Kasetsart J. (Nat. Sci.) 42 : 321 - 327 (2008)

Enumeration and Identification of Microorganisms in Plantation White Sugar from Factories in Thailand

Sirivatana Chittrepol[1], Malai Boonyaratanakornkit[2]* and Klanarong Sriroth[1]

ABSTRACT

Enumeration of mesophilic bacteria and thermophilic bacterial spores from thirty-seven plantation white sugar factories were investigated. The total mesophilic count at 35°C and thermophilic flat sour spores count at 55°C were range from 0-670 CFU/10 g of sugar and 0-1,470 spores/10 g of sugar respectively. Thermophilic anaerobes were not observed where as yeast and mold count and pathogenic bacteria were undetectable in all samples. Eighty-four isolates of thermophilic bacteria were found in samples of twenty-seven factories. Based on physiological, biochemical and phenotypic characteristics, most of isolates were classified as *Bacillus coagulans* which were being the dominant species. *B.circulans* and *B.brevis* were found in the samples of two factories where as *B.macerans* was found in the only one factory.

Key words: plantation white sugar, thermophilic bacteria, *Bacillus* sp.

INTRODUCTION

Plantation white, or mill white, sugar is commonly produced for local consumption in sugarcane growing countries. It is produced at the factory without remelting and refining of raw sugar. If a white product is desired, sulfur dioxide may be bubbled through the cane juice before evaporation. Sugar bleached white by this sulfitation process is called “mill white”, “plantation white”, and “crystal sugar” (http://en.wikipedia.org/ wiki/Sugar_cane).

Sucrose, the most commonly used sweetener, is extracted and purified from cane (*Saccharum officinarum*) and beet (*Beta vulgaris*) (Muller, 1986). Other sweeteners are derived from enzymatic and acid hydrolysis of corn starch to yield corn syrups with various concentrations of reducing sugars. Microorganisms such as *Bacillus stearothermophillus*, *B.coagulans*, *Clostridium thermosaccharolyticum*, *C.nigrificans*, certain mesophilic bacteria, yeast and molds may be present but not grow in starch, sugars and syrups (Clark and Tanner, 1937). However, these organisms can cause spoilage of products when they are present in starches and sweeteners used as ingredients for other foods.

Low water activity is largely responsible for the microbial stability of natural sweeteners (Tilbury, 1976). Sugar will remain microbially stable if they are kept dry. Microorganisms in sweeteners come from the raw source materials or from the manufacturing processes (Kramer and Gilbert, 1989). High processing temperature or

1 Department of Biotechnology, Faculty of Agro-industry, Kasetsart University, Bangkok 10900, Thailand.

2 Department of Applied Microbiology, Institute of Food Research and Product Development, Kasetsart University, Bangkok. 10900, Thailand.

* Corresponding author, e-mail : ifrmlb@ku.ac.th

Received date : 22/11/07 Accepted date : 01/02/08

low water activity of natural sugar afford little opportunity for microbial survival and growth, but recontamination may occur after heat treatment. Plant and equipment sanitation as well as water quality have been identified as factors affecting microbial contamination (Whistler and Paschall, 1967). As a rule, there are usually $<10^2$ microorganisms per gram of sweetener (Scarr, 1968) and there is usually <1 yeast per gram (Tilbury, 1976). Organisms that are likely to survive are sporeforming mesophilic aerobic and thermophilic bacilli.

Sporeforming bacteria in Thai plantation white sugar are not seriously concerned as a part of microbiological quality as mentioned in Thai Standard for Sugar UDC 664.11 (Thai Industrial Standard Institute, 1973) . In this standard for plantation white sugar, pathogenic bacteria should be undetectable and coliform bacteria are not more than 1 per 100 gram sugar. Our aim of this study was to present the microbiological diversity which could be fundamental data for food factories.

MATERIALS AND METHODS

Sample sources

Samples of plantation white sugar were collected from thirty seven factories in Thailand.

Enumeration of microorganisms

Sample preparation and analysis methods were performed by following the Canners' Microbiological standards for sugar (Junk and Pancoast, 1973). The lists of analysis were as follows:

1. Total Mesophilic Count
2. Yeast and Mold Count
3. Thermophilic Spores Count
   - 3.1 Flat Sour spores
   - 3.2 Thermophilic anaerobes not producing H_2S
   - 3.3 Thermophilic anaerobes producing H_2S

Detection of coliform and pathogenic bacteria

Methods of analysis for the MPN/g of Coliform bacteria and pathogenic bacteria (Staphylococcus coagulase positive, *Streptococcus* and *Salmonella* spp.) were analysed by following Thai Industrial Standard for Sugar (UDC 664.11, 1973).

Bacterial strains

Strains studied were isolated from plantation white sugar of thirty-seven factories. The isolates were selected by morphological variation of colonies on enumerating media. Colonies were selected based on colony appearance and on microscopic inspection of the cells.

Cultural conditions

All isolates were grown and maintained on nutrient agar. The incubation temperature was 55°C for optimum growth and kept at 4-6°C in refrigerator for maintenance of the cultures.

Bacterial identification

Several thermophilic flat sour bacterial isolates were identified. Bacterial cells were observed under light microscopy after Gram's and endospore stainings. Physiological and biochemical characteristics were studied by following the Bergey's manuals of determinative bacteriology (Buchanan and Gibbons, 1974).

RESULTS AND DISCUSSION

Enumeration of mesophilic and thermophilic bacteria

Samples of plantation white sugar collected from thirty-seven factories were examined for mesophilic baeteria count and thermophilic spore count. The results showed that the samples of plantation white sugar from thirty-seven factories were contaminated with mesophilic bacteria in the range of 0-670 CFU/10 g of sugar

Table 1 Mesophilic bacteria count and thermophilic flat sour spores count in plantation white sugar from thirty-seven factories.

Factories	Mesophilic bacteria CFU/10 g	Thermophilic flat sour spores/10 g	Factories	Mesophilic bacteria CFU/10 g	Thermophilic flat sour spores/10 g
1	160	170	20	60	120
2	50	150	21	70	30
3	120	140	22	670	0
4	20	30	23	450	740
5	50	100	24	130	70
6	50	160	25	0	140
7	160	30	26	0	20
8	60	40	27	0	140
9	50	0	28	40	20
10	60	40	29	30	30
11	30	0	30	10	10
12	40	10	31	520	0
13	60	0	32	30	0
14	80	0	33	60	320
15	670	1,470	34	0	840
16	50	30	35	0	1,020
17	80	10	36	0	20
18	210	0	37	0	0
19	70	0			

(Table 1). There was no standard for the mesophilic bacteria count of plantation white sugar. There microorganisms may be found in white sugar by post contamination process and they are minor important to a canner because heat treatment would inactivate them (Junk and Pancoast, 1973).

Sugar as a source of thermophilic spoilage was firstly reported by Cameron *et al*. (1928). This work was examined the amount of thermophilic spores count in plantation white sugar samples of thirty-seven factories. The results showed that thermophilic flat sour spores were contaminated in plantation white sugar of twenty-seven factories in the ranges of 0-1,470 spores/10 g of sugar (Table 1). Most of samples contaminated with thermophilic flat sour spores of 100-200 spores/10 g of sugar (Table 1). Standard of white sugar by the National Food Processors Association's Bacterial Standard for Sugar (Horwitz, 1975) mentioned that in the five samples examined there shall be a maximum of not more than 75 spores of thermophilic flat sour in any one sample and an average of the five samples of not more than 50 spores per 10 gram of sugar. The total thermophilic spore count standard is, for the five samples examined there shall be a maximum of not more than 150 spores and an average of not more than 125 spores per 10 gram of sugar.

From this experiment, white sugar samples of some factories were under the microbiological standard due to the high count of spore loads. The high load of thermophilic spores in the samples might come from the raw materials or the poor sanitation of the processes. Detection of thermophilic anaerobic spore formers, coliform

Table 2 Isolates of thermophilic flat sour spores from plantation white sugar factories in Thailand.

Factories	No. of isolates	Factories	No. of isolates
1	A1 A2 A3 A4 A5	20	D13 D14 D15
2	A6 A7 A8 A9 A10	21	D16 D17
3	A11 A12 A13 A14 A15	22	-
4	A16 A17 A18	23	D18 D19 D20 D21 D22 D23
5	A19 A20 A21 A22	24	D24 D25
6	A23 A24 A25	25	D26 D27
7	A26 A27 A28	26	G1
8	A29 A30 A31	27	G2 G3
9	-	28	G4 G5
10	D1 D2	29	G6 G7 G8
11	-	30	G9
12	D3	31	-
13	-	32	-
14	-	33	G10 G11 G12 G13 G14
15	D4 D5 D6 D7 D8 D9	34	G15 G16 G17 G18 G19
16	D10 D11	35	G20 G21 G22 G23 G24
17	D12	36	G25 G26
18	-	37	-
19	-		

Note : The letters A1 to G26 represented for the number of isolates
- = not detected

and pathogenic bacteria were undetectable in all samples.

Isolation of thermophilic flat sour spores

In order to isolate thermophilic flat sour spores which could produce acid, samples from different plantation white sugar factories in Thailand were prepared by weighing 20 g into 100 ml steriled water and boiled for 5 minutes, cooled and plated on a solid medium of glucose tryptone agar containing 0.004% bromcresol purple. Agar plates were incubated at 55°C. Table 2 showed the source and distribution of all isolates that had ability to grow at high temperature and produce acid. A total of 84 isolates was selected as described in materials and methods.

The distribution of all isolates was widespread. Samples of some factories were not detected. It should be noted that extremely heat resistance thermophilic flat sour bacteria required high temperature for destruction spores. The presence of thermophilic flat sour spores in ingredients were generally the one of concerns in food canning operations. The flat sour bacteria had no public health significance (Stumbo, 1973).

Identification of selected isolates

From preliminary study, all isolates were identified. They occured singly or in chains and were straight rods (0.6 to 1.2 μm wide by 2.5 to 5.0 μm long in size), gram positive and endospore-forming strain. They were aerobic, motile, catalase positive. From the morphological studies, stainings and biochemical examinations, all isolates were identified as the genus *Bacillus* (Buchanan and Gibbons, 1974).

For further study, all of isolates could be classified by physiological and biochemical characteristics into fours groups. Group I were

Table 3 Phenotypic characteristics of the group I isolates and a species of *Bacillus coagulans*.

Characteristics	Isolates of group I			*B.coagulans*
	A1 to A31	D1 to D15	G7 to G25	
Rod-shaped	+	+	+	+
Gram reaction	+	+	+	+
Motility	+	+	+	+
Catalase	+	+	+	+
Voges-Proskauer reaction	-	-	-	-
Indole production	-	-	-	-
Nitrate reduction	+	d	-	d
Growth at 37°C	+	+	+	+
50°C	+	+	+	+
65°C	-	-	-	-
Growth in 7% NaCl	-	-	-	-
Starch hydrolysis	+	+	+	+
Casein hydrolysis	-	-	-	d
Tyrosine decomposition	-	-	-	-
Carbohydrate fermentation				
Glucose	+	+	+	+
Arabinose	+	d	+	+
Xylose	d	d	d	d
Mannitol	d	d	d	d
Growth in anaerobic agar	+	+	+	+
Citrate Utilization	+	-	-	+

Symbols : + = positive ; - = negative ; d = substantial proportion of reactions differ

the isolate numbers A1 to A31 except A18 and A25, D1 to D15 except D11 and G1 to G25 except G7 and G25. The biochemical characteristics of these isolated were aerobic and exhibited positive catalase reaction, growth at 50 – 55°C, and did not grow at 7% NaCl. They could ferment carbon sources listed in Table 3. Based on the definition of Buchanan and Gibbons (1974), isolates of Group I were closely related to the species of *Bacillus coagulans*. Belamri, *et al.* (1992) reported that there were several species of bacillus in sugarcane juice such as *Bacillus stearothermophilus, B. subtilis, B. brevis, B. cereus and B. coagulans*. During this study, *B. coagulans* was clearly observed. It contaminated from the raw juice and detected in plantation white sugar of many factories.

Group II consisted of the isolate numbers G7 and G25. The physiological and biochemical characteristics were classified as *Bacillus circulans*. Group III consisted of the isolate numbers A18 and D11 and Group IV had only one isolate, which was A25. These isolates were classified as *Bacillus brevis* and *Bacillus marcerans*, respectively and shown in Table 4. *Bacillus* species encountered in foods were generally widespread. Spores and also vegetative cells were found in foods, water, soil and decomposing vegetation. Resistance to extremes of temperature and chemical substances was greater in spores than in vegetative cells. Therefore, it was not surprising that spores were detected readily in many foods and ingredients such as sugar, starch, cereal grains and spices.

Table 4 Phenotypic characteristics of the group II isolates (*B.circulans*), group III isolates (*B.brevis*) and group IV isolates (*B.marcerans*).

Characteristics	Isolates of group II		Isolates of group III		Isolates of group IV
	G7	G25	A18	D11	A25
Rod-shaped	+	+	+	+	+
Gram reaction	+	+	+	+	+
Motility	+	+	+	+	+
Catalase	+	+	+	+	+
Voges-Proskauer reaction	-	-	-	-	-
Indole production	-	-	-	-	-
Nitrate reduction	-	+	-	-	+
Growth at 37°C	+	+	+	+	+
50°C	+	+	+	+	+
65°C	-	-	-	-	-
Growth in 7% NaCl	+	+	-	-	-
Starch hydrolysis	+	+	-	-	+
Casein hydrolysis	-	-	+	+	-
Tyrosine decomposition	-	-	+	+	-
Carbohydrate fermentation					
Arabinose	-	-	-	-	+
Xylose	-	-	-	-	+
Mannitol	-	-	+	+	+
Growth in anaerobic agar	-	-	-	-	+
Citrate utilization	-	-	+	+	+

Symbols : + = positive ; - = negative

CONCLUSION

In conclusion, the microbial contamination in the plantation white sugar samples collected from thirty-seven factories in Thailand was closely related to genus *Bacillus*. It was thermophilic flat sour sporeformers which could be classified into four species as *Bacillus coagulans*, *Bacillus circulans*, *Bacillus brevis and Bacillus marcerans*. The percentage of predominant *Bacillus coagulans* was 71.43% based on the total of eighty-four isolates. *Bacillus coagulans* could contaminate canned food and gave a flat sour taste. These bacteria were common soil organism. Their spores had $D_{120°C}$ of 0.1 min. (Adams and Moss, 1995). These studies demonstrated that thermophilic aerobic sporeformers in plantation white sugar may cause spoilage of canned foods and fruit drinks, since such products practically are received only a hot – fill or pasteurization heat treatments.

ACKNOWLEDGEMENTS

We would like to express our thanks to thirty - seven plantation white sugar factories for their kindness of providing all samples. This research was partially supported by the Graduate School of Kasetsart University.

LITERATURE CITED

Adams, M.R. and M. O. Moss. 1995. The microbiology of food preservation, pp. 18-102. *In* **Food Microbiology.** Cambridge : The Royal Society of Chemistry.

Belamri, M., L. Fakhereddine and A. Tantaoui – Elaraki. 1992. Determination of optimal growth conditions for saccharolytic bacteria from diffusion juice. **Int. Sugar J.** 97 (1119) : 57 – 60.

Buchanan, R.E. and N.E. Gibbons. 1974. **Bergey' s Manual of Determinative Bacteriology**. 8th ed. The Williams and Wilkins Company / Baltimore. 1246 p.

Cameron, E.J., C.C. Williams and R.J. Thompson. 1928. Bacteriological field studies in canning : Thermophilic contamination of peas and corn. **Natl. canners Assoc. Bull**. 25-L.

Clark, F.M. and F.W. Tanner. 1937. Thermophilic canned food spoilage organisms in sugar and starch. **Food Res**. 2 : 27-39

Horwitz, W. 1975. Thermophilic bacterial spores in sugar : official first action, pp.920-921. *In* **Offical methods of analysis of the Association of official Analytical chemists. AOAC**. Washington. D.C., USA.

Junk, W.R. and H.M. Pancoast. 1973. **Handbook of Sugar for Processors, Chemists and Technologists**. The AVI Publishing Co., Inc. Westport, Connecticut. 327 p.

Kramer, J.M. and R.J. Gilbert. 1989. *Bacillus cereus* and other Bacillus species, pp. 21-70. *In* M.P. Doyle (ed.). "**Food-born bacterial pathogens**". Marcel Dekkar. New York.

Muller, E.G. 1986. The sugar industry, pp. 141-164. *In* "**Quality Control in the Food Industry**" Vol. 3. 2nd ed. Academic Press. New York.

National Food Processors Association. 1972. Bacterial standards for sugar. **Natl. Food Processors Assoc**. Washington, D.C.

Scarr, M.P. 1968. Symposium on growth of microorganism at extremes of temperature : Thermophiles in sugar. **J. Appl. Bacteriol**. 31 : 66 – 74.

Stumbo, C. R. 1973. **Thermobacteriology in Food Processing.** 2 nd ed. Academic Press. New York. 329 p.

Tilbury, R.H. 1976. The microbial stability of intermediate moisture foods with respect to yeasts, pp. 138-165. *In* **Intermediate Moisture Foods.** Applied Science Publishers, London.

Thai Industrial Standard Institute. 1973. **Standard for Sugar.** UDC 664. 11. Ministry of Industry, Bangkok. 65 p.

Whistler, R.L. and E.F. Paschall. 1967. **Starch : Chemistry and Technology**. Vol. II. Academic Press. New York. 733 p.