plated in duplicate sets using sterile molten lukewarm nutrient agar which was amended with 1%. The poured plates were allowed to set and were incubated at 37^0C for 48hours. Discrete colonies that developed after incubation were counted and enumerated as colony forming unit (cfu/g) after multiplying with the dilution factor (10^{-4}).

Isolation of fungi (yeasts/moulds)

The pour-plate method also was used for the isolation of fungi following the method of Barnett and Hunter (1987). The BECTO Sabouraud Dextrose agar and potato dextrose agar were used. The diluents from the 4^{th} test-tube were aseptically transferred to sterile Petri dishes and about 15 to 20ml of sterile-molten lukewarm-SDA/PDA was poured into the plate, allowed to set and incubated at room temperature (28 ± 2^0C). Colonies that developed after incubation were counted,

enumerated in colony forming unit per gram (Cfu/g) samples.

Purification (subculture) of bacterial isolates

Colonies from the primary plates were aseptically picked with a sterile wire loop and transferred onto freshly prepared sterile nutrient agar plate, with a streaking technique such that discrete colonies appear at the ends of streaked lines after incubation. The subculture plates were incubated at 37°C for 24 hours to 48 hours. Discrete colonies from the subculture plates were aseptically transferred and streaked on slant and incubated for another 24 hours at 37°C.

Purification of fungal isolates

Colonies from the primary plates were aseptically picked with a sterile inoculation needle and transferred onto a freshly prepared sterile SDA plate with a streaking method and incubated for 5-7days at 28^0C-30^0C. Discrete colonies were aseptically transferred and stocked on slant and incubated for another 5 days at 28^0C-30^0C. Pure colonies were stored in the refrigerator at 10^0C-15^0C until needed for characterization and identification.

Characterization and identification of bacterial isolates

All bacterial isolates were characterized and identified considering their cultural, morphological, microscopic examination and biochemical characteristics following the methods prescribed by Holt *et al*, (1994). Biochemical test conducted include the following: Gram stain, Catalase test, Oxidase test, Motility test, Methyl red test, Citrate test, Urease test, Spore formation and Sugar fermentation test.

Results and Discussion

The results on the isolation of microorganisms associated with deterioration of tomato and pawpaw fruits are described as follows: the microbial densities during the four day deterioration process of tomato are shown in table 1. Total bacterial count increased from day 1 to day 2 with an observable decrease in day 3. Total fungal count increased from 3.2 x 10^3 cfu/g to 9.8 x 10^7 cfu/g on the first day to the fourth day respectively. Table 2 shows microbial count in pawpaw fruit under deterioration process for four days. There is an increase in total number of bacteria from day 1 to day 2 and a sudden decrease on day 3 while total fungal count increased drastically from 3.5 x 10^3 cfu/g to 1.50 x 10^8 cfu/g. Microscopic and morphological characteristics of bacteria are shown in table 3. Bacterial species including *Rothia, Listeria monocytogenes* etc were isolated from both fruit within the four days of deterioration process. Thirteen (13) fungal isolates (filamentous fungi) were identified as shown below in table 4. The morphological and microscopic features for each of the isolates are presented accordingly in the table. Unlike other fungal species, members of *Aspergillus* and *Penicillium,* major producers of aflatoxin and mycotoxin as well as stomach inflammation were mostly isolated in both fruit during the 4 days deterioration process. Table 5 describes the colour, morphological and microscopic structures of non-filamentous fungi (yeasts). Only three (3) of them were isolated *Saccharomyces* seems to be the most frequently isolated one.

In table 6 there are 17 microorganisms, 6 bacteria such as *Lactobacillus fermenti, Pseudomonas stutzeri*, *Leuconostoc* sp,

Rothia sp, *Listeria monocytogenes* (dangerous food-borne pathogen) and 11 fungi such as *Rhizopus stolonifer, Botrytis cinerea*, *Penicillium notatum* etc isolated from tomato fruit.

Table.1: Microbial densities in tomato fruit under deterioration process for four days (cfu/g)

	Day 1	**Day 2**	**Day 3**	**Day 4**
Total bacterial count	$2.6x10^3$	$2.50 x10^6$	$4.10 x10^5$	NIL
Total pathogenic bacteria count	$1.8 x 10^3$	$1.0 x 10^4$	NIL`	NIL
Total lactobacillus bacterial count	NIL	$2.4 x 10^5$	$7.8 x10^7$	$3.7 x10^7$
Total yeast count	$3. 0x 10^3$	$7.0x 10^4$	$3.2 x10^7$	$9.8 x10^7$
Total mold count	$3.2 x 10^3$	$1.8 x10^5$	$3.8 x10^7$	$7.8 x 10^7$

Table.2 Microbial densities in pawpaw fruit under deterioration process for four days (cfu/g)

	Day1	**Day2**	**Day3**	**Day4**
Total bacterial count	$5.00 x 10^3$	$1.90 x 10^6$	$1.30x 10^5$	NIL
Total pathogenic bacterial count	$2.1 x 10^3$	NIL	NIL	NIL
Total lactobacillus bacterial count	NIL	$4.50 x10^5$	$1.8 x 10^7$	$6.0x 10^6$
Total yeast count	$3.50 x 10^3$	$5.00 x 10^4$	$3.2 x 10^7$	$1.01 x 10^8$
Total mold count	NIL	$5.00x 10^4$	$2.1 x10^7$	$1.50 x 10^8$

Table.3 Morphological, cultural, biochemical characteristic and microscopic examination of the bacterial species.

No of 1^{so}ate	Morphological appearance	Cell shape	Gram reaction	Catalase	Oxidase	Coagnlase	Citrate	Urease	Methyl red	motility	Spore formation	Lactose	Maltose	Glucose	Sucrose	Mannitol	Probable organism
1.	Creamy, flat, irregular, translucent	Rod	-	+	+	-	-	+	+	+	-	00	A0	A0	00	00	*Pseudomonas stutzeri*
2	Milky, flat, irregular, translucent	Rod	+	+	-	-	+	+	+	-	-	AG	AO	AG	AG	00	*Lactobacillus fermenti*
3	Milky, flat, irregular, translucent	Rod	+	+	-	-	+	+	+	+	-	00	AO	AG	AG	AG	*Listeria monocytogenes*
4	Milky, flat, irregular, translucent	Cocci	+	+	+	-	+	+	+	-	-	AG	AG	AG	AG	AG	*Leuconostoc sp.*
5	Milky, raised, circular, translucent	Rod	+	+	+	-	+	+	+	-	-	AO	AO	AG	AG	AO	*Rothia sp.*
6	Creamy, raised, circular, translucent	Cocci	+	+	-	-	-	+	+	-	-	00	AO	AG	AO	OO	*Micrococcus varians*
7	Milky, raised, irregular, translucent	Rod	+	+	-	-	+	+	+	+	+	AG	AG	AG	AG	AG	*Bacillus subtil*is

Key: + Positive reaction; 00 – No reaction

- Negative reactive; AG – Acid, gas production

Table.4 The taxonomic structure, morphological and microscopic examination as well as name of fungal species

Colony colour	somatic structure	Nature of hyphae	Special vegetative structure	Asexual spore	Special reproductive structure	Conidial head	Vesicle shape	Probable organism
White becoming pale green	Filamentous	Septate	Broom-like appearance	Globose chained conidia	Brush-like conidiophores	-	-	*Penicillium nalgiovense*
Bluish green	Filamentous	Septate	Broom like appearance	Globose chained conidia	Brush-like conidiophore	-	-	*Penicillium notatum*
Cottony white	Filamentous	Coenocytic	Stolons, rhizoids	Ovoid sporangio-spores	Tall sporangiophores in groups	-	-	*Rhizopus stolonifer*
Greenish brown	Filamentous	Septate	Sclerotia, swollen conidogenous	Abundant short conidiophores	-	-	-	*Botrytis cineria*
Brownish colony	Filamentous	Septate	Footcell	Globose conidia	Smooth walled erect conidia	Globose	Globose	*Aspergillus niger*
Blue green colony	Filamentous	Setate	Broom like appearance	Subglobose conidia	Highly 3-stage branched conidiophores	-	-	*Penicillium expansum*
Creamish yellow	filamentous	Coenocytic	-	Sporangiospore	Sympodially branched sporan giophore, zygospore	-	-	*Mucor mucido*
Cottony white	Filamentous	Septate	-	One celled conidia in heads (cylindncal in shape)	Solitary phialides	-	-	*Verticillium albo-atrum*
Blackish colony	Filamentous	Septate	-	Phialospores in chains	Erect conidiophores with short branches	-	-	*Humicola sp*
Gray green	Filamentous	Septate	Footell	Globose conidia	Short conidiophores	Dome-shaped broadly clavete		*Aspergillus fumigatus*
Fast growingwhite colony with irregular tufts	Filamentous	Septate	-	1celled conidia in chains	Conidiogenous hyphae	-	-	*Monilia sp.*
Yellow green colony	Filamentous	Septate	Footcell	Globose conida	Phialides borne directty on vesicle, sclerotia	Radiate	Subglobose	*Aspergillus flavus*

Table5 Morphological and microscopic examination and identification of yeasts in fermenting fruits

Colony color	Somatic structure	Nature of hyphae	Pseudomycelium	Asexual reproductive spore	probable organism
Moist milky colony	Unicellular	-	Rudiment pseudomycelium	Budding cells	*Saccharomyces sp*
Reddish colony	Unicellular	Absent	Absent	Blastoconidia	*Rhodotorula sp*
Milky colony	Pseudohyphae	Septate	Well developed pseudomycellium	Blastoconida	*Candida tropicalis*

Table.6 Prevalence frequency occurrence of microbial isolates in tomato fruit

Microbial species	Day 1	Day 2	Day3	Day 4
Lactobacillus fermenti	-	1	4	6
Pseudomonas stutzeri	3	-	-	-
Listeria monocytogenes	1	-	-	-
Leuconostoc sp	1	-	-	-
Rothia sp	20	-	-	-
Rhizopus stolonifer	2	3	3	3
Botrytis cineria	3	-	-	-
Penicillium notatum	14	-	-	-
Saccharomyces sp	15	-	-	-
Rhodotorula sp	1	-	-	3
Aspergillus niger	2	2	1	1
Verticillium albo-atrum	3	4	-	-
Penicillium expansium	1	1	2	9
Mucor mucido	3	1	-	-
Aspergillus flavus	-	-	-	7
Monilia sp	-	-	1	3
Asperigillus fumigatus	3	23	27	3

Key:

— Not isolated

Values Number of colonies

x 10^x Cell population as isolated

Table.7 Prevalence frequency of occurrence of microbial isolates in pawpaw fruit

Microorganisms isolated	Day 1 ($x10^2$)	Day 2 ($x10^4$)	Day 3 ($x10^6$)	Day 4 ($x10^6$)
Micrococcus varians	11	-	-	-
Bifidobacterium bifidum	2	-	-	-
Bacillus subtilis	37	18	8	-
Lactobacillus fermenti	2	2	3	80
Rothia sp	12	4	-	-
Penicillium nalgiovense	1	-	-	-
Saccharomyccs sp	32	-	-	-
Rhizopus stolonifer	1	-	-	-
Aspergillus niger	-	-	4	1
Candida tropicalis	-	-	-	93
Humicola sp	2	-	-	-

Key:

—	Not isolated
Values	Number of colonies
x 10^x	Cell population as isolated

Table 7 shows prevalence frequency of occurrence of microbial isolates in pawpaw fruit. *L. fermenti* were the most frequent because they occurred daily and its colonies were found increasing from day 3 to day 4. Unlike *L. fermenti, M. varians, Penicillium nalgiovense, Saccharomyces* sp, *R. stolonifer* were found only on day 1.

Studies conducted on microorganisms associated with spoilage of tomato and pawpaw fruits show that, in tomato, total

bacterial count on the first day was 2.60x103cfu/g, increased to $2.50x10^3$cfu/g on the second day, decreased to $4.1x10^5$cfu/g on the third day but were absent on fourth day while total fungal count increased continuously from $3.0x10^3$cfu/g on day1 to 9.8 $x10^7$cfu/g on the fourth day as reported by Chukwuka *et al*., (2010). There was no *Lactobacillus* on day1 but increased from $2.4x10^5$cfu/g on day2 to $3.7x10^7$cfu/g on day4. Pathogenic bacteria such as *Listeria monocytogenes* present on day1 and day2 but absent on day3 and day4.

In pawpaw, there was an observable increase in the total number of bacteria colonies from day1 to day2, that is, from $5.0x10^3$cfu/g to $1.9x10^6$cfu/g but decreased to $1.3x10^6$cfu/g on the third day, whereas total fungal count ranged from $3.5x10^3$cfu/g on day1 to $5.0x10^4$cfu/g on day2 to $3.2x10^7$cfu/g on day3 and further increased to $1.50x10^8$cfu/g on the last day (Barth *et al,* 2009). Mould and

Lactobacillus were absent in the first day. No pathogenic bacterium on the day2, 3&4 but was isolated on the first day.

Eleven fungal isolates from tomato included: *Rhizopus stolonifer, Botrytis cineria, Penicilium notatum, Saccharomyces species, Rhodotorula species, Aspergillus niger, Vorticillium albo-atrum, Penicilium expansium, Mucor mucido, Aspergillus fumigatus, Aspergillus flavus.* These agree partly with the findings of Li-Cohen and Bruhn (2002) who discovered that species of fungi associated with the spoilage of some edible fruits including tomatoes include species of *Aspergillus, fusarium, Penicillium and Rhizopus*. The isolation of *Aspergillus niger, Rhizopus stolonifer, Mucor species* from rotten tomato confirmed the studies of Chuku *et al* (2008), Akinmusire (2011) who reported that *A. flavus* and *A. fumigatus* caused tomato spoilage. Spores of *Vorticillium sp* and *Botrytis cineria* are air-borne and may be endemic to the area that this research was conducted.

In paw-paw, seven (7) fungal species such as *Penicillium nalgiovense, P. notatum, Saccharomyces sp, R. stolonifer, Humicola sp, A. niger and Candida tropicalis* were found. Akintobi *et al* (2011) reported that *Rhizopus sp, Aspergillus sp, Penicillium sp* and *Candida tropicalis* as responsible for pawpaw fruit rot in Ibadan south west Nigeria. In this study, *Humicola sp, Saccharomyces sp* were found contrary to the work of Akintobi *et al*., (2011). *Humicola sp* are soil and plant - borne thus could be as a present in the fruit. *Saccharomyces sp* is natural fermenting yeast in sugary foods.

The preponderance of the isolated moulds from tomato and pawpaw belongs to *Aspergillus sp* and *Penicilium sp* and these confirms their prevalence in fruits and foods exposed to tropical humid climate, thus consisting potential health risks to consumers of these fruits and its by products. Six (6) bacterial isolates- *Lactobacillus fermenti, Pseudomonas stutzeri, Listeria monocytogenes, Leuconostoc species, Rothia species*, were found in tomato. Ajayi (2013) isolated *Lecuconostoc sp* and *Lactobacillus sp* as tomatoes natural flora which could participate in spoilage of such fruit. Five (5) bacteria species- *Micrococcus varians,, Bacillus subtilis, L. fermenti, Rothia sp*. were isolated from pawpaw fruit. Presence of *M. varians* in foods causes dental decay and *Bacillus subtilis* causes flat sour of fruits and denaturing of body DNA (Nester *et al.,* 1995).

In pawpaw, *L. fermenti* was present in day 2 uptil day 4. *A. niger* was found on the third day and last day while *C. tropicalis* was seen only on the last day (day 4). *Micrococcus varians, P. nalgiovense, Saccharomyces sp*. and *R. stolonifer* were encountered only on day 1. In tomato, *P. stutzeri, L. monocytogenes, Leuconostoc sp., Rothia sp., B. cineria, Saccharomyces sp P. notatum* were present on day 1 but absent in day 2, day 3 and day 4. *Rhizopus stolonifer, A. niger, Penicillium expansium* and *Aspergillus fumigatus* were present from day 1 to day 4. *Aspergillus flavus* was not found in day 1 to day 3 but was present on day 4.

In previous research works that were accessible although analysis were not conducted on daily basis as done in this study, some microorganisms that were isolated on day 4 and/or day 5 have been reported as the spoilage organisms in tomato and pawpaw fruits in the previous researches. However, spoilage of tomato

and pawpaw are mostly associated with fungi (Singh and Sharma, 2007).

Fruits and vegetables are very important and have high dietary and nutritional qualities. The importance of these fruits with its nutritional and other importance cannot be over emphasized, as its spoilage often result to wastage of economical resources as well as food poisoning.
From the results obtained in this study, it was discovered that few organism encountered are food borne pathogens. It is also revealed that some spoilage microorganism (mostly fungi) gained access into these fruits during the processes of cultivating, harvesting, grading and packing and environmental contaminant which have in one time or the other been involved in food poisoning. The prevalence frequency of occurrence of fungi was higher than that of bacteria in both fruits. The high prevalence of fungi and however, bacteria demand that appropriate control measures against infection should be employed. Adequate microbiological knowledge and handling practices of these produce would help minimize wastes due to deterioration.

It is therefore, important that both the farmers who harvest the fruits into bags for transportation, the marketers, and consumers take necessary precautions in preventing contamination and eating contaminated fruits. This will however, enhance reduction in the risk of microbial toxins that are deleterious to human health which are produced form these microorganism that have been isolated.

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