Identification of Various Micro-organisms Found in Sweetpotatoes and Guava

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INTRODUCTION

The processing of tropical fruits and vegetables is being extensively investigated at the Food Technology Laboratory of the Agricultural Experiment Station of the University of Puerto Rico. Many of these fruits and vegetables can either be hot-packed in cans or frozen. Information is lacking on suitable processing schedules for most of these products. In order to develop proper processing schedules, as well as adequate assay procedures to determine the contamination of frozen products, a research project is being carried out in this Laboratory to identify the natural flora generally present in the fruits and vegetables grown in Puerto Rico. This paper deals with the isolation and identification of organisms present in sweetpotatoes and guavas.

MATERIALS AND METHODS

SWEETPOTATOES AND GUAVAS

Sweetpotatoes of the U.P.R. 3 and Rico varieties were used. All samples were obtained from the Isabela Substation and harvested at 4½ months after planting.

Fresh guavas, at different degrees of ripeness were obtained from various orchards through the eastern portion of the Island.

CULTURE MEDIA

Tryptone Glucose Extract Agar (Difco) and Nutrient Agar (Difco), respectively, were used for the isolation and storage of the micro-organisms obtained from guavas and sweetpotatoes. Special differential media were used for the characterization of the micro-organisms isolated.

EQUIPMENT

A Waring Blendor was used to disperse the guava and sweetpotato in sterile water.

Inoculated media were incubated at 27°, 37°, and 55° C.

SAMPLE PREPARATION

Guavas and sweetpotatoes were peeled under aseptic conditions and the cortex and flesh collected in separate sterile Petri dishes. The cortex was

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transferred to a sterile stainless steel Waring Blendor jar and dispersed in sterile water for 2–3 minutes. Two parts of sterile water was added per part of cortex.

Using this homogeneous suspension as the starting material, serial dilutions were prepared. Aliquots from the various dilutions were seeded in sterile Petri dishes to which warm Tryptone Glucose Extract Agar (Difco) was added. The medium was allowed to solidify and was incubated at 27°, 37°, and 55° C. for over 24 hours.

The same method described above was repeated with the flesh of both products. Guavas were studied at different degrees of ripeness.

TABLE 1.—*Characteristics of individual cells of organisms isolated from sweetpotato*

Item	Characteristics of organism—			
	A	B	C	D
Forms	Long, thin rods	Long rods	Long rods, may be encapsulated	Long, thin rods
Size[1]				
l.	1.75–2.25	1.5–3.5	1.5–3.5	1.5–3.5
w.	0.5 –0.75	0.5–1	0.45–0.75	0.4–1
Gram stain	+	+	+	+
Acid-fast stain	–	–	–	–
Movement	+	+	+	+
Spores	Ellipsoidal subterminal	Ellipsoidal subterminal. Sporangia may be swollen	Ellipsoidal central	Ellipsoidal central to para-central

[1] Size in microns.

All colonies of apparently different organisms were separated and purified after the period of incubation was over.

Morphological and physiological characteristics of each micro-organism were determined. Final classification of the different organisms was carried out in accordance with Bergey's Manual of Determinative Bacteriology.

RESULTS AND DISCUSSION

The results of the tests made for the classification of the bacteria isolated from sweetpotatoes are summarized on tables 1 to 4. It was possible to isolate four apparently different organisms identified by letters A to D.

Studying the characters of the individual cells (table 1) it was found that the four organisms appeared as long, motile, sporogenous, Gram-positive bacilli. Although the colonies belonging to the four organisms were similar in color and form (table 2) they varied in elevation, consistency,

and margin. All the organisms except B produced a white pellicle when grown on Nutrient Broth (Difco). It was found that the optimum growth temperature was around 30° C. These characteristics indicated that the organisms isolated from sweetpotatoes belonged to the Genus *Bacillus*.

Specific biochemical reactions were then studied to determine as closely as possible the species to which each of the four isolates belonged. The diameter of the vegetative rods (table 1); appearance of the protoplasm of young cells growing on glucose agar; growth on soybean agar (*2*)[2], 7-percent NaCl broth (*3*, *4*), tyrosine agar (*5*); citrate utilization; casein and starch hydrolysis; production of catalase, acetyl-methyl-carbinol and lecithinase (*6*) (table 4); and the fermentation of carbohydrates—primarily

TABLE 2.—*Morphology of the colonies of organisms isolated from sweetpotato*

Item	Characteristics of organism—			
	A	B	C	D
Form	Small, circular irregular	Confluent	Circular, confluent	Circular, irregular, confluent
Elevation	Raised	Raised	Effuse or umbonate	Effuse or umbonate
Margin	Entire	Ramose	Entire or erose	Crenate or erose
Consistency	Opaque, smooth	Smooth	Finely granular	Coarsely granular
Color	White	White	White	White
Growth on Broth	White pellicle	Faintly diffused; sediment	White, rough pellicle	White, smooth pellicle

glucose, lactose, sucrose, mannitol, arabinose, xylose, and glycerol—(table 3), were the main criteria used for differentiation into species.

Organisms A, C, and D have a diameter of less than 0.9 μ. They show a good, spreading growth on glucose agar with young cells staining uniformly. They grow well on soybean agar, 7-percent NaCl broth, tyrosine agar, citrate medium, and anaerobic glucose broth under anaerobic conditions. They have a positive reaction to hydrolysis of gelatin and casein and a negative reaction to starch hydrolysis. They produce catalase and acetyl-methyl-carbinol, but give a negative lecithinase test. Organisms A, C, and D appear very similar to *Bacillus licheniformis*.

Organism B has a diameter of over 0.9 μ. It grows well on glucose agar, producing large, vacuolated cells. It produces catalase and acetyl-methyl-carbinol. It has the ability to reduce nitrates and hydrolyze gelatin, casein,

[2] Italic numbers in parentheses refer to Literature Cited pp. 289–90.

and starch, and gives a positive lecithinase test. This organism does not produce acid from arabinose, xylose, or mannitol. It can be concluded that organism B is similar to *Bacillus cereus*.

TABLE 3.—*Fermentation reactions of organisms isolated from sweetpotato*

Carbohydrate	Characteristics of organism—			
	A	B	C	D
Arabinose	−	−	−	−
Rhamnose	−	−	−	−
Xylose	−	−	−	−
Glucose	+	+	+	+
Fructose	+	+	+	+
Galactose	+	+	−	−
Mannose	+	±	−	+
Lactose	+	±	+	−
Sucrose	±	−	±	±
Maltose	−	+	−	±
Trehalose	±	+	−	−
Melibiose	−	−	−	−
Raffinose	−	−	−	−
Melezitose	−	−	−	−
Starch	−	+	−	−
Inulin	−	−	−	±
Dextrin	−	+	−	−
Glycogen	−	+	−	−
Glycerol	±	−	−	±
Erythritol	−	−	−	−
Adonitol	−	−	−	−
Mannitol	+	−	−	±
Sorbitol	−	−	−	±
Dulcitol	−	−	−	−
Salicin	+	+	−	±
Aesculin	+	+	−	±
Alpha-methyl glucoside	−	−	−	−

SWEETPOTATOES

GUAVAS

The results of tests carried out on guavas are summarized on tables 5 to 8. It was possible to isolate nine cultures, seven of which were motile, sporogenous Gram-positive or Gram-variable rods of varying sizes; and two Gram-positive cocci with cells arranged in irregular masses (table 5). The rods numbered from 1 to 7 can grow under aerobic or anaerobic conditions preferably at temperatures close to 30° C. The cocci presented a more

TABLE 4.—*Other biochemical reactions of organisms isolated from sweetpotato*

Reaction	Characteristics of organism—			
	A	B	C	D
Gelatin hydrolysis	+	+	+	+
Indole production	−	−	−	−
Nitrate reduction	+	+	+	+
Catalase production	+	+	+	+
Decomposition of urea	−	−	−	−
Citrate utilization	+	+	+	+
Casein hydrolysis	+	+	+	+
H_2S production	−	−	−	−
Litmus milk reaction	Peptonization, slow reaction	Peptonization, fast reaction	Peptonization, fast reaction	Peptonization, fast reaction
Methyl-red test	±	+	−	−
Voges-Proskauer test	+	+	+	+
Growth on 7-percent NaCl broth	Good; granular pellicle	Good	Good; pellicle	Good
Growth on Anaerobic Glucose Broth	Good; pellicle	Good; diffused	do.	Good; pellicle
Growth on alkaline anaerobic nitrate agar	Good; z+	Good; +	Good; z+	Good; +
Growth on Proteose Peptone Acid Agar	Fair	Very good	Very good	Do.
Growth on Tyrosine Agar	Good; white colonies	Good	Very good; white colonies	Very good; white colonies
Growth on soybean agar	Good	do.	Good, flat, dry growth	Good, flat, dry growth
Growth on Glucose Agar	Good; spreading	do.	Very good; spreading	Good; spreading
Gram-stain reaction on Glucose Agar	Small Gram + rods; stained uniformly	Large, vacuolated Gram + rods	Small, encapsulated, Gram variable rods	Small, Gram + rods, stain uniformly
Lecithinase test	−	+	−	−
Growth on potato plugs	None	Flat, moist, off-white color	Dry, rough wrinkled, beige growth	Dry wrinkled cream-pink color

TABLE 5.—*Characteristics of individual cells of organisms developed from guava*

Item	Characteristics of organism—								
	1	2	3	4	5	6	7	8	9
Form	Long, thin rods, beaded	Long rods	Small rods	Long, thin rods	Long, thick rods	Large rods, beaded	Small rods	Cocci	Cocci
Size[1]									
l.	4–7	1.5–3.5	1–2.5	2.5	2.5–6	3–4	1–2.5		
w.	1	0.5–1	0.4–0.5	0.5–0.75	0.75–1.25	0.75–1	0.35–0.5		
Gram stain	Variable	+	Variable	Variable	+	+	+	+	+
Acid-fast stain	−	−	−	−	−	−	−	−	−
Movement	+	+	+	+	+	+	+	−	−
Spores	Ellipsoidal; subterminal	Spherical to ellipsoidal; subterminal	Ellipsoidal; subterminal	Ellipsoidal; subterminal; sporangia may be swollen	Spherical; subterminal	Ellipsoidal subterminal; sporangia slightly swollen	Ellipsoidal; central		

[1] Size in microns.

TABLE 6.—*Morphology of the colony of organisms isolated from guava*

Item	Characteristics of organism—								
	1	2	3	4	5	6	7	8	9
Form	Large, circular	Large, circular	Tiny, circular, confluent	Circular, small	Large, circular, confluent	Large, circular, confluent	Spreading	Circular, small	Circular, small
Elevation	Low convex or convex	Low convex or convex	Raised	Low convex	Raised	Raised or convex	Raised	Convex	Low convex
Margin	Entire or erose	Entire or erose	Entire or undulate	Entire or irregular	Entire	Undulate or ramose	Entire or curled	Entire	Entire
Consistency	Smooth	Smooth	Rough, dry, or smooth, opaque	Smooth, opaque	Opaque, smooth	Opaque	Opaque or translucent	Smooth	Smooth
Color	Yellow	Yellow	White or yellow	Yellow	Beige to yellow	White	White	Creamy yellow	White
Growth on broth	Granular, scant	Diffused, scant	Granular, scant	Diffused, scant	Diffused, scant	Diffused, scant	Diffused, scant	Diffused	Granular, diffused

luxuriant growth at 37° C. under aerobic conditions, although they developed under anaerobic conditions on glucose broth.

Observing the cellular morphology (table 5) and the characteristics of growth on solid and liquid media (table 6) it was evident that the rods

TABLE 7.—*Fermentation reactions of organisms isolated from guava*

Carbohydrate	Characteristics of organism—								
	1	2	3	4	5	6	7	8	9
Arabinose	±	+	−	−	±	±	−	±	±
Rhamnose	−	−	−	−	−	−	−	−	−
Xylose	±	±	−	−	±	−	−	±	±
Glucose	+	+	+	+	+	+	+	+ (pH 5.0)	+ (pH 5.0)
Fructose	±	+	+	±	+	+	+	+	+
Galactose	+	+	−	±	±	±	+	±	+
Mannose	+	±	±	−	±	−	+	+	+
Lactose	+	+	−	±	+	±	−	+	+
Sucrose	±	±	−	±	+	+	±	±	+
Maltose	+	+	−	+	+	+	−	+	+
Trehalose	−	±	−	−	±	+	±	±	−
Melibiose	−	−	−	+	±	−	−	−	−
Raffinose	+	+	−	−	+	−	−	−	−
Melezitose	−	−	−	−	−	−	−	−	−
Starch	±	±	−	±	+	−	−	−	±
Inulin	−	±	−	−	−	−	−	−	±
Dextrin	+	+	−	±	+	+	−	−	±
Glycogen	−	+	−	+	+	−	−	−	+
Glycerol	−	−	−	+	−	−	−	+ (Slow)	+ (Slow)
Erythritol	−	−	−	−	−	−	−	−	−
Adonitol	−	−	−	−	−	−	−	−	−
Mannitol	±	−	−	−	±	−	±	+	−
Sorbitol	+	−	−	−	−	−	−	−	−
Dulcitol	−	−	−	−	−	−	−	−	−
Salicin	−	−	+	−	−	−	+	−	−
Aesculin	−	±	±	−	±	−	±	−	±
Alpha-methyl glucoside	−	−	−	−	−	−	−	−	−

isolated from guavas belonged to the Genus *Bacillus*; and the cocci were members of the Family Micrococcaceae.

To differentiate the organisms into species a study of their biochemical reactions was carried out. Fermentation reactions on carbohydrates (table 7); hydrolysis of gelatin, casein, and starch; production of indole, hydrogen sulfide, catalase, and acetyl-methyl-carbinol; reaction on nitrates, citrate,

TABLE 8.—*Other biochemical reactions of organismus isolated from guava*

Reaction	Characteristics of organism—								
	1	2	3	4	5	6	7	8	9
Gelatin hydrolysis	+	+	+	+	+	+	+	−	−
Indole production	−	−	−	−	−	−	−	−	−
Nitrate reduction[1]	Z+	Z+	+	Z+	Z+	+	Z+	+	Gas Z+
Catalase production	+	+	+	+	+	+	+	+	+
Decomposition of urea	−	−	−	−	−	−	−	+	+
Citrate utilization	+	+	+	+	+	+	+	−	−
Casein hydrolysis	+ (fast)	+	+	+	+	+	+	−	−
H_2S production	−	−	−	−	−	−	−	−	−
Litmus milk reaction	Peptonization, alkaline	Peptonization	Peptonization, alkaline	Peptonization, alkaline	Peptonization	Peptonization	Peptonization	Acid	Acid, coagulation
Methyl-red test	±	−	±	±	±	+	±	−	+
Vogues-Proskauer test	−	−	+	−	−	−	+	+	−
Growth on 7-percent NaCl broth	Good	Good	Good, pellicle	Good	Poor	Fair	Fair	Diffused, ring	Diffused
Growth on Anaerobic Glucose Broth	Poor	Poor, sediment	Fair, pellicle	Poor	Poor, sediment	Good, sediment	do.	Flocculation sediment, pellicle	Do.
Growth on Alkaline Anaerobic Nitrate Agar	Z+	Z+	Z+	Z+	Z+	+	Z+		
Growth on Proteose Peptone Acid Agar	Good	Better than on nutrient agar	Fair	Fair	Good	Good	Poor		

Growth on Tyrosine Agar	Good, white colony, dark agar	Good, smooth white colony, dark agar	do.	Good, dark agar	Good, amber color agar	do.	Fair		
Growth on Soybean Agar	Good	Good, moist	Good	Good	Good	do.	Good		
Growth on Glucose Agar	Fair, less than on nutrient agar	Fair	Good, spreading	Poor	Good, mucoid	do.	Good, spreading		
Gram stain from Glucose Agar	Large, thick Gram ± rods, beaded	Large, Gram + rods, uniformly stained, capsule	Large, Gram-variable rods, beaded	Large, Gram-variable rods, stain uniformly	Short, thick Gram ± rods bipolar-stain, capsule	Long, thick, Gram + rods, beaded	Small, Gram ± rods stain, uniformly		
Lecithinase test	Undetermined	−	−	−	−	+	−		
Growth on potato plugs	Moist, abundant yellowish white	Moist, yellow	−	Flat, moist white	Abundant moist, beige-white	Moist, white, abundant	−		
Starch hydrolysis	−	+	−	+	−	+	−	−	−
Coagulate test								−	−
Blood hemolysis								−	Beta-hemolysis
Growth on $NH_4 \cdot H_2PO_4$ agar								−	−
Sodium hippurate utilization								−	−

[1] Nitrate-reduction test was done on Nitrate Agar (Difco) incubated at 30° C. A distinct pink or red color (+) indicated the presence of nitrite. If no color appears in tubes with abundant growth, zinc dust is added. The presence of the distinct red color (Z+) indicates the reduction of nitrate to nitrite.

urea, and litmus milk; and growth on a salt-containing medium (table 8) were determined for each culture.

To characterize the species of the Genus *Bacillus* other tests besides the ones mentioned above were carried out. The ability to grow on such media as proteose peptone acid agar (Thermoacidurans Agar Difco), tyrosine agar, soybean agar, glucose agar, and anaerobic glucose broth (table 8) was determined.

Organisms 1 and 5 with a diameter of over 0.9 μ, with cells appearing vacuolated when grown on glucose agar, unable to produce acetyl-methyl-carbinol, and lecithinase (table 8), and fermenting glucose, sucrose, and mannitol (table 7) appeared to be similar to *Bacillus megatherium.*

Organism 6 is also a large bacillus with a diameter of over 0.9 μ and vacuolated protoplasm when grown on glucose agar. The facts that it did not ferment mannitol or xylose (table 7), and that it produced acetyl-methyl-carbinol and lecithinase (table 8) indicated its similarity to *Bacillus cereus.*

Organisms 2, 3, 4, and 7 are all smaller with a diameter of less than 0.9 μ and all stain uniformly when grown on glucose agar. Organisms 3 and 7 vary in size from 1 to 2.5 μ in length by 0.35 to 0.5 μ in width. Both of them grow well on soybean agar and on 7-percent NaCl broth. They do not hydrolyze starch, do not produce acetyl-methyl-carbinol; but produce lecithinase. It can be said that organisms 3 and 7 are similar to *Bacillus pumilus.*

Organisms 2 and 4 grow well on soybean agar and 7-percent NaCl broth, but show poor development on anaerobic glucose broth. When grown on tyrosine agar they produce a pigment which turns the agar dark. These organisms are classified as *Bacillus subtilis* var. *niger.*

Organisms 8 and 9 appeared as nonmotile, Gram+ spherical cells arranged in irregular masses. Both of them produce small, circular, smooth colonies with entire margin, creamy yellow to white in color (table 6). Both give an acid reaction in litmus milk and organism 9 caused coagulation also. Organism 8 showed no hydrolysis of starch, aesculin, and sodium hippurate (*7*). Both of them reduced nitrate, and give a positive reaction to production of catalase and decomposition of urea. Neither one utilized $NH_4H_2PO_4$ (*8, 9*) as a source of nitrogen. Organism 9 shows beta-hemolysis on blood agar plates; not so organism 8. The coagulase test for both of them was negative. Although various characteristics shown by organisms 8 and 9 appear to diverge from those expected of a *Staphylococcus aureus*; these organisms appear closer to this Genus than to any other of the Genera encountered in the Family Micrococcaceae.

All the organisms isolated from guavas and sweetpotatoes were encountered in the cortex. This is to be expected inasmuch as the isolates

are organisms commonly found in nature whose habitat is the soil, dust particles, and plant surfaces.

SUMMARY

A study was undertaken for the purpose of obtaining information about the flora normally encountered in sweetpotatoes and fresh guavas.

Homogeneous suspensions of the cortex and flesh of both products were prepared in sterile water and used for the isolation of micro-organisms. Isolations were carried out on Tryptone Glucose Extract Agar plates incubated at 27°, 37°, and 55° C.

An extensive study of the morphological and physiological characteristics of each micro-organism was carried out in accordance with the 7th edition of the Bergey's *Manual of Determinative Bacteriology* (1957).

It was found that the organisms isolated from sweetpotatoes are similar to the Genera *Bacillus cereus* and *B. lichemformis.* Those isolated from guava were classified as *Bacillus megatherium*; *B. cereus*; *B. pumilus*; *B. subtilis* var. *niger*; and *Staphylococcus aureus.*

RESUMEN

El presente trabajo incluye los datos obtenidos de un estudio que se llevó a cabo para obtener información acerca de la flora que se encuentra normalmente en la guayaba y en la batata fresca.

Suspensiones homogéneas de la corteza y la pulpa de ambos productos fueron preparados en agua estéril, las cuales fueron usadas para aislar los microorganismos.

Se hizo un estudio abarcador de los caracteres morfológicos y fisiológicos de cada organismo para lo cual sirvió de guía la Séptima Edición del *Manual de Bacteriología de Bergey* (1957).

Se encontró que los organismos aislados de la batata son similares a los de los géneros *Bacillus cereus* y *B. lichemformis.* Los de la guayaba se clasificaron como *Bacillus megatherium*; *B. cereus*; *B. pumilus*; *B. subtilis* var. *niger* y *Staphilococcus aureus.*

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